

**EFFECT OF LIME, PHOSPHORUS AND *RHIZOBIUM* INOCULATION ON
BEAN (*Phaseolus vulgaris* L.) GROWTH AND YIELD IN AN ACID MOLLIC
ANDOSOL**

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**A Thesis Submitted to the Board of Graduate School in Partial Fulfillment for the
Requirements of a Master of Science Degree in Soil Science of Egerton University**

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APPROVAL PAGE

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This thesis is my original work and has not previously been published or presented for a degree or a diploma in this or any other university.

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DEDICATION

To my Uncle Clement Onsomu and my late mother Mrs. Eunice Onchabo for their patience, sympathy and both financial and moral support they accorded me during the preparation of this manuscript.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance.
ATP	Adenosine triphosphate.
BNF	Biological Nitrogen Fixation.
CIAT	Centro Internacional de Agricultura Tropical
DM	Dry matter.
DMRT	Duncan's Multiple Range Test.
CEC	Cation exchange capacity.
FAO	Food and Agriculture Organization.
KARI	Kenya Agricultural Research Institute.
MPN	Most Probable Number.
MSTAT-C	Microcomputer statistical program for experimental design and analysis.
SOM	Soil organic matter.
UH ₂	Upper highland.
WFS	Weeks from sowing

ABSTRACT

Bean yields in most small holder farms in Kenya are low. The low bean yields are due to low or non-use of fertilizers, poor soil management practices, inherent acid soil infertility, diseases and pests. In order to determine the potential for increasing bean yields, a field trial was laid out for two seasons in Molo on a mollic *Andosol*. A greenhouse experiment was conducted at Egerton University to determine rhizobial populations at the trial sites. Three factors: lime, phosphorus and *Rhizobium* inoculation at 2, 3 and 2 levels respectively were combined to give 12 treatments which were allocated to plots in a randomized complete block design with three replications. Lime significantly ($p \leq 0.05$) increased soil pH, available soil P, exchangeable Ca and Al, number and weight of nodules and grain yield in both seasons but was only significant on N and P plant contents and rhizobial populations in the second season. Phosphorus significantly ($p \leq 0.05$) increased soil available P, number and weight of nodules, number of pods/plant and seeds/pod, grain yield, plant N and P contents in both seasons and rhizobial populations in the second season. *Rhizobium* inoculation significantly ($p \leq 0.05$) increased plant N content and rhizobial populations in both seasons. There was a significant positive lime by phosphorus interaction on number and weight of nodules in the first season and on yield in the second season. Lime by *Rhizobium* inoculation interaction significantly ($p \leq 0.05$) increased weight of nodules and grain yield in the first season and number of pods/plant and grain yield in the second season. Phosphorus by *Rhizobium* inoculation interaction significantly increased number and weight of nodules in the first season and yield in the second season. The lime by phosphorus by *Rhizobium* inoculation interaction only significantly increased number of nodules in the second season. Lime significantly ($p \leq 0.05$) increased yields and reduced soil acidity stress hence liming is beneficial to bean production in acid soils. Phosphorus increased bean growth and yield. Higher yields were realized at 120 kg P_2O_5 /ha than 60 kg P_2O_5 /ha implying that high rates could be recommended for improved bean yields in acid soils. No yield response was observed with inoculation without P or lime in the mollic *Andosol*. Lime and phosphorus should be applied to enhance bean yields in mollic *Andosols*. In addition, the favourable response to inoculation though to a limited extent in some cases stresses the need for the adoption of these treatments either singly or in combination in acid soils.

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CHAPTER ONE

1.0

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most commonly grown grain food legume in Kenya. There is a wide range of cultivars with varied adaptability to vast agro-ecological zones thereby making common bean a popular and one of the staple food crops in the country. Beans constitute an important source of readily available protein for the rural population of Kenya where animal protein sources are inaccessible and expensive. In addition to other legumes, beans are valuable for improving soil fertility in farming systems because of their ability to fix atmospheric nitrogen (Searle *et al.*, 1981; Pilbeam *et al.*, 1995). Bean production is done by small-scale farmers mainly in a maize-bean intercrop though production under crop rotation is sometimes practiced. About 500,000 hectares in Kenya are under bean production (Kimani *et al.*, 1997).

A major problem with bean production in the country is low yields that range from 400 kg/ha to 600 kg/ha (Kimani *et al.*, 1997) against a potential of up to 3000 kg/ha (Gethi and Muriithi, 1993). The low yields have been attributed to low soil fertility, limited use of both organic and inorganic fertilizers, land deterioration through plant mining, erosion or the deterioration of soil physical conditions (Kimani *et al.*, 1997). Low bean yields are also realized due to widespread use of poor quality planting seed and diseases/pests (Ombiri, 2000). Most tropical acid soils have low amounts of nitrogen and wide spread phosphorus deficiency (Alexander, 1977; Schroder, 1992). In addition to the low soil N and P in acid soils, aluminium and manganese toxicities (Foy, 1984) as well as calcium and magnesium deficiencies (Clark, 1984), have also been reported as important constraints to bean production. The low N problem in tropical acid infertile soils can be sustainably addressed through biological nitrogen fixation (BNF), a symbiotic association between the root nodule bacteria (*Rhizobium* spp.) and a grain legume such as beans (Giller and Wilson, 1991; Schroder, 1992). The

successful use of biological nitrogen fixation process as a means of providing nitrogen has however been met with various obstacles including occurrence of acid soils, aluminium toxicity and phosphorus deficiency (Keyser and Munns 1979a,b; Giller and Wilson, 1991). The establishment of a productive symbiosis with *Rhizobium* spp. is often prevented by microsymbiont susceptibility to acidity stress in the rhizosphere or during the early phases of root nodule formation (Calvalho *et al.*, 1982).

Acid soil infertility in bean production can be corrected through a number of methods including liming and high P fertilizer application (Kamprath and Foy, 1985), *Rhizobium* inoculation and N-fertilization (Giller and Wilson, 1991; Schroder, 1992). Lime raises soil pH and consequently makes P available, besides reducing amounts of aluminium, iron and manganese in soil solution by precipitating them and thus reducing their toxicity (Friesen *et al.*, 1980; Kunishi, 1982). Lime also increases the supply of Ca and/or Mg (Ismael *et al.*, 1993), rhizobial populations and enhances nodulation (Evans *et al.*, 1980; Ssali, 1981). Inoculation with *Rhizobia* facilitates bean nodulation, nitrogen fixation and improves yields (Giller and Wilson, 1991). The combination of lime, phosphorus and *Rhizobium* inoculation would thus help alleviate the acid soil infertility problems favourably and improve bean nodulation, dry matter production and grain yields.

1.1 Statement of the problem

Bean yields in Molo Division situated in central Rift Valley, Kenya, are quite low averaging about 450 kg/ha. The low yields could be attributed to acid soil infertility given that the active pH of the soils is 4.4 whereas the optimum pH for bean growth ranges between pH 5.5 and 6.7. In addition, poor farm management practices such as use of low rates and wrong type of fertilizers. Continuous cropping could also equally contribute to the low yields. There is a potential for soil acidification in the region through the continual use of ammonium-

based fertilizers. It is apparent that no suitable soil management packages have been put in place to curb the soil acidification in the area. Acid soil infertility results to P-fixation, Ca and Mg deficiencies and Al, Mn and Fe toxicities, which are serious soil constraints to achieving high bean nodulation and yields. These soil conditions are also unfavourable for most soil microbes and thus injurious to the nitrogen-fixing bacteria (*Rhizobium* spp.), which are sensitive to low pH and Ca. Consequently, native strains of the *Rhizobia* are usually present in only small numbers, which are ineffective in causing proper bean nodulation.

1.2 Justification of the study

Common bean of the cultivar Rosecoco is widely grown and popular with most farmers in Kenya because of its adaptability, attractive market prices, taste and good cooking properties. Its yields need to be increased so as to provide a cheap source of protein both for the rural and urban Kenyan population. To achieve this, a sustainable strategy for overcoming P deficiency and related soil acid infertility problem in potential bean growing areas needs to be found. It is envisaged that lime, phosphorus and *Rhizobium* inoculation would be effective measures to allow for improved bean growth and yield in acid soils. Lime would overcome the deleterious effects of Al, Mn and Fe toxicities by raising the soil pH and provide for essential nutrients like calcium, magnesium and phosphorus as well as increased rhizobial populations, dinitrogen fixation and general microbial activities that are important in bean nodulation and yield. Phosphorus was expected to give positive yield responses and overcome element P deficiency. *Rhizobium* inoculation would enhance early nodule development, which in turn would provide nitrogen for vigorous vegetative growth, and support high seed yields. The combination of *Rhizobium* inoculation with lime and phosphorus would thus favourably reduce the deleterious effects of soil acidity and provide for favourable conditions that would enhance better bean growth and yield. This study therefore would enhance knowledge on acid soil management for optimum bean production.

1.3 Objectives

The broad objective of this study was to improve bean yields and ensure on farm food security in acid infertile soils. The specific objectives were to determine:

- (1) Yield responses of beans to lime, phosphorus and *Rhizobium* inoculation in a mollic *Andosol*.
- (2) The effect of lime, phosphorus and *Rhizobium* inoculation on the nodulation, N and P uptake of beans in a mollic *Andosol*.
- (3) The effect of lime, phosphorus and *Rhizobium* inoculation on the rhizobial population in a mollic *Andosol*.

1.4 Hypotheses

In order to achieve the above set objectives, the following hypotheses were postulated:

- (1) Lime, phosphorus and *Rhizobium* inoculation would increase bean yields.
- (2) Lime, phosphorus and *Rhizobium* inoculation would increase bean nodulation and N and P uptake.
- (3) Lime, phosphorus and *Rhizobium* inoculation would increase rhizobial population in acid soils.

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Effects of soil acidity on bean production

The common bean (*Phaseolus vulgaris* L.) is the most important food legume for human consumption worldwide (CIAT, 1993). Its production in humid and sub-humid tropics is limited by low soil fertility, particularly soil acidity related nutrient deficiencies (nitrogen, phosphorus, calcium and magnesium) and toxicities (aluminium, iron and manganese) (Foy 1980; Ta *et al.*, 1989; Aggarwal, 1994; Wortmann, 1994). Wortmann (1994) classified low available soil-nitrogen and phosphorus as well as toxicity of aluminium and manganese in that order, as the main soil fertility constraints to bean production in Africa. Under acidic soil conditions, crop production is also likely to suffer from N deficiencies and low microbial populations (Sanchez, 1976; Alexander, 1977). Hydrogen ions have also been reported to affect root growth and functioning at pH values below 5.5 irrespective of Al concentration (FAO, 1984). Even though different bean genotypes respond to soil acidity differently, the majority of genotypes are noticeably affected by pH less than 4.9 (Sanchez and Uehara, 1980; Dwivedi, 1996; Verma and Singh, 1996; Keya, 1997). Although beans can obtain their N requirement from N fixation, the activity of symbiotic *Rhizobium* bacteria is markedly reduced by low soil pH and P deficiency (Keyser and Munns, 1979b; Vadez *et al.*, 1997).

2.1.1 Phosphorus deficiency

Low soil phosphorus is the principle nutritional problem of beans and is probably the most widespread and frequent limitation to bean production in the tropics. Phosphorus availability in soils is the primary limitation to bean production in the tropics, restricting the development of sustainable agriculture in the region (Ssali and Keya, 1986; Yan *et al.*, 1996). It is estimated that over 50 % of common bean production in the tropics is limited by P deficiency (CIAT, 1987; CIAT, 1992). The P deficiency is probably the most important nutritional

factor limiting nodulation, nitrogen fixation and yield of the beans in many tropical soils (Vidor *et al.*, 1979; Graham, 1981). It causes a considerable reduction in net photosynthesis, short, sometimes dwarfed plants with thin stems and shortened internodes and consequently shoot growth in beans (Haustchild *et al.*, 1996). It also causes a prolonged vegetative period and a shortened reproductive phase. Flowering is delayed, few flowers are produced, and the level of aborted blossoms is high, thus few pods form and contain only a small number of seeds consequently reducing the grain yields (Malavolta *et al.*, 1981; Howler, 1991). The deficiency of P also limits nitrogenase activity and nodulation (Vadez *et al.*, 1997). Similar observations have been reported with different legume plants such as a decrease in the number of pods and seeds of faba bean (Xia, 1997) and seed weight of soybean (Wang, 1981).

2.1.2 Aluminium toxicity

Under a tropical climate, soil acidity and, consequently, aluminium toxicity, is a major constraint to the production of food crops (Horst and Klotz, 1990; Manrique, 1993). Aluminium toxicity has been implicated as a contributing factor in poor bean yields observed in acid soils (Reeve and Sumner, 1970). It has been ranked as the second principal nutritional problem of beans (Abruna *et al.*, 1974) and is strongly related to phosphorus and calcium deficiencies. High aluminium concentrations inhibit bean growth and reduce the capacity to exploit soil water and nutrients (Mugai and Agong, 1996). With lime applications to neutralize the aluminium, only the first 20 cm of soil are affected and this causes roots to grow horizontally rather than deeper into the non-affected soil. The plant is therefore stunted and grows poorly. Aluminium toxicity inhibits cytokinin production by root tips and suppresses its transport to beans (Massot *et al.*, 1994) and soybean (Pan *et al.*, 1988) shoots and hence reduces shoot growth. The inhibition of the uptake, translocation and utilization of phosphates (Haynes, 1982; Foy and Fleming, 1978) also characterize aluminium toxicity.

Aluminium also binds P in soil solution, at root surfaces and cell walls, making it unavailable for cell metabolism (Haynes and Naidu, 1991). In addition, phosphate ions present in acidic soils react with Al, Fe, and Mn, rendering P unavailable for plant growth (Hue *et al.*, 1991). These stresses interact with other soil and plant factors to reduce growth, poor germination and seedling establishment, poor nodulation and low yields.

Aluminium has been shown to strongly affect nodulation by delaying nodule appearance (Calvalho *et al.*, 1981). The effect of aluminium on nodulation occurs before any significant effect on growth of tops, root growth or root elongation (Calvalho *et al.*, 1981). Mugai and Agong (1996) found that high aluminium concentration (10 ppm and above) markedly reduced bean nodulation. They also noted that there was a consistent reduction in the shoot yield from Al concentration of 20 ppm and above. Root yield also displayed a trend similar to that of the shoot and the reduction in root dry matter yield related strongly to both fresh and dry shoot yields. It is apparent that Al toxicity affected plant growth and development primarily through impairment of the root system. Similar observations have been reported in groundnuts (Syed-Omar *et al.*, 1991) and soya bean (Pan *et al.*, 1988).

2.1.3 Calcium and magnesium deficiency

Calcium deficiencies are almost always found in plants suffering from aluminium toxicity or a combination of aluminium and manganese toxicities. Magnesium deficiency usually occurs in acid soils with low base levels and on volcanic ash soils with low levels of potassium and calcium. Acid soils with pH levels below 5.5 normally have low levels of calcium and magnesium (Sanchez, 1976; Clark, 1984; Samson *et al.*, 1986). While calcium deficiency results in poor root development and sometimes collapse of the hypocotyl in beans (Helms, 1971), magnesium deficiency causes interveinal chlorosis of lower leaves. Calcium and magnesium deficiencies affect nodulation and nodule growth much more than root and shoot

growth in bean plants (Cassman *et al.*, 1980). Lowther and Loneragan (1968) also found that root infection and nodule initiation in subterranean clover have a much higher calcium requirement than the root and shoot growth. Magnesium deficiency may be assumed to have a corresponding detrimental effect on carbohydrate supply to the root nodule and thus on the nitrogen fixation rate. Calcium deficiency has also been found to affect plant height and production of dry matter in beans (Malavolta *et al.*, 1981), limit nodulation in soybean (Franco and Munns, 1982), dry matter production in subterranean clover and podding in groundnuts (Syed-Omar *et al.*, 1991).

2.2 Effect of soil acidity on bean growth and yield

Inhibition of nodulation in beans can be caused by limited persistence of *Rhizobium* strains in acid soils. Successful establishment and subsequent persistence of legumes depend largely upon development of an effective nitrogen fixing symbiosis between the host plant and the appropriate *Rhizobium* species (Graham, 1981; Weaver and Graham, 1994). Colonization of soil or the legume host rhizosphere by the homologous *Rhizobium* can markedly affect nodulation and dinitrogen fixation. With annual legumes, certain aspects of the nitrogen fixing (N₂ fixing) symbiosis with root nodule bacteria are determined very early in the life of the plant and therefore, root (rhizosphere) colonization by the bacteria is a prerequisite step in the processes leading to nodule formation and nitrogen fixation (Vincent, 1980). Hence, the presence and supply of an appropriate *Rhizobium* strain, specificity between *Rhizobium* strain and the host and competition between native *Rhizobia* and introduced high-efficiency strains are important factors for nodulation and fixation of nitrogen (Graham, 1978 and 1981). For the inoculant strain to be effective, it must be more competitive than strains in the native populations (Weaver *et al.*, 1972; Weaver and Frederick, 1974; Weaver and Graham, 1994). Bean nodulation is however more dependent on soil conditions especially associative soil acidity than *Rhizobia* survival when added in high concentrations to the soil (Lovato, 1984).

Munns (1968) observed fewer and delayed appearance of nodules at higher acidity levels even after inoculation. Elsewhere, in the field as well as in pots in a greenhouse, Wolff *et al.* (1993) found that nodule numbers of *Phaseolus vulgaris* at early stages of plant development were reduced by 30-50 % in acid soils compared with neutral soils.

The *Rhizobium* species are sensitive to low pH and soil calcium levels (Mulder, 1968). Consequently, native strains of *Rhizobia* are usually present in only small numbers, if at all, in an acid soil. An increase in soil pH may increase the population of the native strains or create conditions more favourable for their effective functioning (Evans *et al.*, 1980; Karanja *et al.*, 1995). When bacteria have to multiply extensively from a small inoculum, acidity could conceivably limit nodulation by slowing the development of the rhizosphere population. This may explain benefits to nodulation from heavy inoculation in acid soils. Nevertheless, even when a large rhizobial population accumulates rapidly, nodulation remains pH dependent (Munns, 1968). Carter *et al.* (1995), observed that the persistence and competitiveness of *Rhizobium leguminosarum* strains in acid soils with high naturally occurring populations of *Rhizobia* was poor.

2.3 Effect of lime on soil fertility, bean nodulation and yield

Lime is used to correct acid soil infertility and P fixation in tropical acid soils (Sanchez, 1976; Kamprath and Foy, 1985). Dwivedi (1996) observed that liming an acid soil increased the soil pH linearly to near neutrality and reduced exchangeable H, Al, Fe, and Mn thereby raising the exchangeable Ca and Mg status of the soil. Aluminium, Fe and Mn uptake by crops were also greatly reduced.

Maximum phosphate availability to plants occurs when soil pH is maintained in the range of 6.0-7.0 (Kamprath and Fox, 1985; Sutton and Hall, 1987; Nurlaeny *et al.*, 1996). Lime often

increases the utilization of soil phosphates by plants through amelioration of aluminium toxicity (Haynes, 1982). Extractable aluminium is reduced to low levels by liming (Naidu *et al.*, 1990) and this enhances formation of more soluble calcium phosphate, increases mineralization of organic phosphates and decreases P fixation thereby availing more P to plants (Miranda and Rowell, 1987). Enhancement of root growth as a result of aluminium detoxification is another means by which lime may enhance P uptake and growth (Haynes, 1984). Improved root growth is attributable to improved soil calcium status and formation of less toxic Al species; AlSO_4^- or Al^{3+} precipitation (Oades, 1979; Pavan *et al.*, 1982; Noble and Sumner, 1988). Fox *et al.* (1991) found that by increasing the pH of volcanic ash soils in Hawaii by 0.5 units through liming, the exchangeable aluminium decreased to a half of its value accompanied by a decrease in P fixation. Verma and Singh (1996) observed that liming an acid *Alfisol* was effective in increasing exchangeable and extractable P, P uptake and yield of soya beans.

There are however conflicting views on the effect of lime on P availability in highly weathered acidic soils (Friesen *et al.*, 1980; Haynes, 1984). Liming can increase (Dalal, 1986), decrease (Amarasiri and Olsen, 1973) or have no effect (Miranda and Rowell, 1987), on the availability of P. Amarasiri and Olsen (1973), found that lime decreased P in solution and labile P, and increased P sorption capacity of the soil. This is attributable to increased sorption capacity to reactions of the added P with freshly precipitated iron and aluminium hydroxides. The effect of lime therefore on P availability may depend on the extent to which P is sorbed by reactions with the exchangeable aluminium and adsorbing surfaces.

Bean performance in terms of yield in acid soils is poor and the legume–rhizobium symbiosis is often limited (Andrew, 1978; Yost *et al.*, 1985). Suppression of exchangeable aluminium in such soils through liming enables better nodulation of roots and thus results in higher

yields (Ssali, 1981). Lime promotes legume nodulation, as root infection is favoured by improved rhizosphere pH and Ca contents (Andrew and Johnson, 1976; Samson *et al.*, 1986). Significant increases in nodule number, pod number (Syed- Omar *et al.*, 1991), plant growth parameters and yield (Gracia, 1995), have been observed in tropical and temperate legumes due to liming. Lime pelleting of inoculated seeds has also been sown and used widely in acid soils, and usually improves nodulation (Morales *et al.*, 1973; Cordero and Blair, 1978; Graham, 1981). Graham and Donowa (1981), found that when an acid soil was amended from an initial pH of 4.6 to pH 6.5 by liming with CaCO_3 (4 g/kg soil), the shoot weight of groundnuts increased by 30 % and nodule numbers by 112 %.

2.4 Effect of phosphorus fertilizer on soil fertility, bean nodulation and yield

Phosphorus fertilization in acid soils is important in overcoming acidity induced element deficiency. Large additions of P fertilizer to soils could reduce the adverse effects of aluminium toxicity due to direct precipitation of Al-phosphates. Also, soluble, yet non-toxic Al-P complexes may form in the soil solution (Alva *et al.*, 1986). An indirect role of P in alleviating soil acidity is plant-root mediated. Phosphorus promotes root extension and enhances the ability of plant roots to penetrate deeper into acidic zones (Mckenzie and Nyborg, 1989). Phosphorus may also cause roots to divide and elongate at a rate faster than the rate of diffusion of Al ions to the meristem thus avoiding Al injury (Aniol, 1991). High rates of P however are required to mitigate Al toxicity (Robson, 1989), since sub optimal supply of P to plants, even at levels sufficient to preclude normal nutritional P-stress, may seriously reduce tolerance to Al in Al-toxic soils (McLaughlin and James, 1991).

Inorganic P fertilization has been found to increase dry matter production of beans and ultimately the organic matter content in soils, nodule development, dinitrogen fixation (Graham and Rossas, 1979), P uptake and tissue nitrogen yield (Ssali and Keya, 1986). Grain

yield and yield components (pods/plant and seeds/pod) are also affected favourably (Fagaria and Carvalho, 1996). According to Gobara *et al.* (1993), phosphate fertilization resulted in increased dry matter content of beans significantly at early stages. The importance of P for nodulation as well as for metabolism has been demonstrated and discussed by Giller and Wilson (1991). Low available P in soils has been shown to be responsible for poor bean nodulation and consequently, low dinitrogen fixation (Karanja *et al.*, 1997). Ssali and Keya (1983) obtained a three-fold increase in bean nodulation when 150 kg P/ha was applied to a *Nitisol* in Kabete, central Kenya. Floor (1985), showed through field experiments that application of phosphorus increased nodule mass, dry matter yield, nitrogen yield and nitrogen fixation. Beans require more phosphorus when dependent on nitrogen fixation than when they depend on fertilizer nitrogen (Keya *et al.*, 1981; Floor, 1985). Nodules have been shown to have a high P requirement, with P contributing to an increase in the number and size of nodules (Bonetti *et al.*, 1984). Phosphorus is an essential element for increased nodulation (weight and number of nodules) (Saito and Ruschel, 1976).

2.5 Effect of *Rhizobium* inoculation on bean growth and yield

The introduction of large numbers of effective *Rhizobia* into the rhizosphere through legume inoculation procedures will promote, but not ensure, establishment of the symbiotic relationship. Inoculation failures often occur in soils having low populations of effective *Rhizobia* (Graham, 1981; Weaver and Graham, 1994). Moreover, there is a keen competition for nodule sites among tropical native *Rhizobia* and inoculated strains (Vidor *et al.*, 1979). Therefore, large numbers of rhizobial cells would seem necessary to ensure sufficient nodulation especially if there is a large native *Rhizobia* species (Weaver *et al.*, 1972). The relative competitive abilities for nodulation of native strains irrespective of the population size, may be a factor in determining inoculum success.

Inoculation of beans (*Phaseolus vulgaris* L.) with nodulating bacteria (*Rhizobium phaseoli*) is not a common practice in East Africa (Ssali, 1981). However, positive nodulation and yield responses are occasionally observed with proper cultivars, inoculum strains, carriers and application rates (Ssali, 1981; Sparrow and Hem, 1983). Inoculation with commercial strains compared with uninoculated controls has been shown to increase bean yields (Weiser *et al.*, 1985; Karanja *et al.*, 1997). *Phaseolus vulgaris* was found to respond to inoculation at low nitrogen levels in terms of grain yields (Sakala, 1985). Similarly, Abebe and Abegazi (1985) and Abebe (1987) observed significant increases in grain yields of beans but noted that response of *Phaseolus vulgaris* to inoculation differs from place to place and from variety to variety. Inoculation of late maturing bean varieties has been found to produce significantly higher dry matter and grain yield at maturity compared to early maturing cultivars (Abebe and Abegazi, 1985; Ssali and Keya, 1986).

Nevertheless, successful nodulation and seed yield increases of dry beans are not always observed after inoculation with *Rhizobium phaseoli* in the bean producing regions (Weiser *et al.*, 1985). In East and South African regions, this lack of yield response to inoculation has been reported by Ssali and Keya (1983) and Ssali (1988). This lack of response is due to the presence of large populations of compatible indigenous *Rhizobia* effective in nitrogen fixation (Lalande *et al.*, 1995). Further, poor response of *Phaseolus vulgaris* to inoculation in Africa has been attributed to lack of available phosphorus and other environmental factors such as aluminium toxicity and low pH (Ssali and Keya, 1983; Giller, 1990; Giller and Wilson, 1991). Chege (1994) observed no response in *Phaseolus vulgaris* grown in acid soils of Uasin Gishu (Kenya) inoculated with a commercial *Rhizobium* inoculant. This lack of response could have been due to low soil pH and aluminium toxicity that could have affected the efficiency of the symbiotic *Rhizobia* used in the study.

Colonization of the legume host rhizosphere by its homologous *Rhizobium* can enhance nodulation and thus dinitrogen fixation. Lovato *et al.* (1985) obtained better nodulation, dry matter accumulation and total nitrogen in the shoots in soils with higher pH. These results indicate that bean nodulation and nitrogen fixation can considerably be affected by adverse soil conditions such as soil acidity, rather than by *Rhizobium phaseoli* survival in the rhizosphere. Herridge *et al.* (1984) and Brockwell *et al.* (1989) have confirmed the effectiveness of indigenous *Rhizobium* in the soil for nodulating beans.

2.6 Nitrogen and phosphorus uptake and accumulation in beans

The pattern of N accumulation in the plant will vary to some extent with nutrient levels and their balance in soil, tillage practices, temperature, soil type, and moisture availability (Tisdale *et al.*, 1990; Hardter and Horst, 1994). Beans need more nitrogen than any other nutrient. Relatively high nutrient concentrations in the plant are necessary for maximum growth during the vegetative phase. Friedrich and Schrader (1979) demonstrated that the amount of N redistributed from vegetative tissue is highly dependent upon soil N availability during growth. Maximum N content in plant tissue may be constrained by soil N availability and plant N uptake. Normal unfurled, trifoliate leaves of beans contain about 5 % N, but if these leaves are deficient in nitrogen, they may contain about 3 % N. At first, beans fulfil their nitrogen requirements from the reserve present in cotyledons. However, beans begin to exhibit symptoms of nitrogen deficiency 14-20 days after emergence if they do not receive nitrogen fertilizer. It is at this stage of development that beans form nitrogen-fixing nodules. From about day 30 to 60, the nitrogen requirement increases almost linearly, with maximum absorption occurring at about day 56. With the formation of pods, a great part of the plant's nitrogen passes to the developing seeds. By harvest time, almost 90 % of the nitrogen in a bean plant is found in the seeds (Graham, 1979). Nitrogen in senescing aboveground parts of a crop is made available to the growing grain while root nitrogen appears in soils as roots

decompose (Swinnen, 1994). Without fertilization, N uptake will depend on mineral N reserve in the soil and the rate of N mineralization (Gallagher, 1984).

Phosphorus is one of the essential plant nutrients. It is a component of every living cell as part of the nucleic acids. It is also vital in energy transfer needed by plants for metabolism (Troeh, 1993). This element is present in plant tissues and soils in smaller amounts than are N and K. Phosphorus is taken up from soil in H_2PO_4^- and HPO_4^{2-} forms by plants and unless the soil contains adequate phosphorus or it is supplied to soil from external sources, plant growth will be restricted. Little of this element is lost by leaching and crop removal is generally small (Tisdale *et al.*, 1990). Plant accessible soil phosphorus levels can undergo a gradual decline where crop removal exceeds the amount being returned to soil by fertilizers, animal manure and crop residues (Tisdale *et al.*, 1990). Phosphorus uptake by crops is influenced by soil available P, soil pH and form and method of fertilizer P application (Bekunda, 1990). Small additions of lime to acid Hawaiian *Latosols* greatly increased the uptake of P fertilizer (Fox *et al.*, 1964). But liming to pH 7.6, drastically reduced P uptake when the initial pH was 5.8 (Sumner, 1979).

CHAPTER THREE

3.0

MATERIALS AND METHODS

This study consisted of field and greenhouse experiments. A field trial was conducted in the short and long rain seasons of the years 1999 and 2000 respectively while a greenhouse experiment for the quantification of rhizobial population was carried out before first planting and after first and second harvesting.

3.1 Field trial

The field trial was conducted in a farmer's field located 5 km South East of Molo Town ($0^{\circ}12'S$, $35^{\circ}41'E$), Nakuru District. The altitude at the trial site was about 2680 metres above sea level. The mean annual rainfall is about 1250 mm and is distributed into long rain (April to June) and short rain (September to November) seasons. The long rain season receives about 450 mm to 650 mm of rainfall whereas the short rain receives about 300 mm to 500mm of rainfall annually. The mean maximum and minimum air temperature is $20.6^{\circ}C$ and $6.9^{\circ}C$ respectively (Jaetzold and Schmidt, 1982). The rainfall and temperature data recorded in a nearby Molo National Pyrethrum Research Sub-Centre during the experimental period are shown in Appendix 1 and 2 respectively. In 1999 and 2000, the total number of wet days were 108 and 124 respectively giving a total annual rainfall of 1966 mm and 2237 mm respectively. The mean maximum and minimum air temperature was $20.7^{\circ}C$ and $6.6^{\circ}C$ in 1999 and $21.5^{\circ}C$ and $6.8^{\circ}C$ in 2000 respectively. The experimental site lies in the wheat-pyrethrum agro-ecological zone (UH₂), characterized by a long cropping season that has two distinct variable cropping seasons. The long rain season is utilized for growing wheat, maize, beans, peas, cabbage, kales, carrots and potatoes while the short rains season is used for growing peas, vegetables, beans, and potatoes. Pyrethrum is grown throughout the year (Jaetzold and Schmidt, 1982).

A representative soil profile pit was dug and described within the farm in which the trial was conducted (Table 1).

Table 1: Soil profile pit characteristics

Horizon symbol	Depth	pH	Texture	Bulk density (g/cm ³)	CEC (cmol/kg)	%OC	Porosity
AP1	0-20	4.5	Loam	0.8	17.5	1.4	0.8
AP2	20-29	4.6	Clay loam	1.2	18.5	1.2	0.6
AP2	29-46	4.5	Clay loam	1.0	20.3	1.1	0.5
BA	46-75	4.2	Clay loam	1.0	18.2	1.3	0.6
BW1	75-109	3.9	Clay loam	1.04	19.0	0.9	0.5
BCW2	109-134	4.0	Clay	1.30	19.7	0.9	0.4
CB	134-204	4.0	Clay loam	1.37	16.8	0.7	0.3

The slope of the site was 6-7 % with a North East slope aspect/direction. The soils at the site have a very deep, well-drained, dark brown to strong brown B-horizon with clear distinctions of the different horizons through its depth especially when moist. It had a big thick topsoil horizon with a smeary consistence. Root distribution was normal with the exception of a plough pan at 20-29 cm deep, most of them being fine and concentrated at the top 20-cm layer. The soils are moderately well drained, humic loam, moderately deep with dark brown sub-soil and a medium textured topsoil, classified as mollic *Andosols* (FAO-UNESCO, 1990). The soil chemical and physical properties of the experimental site determined before liming are given in Table 2.

Table 2: Some chemical and physical properties of the top 20-cm soil of the experimental site

Soil Property	Measure
pH (1:2.5) - Water	4.4
pH (1:2.5) - KCl	3.9
Organic Carbon (%)	1.2
Total Nitrogen (%)	0.09
C/N ratio	13
Available P (Olsen) (ppm)	7.2
Extractable bases (cmol/kg)	
Ca	4.5
Mg	2.4
Na	2.1
K	0.9
CEC (cmol/kg)	17.0
Exchangeable acidity (cmol/kg)	1.9
Exchangeable aluminium (cmol/kg)	0.7
Aluminium Saturation (%)	16.0
Bulk density (g/cm ³)	0.8
Texture	
Sand (%)	28
Silt (%)	31
Clay (%)	41
Textural Class	Clay loam
Rhizobial population (Number/gram of soil)	32

3.1.1 Experimental design and treatments

A randomized complete block design with a 2x3x2 factorial arrangement replicated three times was used to investigate the effect of lime, *Rhizobia* inoculation and phosphorus application and their interactions on bean nodulation, yield and rhizobial populations in a mollic *Andosol*. The treatments were the factorial combinations of two levels of lime (0 and 6 t/ha), three levels of P₂O₅ (0, 60 and 120 kg/ha) and two levels of *Rhizobium* inoculation (inoculation and no inoculation). The treatments and field layout with randomized allocation of treatments was as shown in Table 3 and Figure 1 respectively.

Table 3: Treatments

Treatment No.	Lime (t/ha)	Phosphorus (kg P ₂ O ₅ /ha)	<i>Rhizobium</i> inoculation	Treatment description
1	0	0	No inoculation	Control
2	6	0	No inoculation	Lime
3	0	0	Inoculation	<i>Rhizobium</i> inoculation
4	0	60	No inoculation	60kg/ha P ₂ O ₅
5	0	120	No inoculation	120kg/ha P ₂ O ₅
6	6	0	Inoculation	Lime x <i>Rhizobia</i>
7	6	60	No inoculation	Lime x 60kg/ha P ₂ O ₅
8	6	120	No inoculation	Lime x 120kg/ha P ₂ O ₅
9	0	60	Inoculation	60kg/ha P ₂ O ₅ x <i>Rhizobia</i>
10	0	120	Inoculation	120kg/ha P ₂ O ₅ x <i>Rhizobia</i>
11	6	60	Inoculation	Lime x 60kg/ha P ₂ O ₅ x <i>Rhizobia</i>
12	6	120	Inoculation	Lime x 120kg/ha P ₂ O ₅ x <i>Rhizobia</i>

REP III

T12	T7	T6	T4	T5	T1	T2	T11	T9	T3	T10	T8
P25	P26	P27	P28	P29	P30	P31	P32	P33	P34	P35	P36

REP II

T6	T1	T4	T3	T8	T11	T9	T10	T5	T12	T2	T7
P24	P23	P22	P21	P20	P19	P18	P17	P16	P15	P14	P13

REP I

T7	T3	T9	T1	T5	T10	T4	T8	T2	T11	T6	T12
P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12

Key: T = Treatment and P = Plot number.

Figure 1: Field layout with randomized allocation of treatments

The plot sizes measured 4.0 m by 2.5 m and each replication measured 120 m². There was a 1m path between the blocks and a 50-cm path between the plots. Phosphorus fertilizer was applied as Triple superphosphate (46% P₂O₅). Koru agricultural lime with a chemical composition of 49% calcium oxide (CaO) was used and the *Rhizobium Phaseoli* inoculum (Biofix) supplied by the MIRCEN project of Soil Science Department of the University of Nairobi was used.

3.1.2 Establishment of field experiment

Land preparation was carried out using a hand hoe. The lime treatment was broadcast uniformly on the soil surface at a predetermined rate of 6 t/ha from an incubation experiment and mixed thoroughly with the topsoil. The land was then tilled into a fine seedbed and the lime allowed to equilibrate for two weeks before planting.

Bean seeds (Rosecoco variety) were inoculated with the *Rhizobium* inoculum as follows: Gum Arabic (as a sticking agent) was dissolved in warm distilled water at a rate of 30 g/300 ml of water and then used to uniformly wet the seeds. The seeds were then mixed with the inoculant thoroughly until all the seeds were uniformly covered with the inoculum. The inoculated seeds were protected from direct sunlight by covering the seeds with paper and keeping them under shade before planting. Three bean seeds were sown per hill at a spacing of 50 cm between the rows and 20 cm within the rows to give a total of 8 rows per plot and 12 hills per row. Thinning was done 2 weeks after emergence leaving 2 plants per hill thereby giving a plant population of 200,000 plants per hectare. At planting, P fertilizer at 60 kg P_2O_5 /ha and 120 kg P_2O_5 /ha was applied at respective hills and mixed well with the soil. Weeding and harvesting was done manually.

3.2 Soil and plant sampling

3.2.1 Soil sampling and preparation

Three random soil samples were obtained from different horizons of the profile pit and analyzed for moisture content, soil pH, texture, organic carbon, CEC, and bulk density. In addition, three random soil samples were collected from each block at a depth of 0-20cm and then mixed thoroughly to obtain a bulk composite sample for site characterization. The soil samples were analyzed for soil pH (water and KCl), soil texture, total N, available P, Ca, Mg, CEC, organic carbon, exchangeable acidity, exchangeable aluminium and aluminium saturation. After the first and second harvesting, soil sampling was done for every plot to determine the soil pH, total N, extractable P, Ca, Mg, and exchangeable aluminium. The soil samples were air dried at room temperature and ground to break soil lumps and sieved through a 2mm sieve before analysis. The samples for the analysis of total N and organic carbon were further ground in a mortar and sieved through a 0.3-mm sieve before analysis.

The soil samples for inoculation in the greenhouse were obtained from the field 5 hours prior to inoculation. The soil samples were taken from the surface up to a depth of 20cm using sterilized soil sampling equipment. The samples were put into polyethylene bags and transported in a cool box. In the laboratory, the fresh soil samples were thoroughly mixed on a clean and sterilized working bench and passed through a sterilized coarse sieve to remove stones and large pieces of non-decomposed organic matter. A representative sample was obtained by the quartering system (Okalebo *et al.*, 1993).

3.2.2 Plant sampling

- i. **Seedling emergence:** The number of plants that had germinated in 14 days after planting was established by physical counting and recorded for each plot. Seedling emergence was expressed as a percentage of the number of seeds sown.
- ii. **Nodule number and weight:** Three bean plants per plot were randomly sampled at 50 % flowering by carefully uprooting them. The soil attached to the roots was washed off under tap water and number of nodules counted. The nodules were then carefully detached, oven dried at 60°C and their dry weight determined after attaining a constant oven dry weight.
- iii. **Number of pods/plant and seeds/pod:** Pods were counted on 3 randomly selected plants per plot at 90 days after planting while a random sample of 6 pods per plot was taken at harvesting and the seeds were counted to score the number of seeds per pod.
- iv. **Grain yield:** At harvest all the plants in the middle four rows were uprooted, threshed and the shelled grains dried in an oven at 30°C for 7 days and the grain yield determined at 12 % moisture.

3.3 Soil analysis

3.3.1 Determination of lime requirement

Lime requirement was determined using the incubation method described by Hinga *et al.* (1980). Five grams of dry soil was placed into a series of 7-100 ml beakers and 0. 5, 10, 20, 30, 40 and 50 ml of 0.04N Calcium hydroxide were added. CO₂-free distilled water was added to each of the beakers to bring the final volume to 50 ml. The contents of each beaker were stirred and incubated for three weeks to allow for equilibration. The pH in each beaker was measured after adjusting the solution level to 50 ml with distilled water where necessary. A buffer curve was then plotted and used to determine the lime required to a selected pH (6.5) as follows:

$$\text{Amount of lime (kg/ha)} = (2 \times 10^9 \times K / 100\text{g}) \times 0.037 / 10^6.$$

Where: K = meq of Ca(OH)₂ from the buffer curve (amount of lime required to raise the pH by that margin in 100 g of soil).

$$1 \text{ meq} = 0.037\text{g of Ca(OH)}_2$$

3.3.2 Soil pH

The soil pH was determined by use of a calibrated Fisher Accumet® pH meter model at a soil: solution ratio of 1:2.5 (Okalebo *et al.*, 1993). Ten grams of soil was placed in 60-ml plastic shaking bottle and 25 ml of distilled water/1N KCl added. The mixture was shaken for 10 minutes on a mechanical electric shaker, allowed to stand for 30 minutes and stirred again for two minutes before taking the pH readings.

3.3.3 Exchangeable acidity/aluminium

Exchangeable acidity and aluminium were determined using titration method described by Anderson and Ingram (1993). Twenty-five ml of 1M KCl was added to a 10g-soil sample in 50 ml plastic shaking bottle and mixed by shaking on a mechanical electric shaker for 30

minutes. The mixture was left to stand for 30 minutes and filtered into a 250-ml flask using Whatman No. 42 filter papers. The mixture was leached with 5 successive 25-ml aliquot increments of 1M KCl to a total of 150 ml. Four drops of phenolphthalein indicator was added to the filtrate and titrated with 0.1N NaOH to the first permanent pink colour end point. The titrations were corrected for a blank titration of 150-ml KCl solution. The KCl - extractable acidity was calculated and expressed in cmol/kg of soil as follows:

$$\text{KCl acidity} = \frac{(S - B) \times N \times 100}{W}$$

Where; S = ml of NaOH for sample.

B = ml of NaOH for blank.

N = normality of NaOH (0.1N)

W= Weight of soil sample (g)

To estimate the level of Al, the titre for NaOH was recorded and then 10 ml of 1N KF was added to the 150-ml filtrate solution and titrated with 0.1N HCl until the pink colour disappeared. The contents were left to stand for 30 minutes and additional HCl was added to a clear end point. Exchangeable Al was calculated and expressed in cmol/kg as follows:

$$\text{KCl exchangeable Al} = \frac{V \times N \times 100}{W}$$

Where; V = ml of HCl

N = normality of NaOH(0.1N)

W = Weight of soil sample (g)

3.3.4 Available soil phosphorus

Available soil P was determined using Olsen's method as described by Okalebo *et al.* (1993). The soil P was extracted with 0.5 M solution of sodium bicarbonate at pH of 8.5 at 1: 20 soil to extractant ratio. Soil sample weighing 2.5 g was placed into a 150-ml plastic-shaking bottle, 50 ml of Olsen's extracting solution and a scoop of activated charcoal were added to

each bottle. The bottles were stoppered and shaken on a mechanical-electric shaker for 30 minutes. The suspension was filtered through Whatman No. 42 filter paper. Phosphate standard solutions containing 0, 0.5, 1, 2.5, 5, 7.5, 10 and 12.5 ppm of P were prepared.

Ten millimetres of each P standard solution, 10 ml of sample filtrates and 2 reagent blanks were consequently pipetted into 50-ml volumetric flasks. Five ml of 0.8 M boric acid was added to each flask. Beginning with the standards and the blanks, 10 ml of ascorbic acid reagent was added to each flask and made to the mark with distilled water. The contents were shaken well and the absorbance of the solutions measured at a wavelength setting of 880 nm after 1 hour. A standard P curve was obtained from the absorbance values of the P standard solutions, which was used to estimate the concentration of phosphorus in the filtrates. Correction for the reagent blank P concentration was made by subtracting the reagent P concentration from the sample P concentration, hence:

$$\text{Available P (ppm)} = \text{corrected P in solution (ppm)} \times 100.$$

3.3.5 Total soil nitrogen

Total soil N was determined by the Kjeldahl digestion procedure as described by Okalebo *et al.* (1993). Soil samples weighing 0.3g were placed into digestion tubes, 4.4 ml of digestion mixture added and the contents digested for two hours at 360 °C. Each digest was made up to the 50-ml mark with distilled water, shaken thoroughly and then allowed to settle. A 10 ml aliquot sample solution was steam distilled after adding 10 ml of 40 % NaOH into 5 ml 1% boric acid containing 4 drops of the mixed indicator. The distillate was titrated with N/140 HCl to a definite pink colour end point.

$$\% \text{ N} = \frac{C \times 0.1}{W}$$

Where; C = Corrected ml of N/140 HCl

W = Weight of soil sample

3.3.6 Soil organic carbon

The organic carbon content was determined by the wet oxidation method as described by Okalebo *et al.* (1993). Soil sample weighing 0.5g was placed into a block digester tube into which 5-ml potassium dichromate solution and 7.5 ml concentrated sulphuric acid was added. The tube was placed in a preheated block at 145–155°C for exactly 30 minutes and then removed to cool. The digests were transferred quantitatively to 100-ml conical flasks, 0.3 ml of ferroin indicator added and then titrated with ammonium sulphate solution to a brown end point. The percentage organic carbon was calculated using the formula:

$$\% \text{ Organic Carbon} = \frac{T \times 0.2 \times 0.3 \times 100}{W}$$

Where; T= Corrected titre volumes.

W= Weight of soil sample.

3.3.7 Cation exchange capacity

The cation exchange capacity of the soil was determined by the ammonia saturation method (Hinga *et al.*, 1980). Five grams of soil sample was weighed into a 250 ml conical flask. Twenty five ml of 1.0 N ammonium acetate–pH 7 was added to the soil in the flask, mixed by swirling and then left to stand for 30 minutes with occasional swirling. The mixture was filtered into a 100-ml volumetric flask using Whatman No. 42 filter paper. Further 25 ml of ammonium acetate solution was added to the remaining soil in the conical flask, shaken and let to stand for 15 minutes after which it was transferred to the filter funnel. The ammonium-saturated soil was leached with 25-ml portions of 10 %-acidified sodium chloride at a time until 225 ml had passed through the sample. The leachate was transferred quantitatively into an 800 ml Kjeldahl flask where 25 ml of 1.0 N sodium hydroxide was added. About 60 ml of the solution was distilled into a 150-ml conical flask containing 5.0 ml of 2 % boric acid. 4 drops of a mixed indicator were added and then the boric acid solution titrated with standard

0.1 N sulphuric acid to a pink colour end point. Reagent blanks were also run simultaneously.

The CEC in the soil was calculated as follows:

$$\text{CEC (cmol/kg)} = \frac{B - T}{W} \times 100$$

Where; B = Blank titre

T = Titration value

W = Weight of soil sample

3.3.8 Exchangeable calcium and magnesium

The cations were extracted with excess ammonium acetate solution (pH 7) and their amounts determined by atomic absorption spectrophotometry (Okalebo *et al.*, 1993). Five grams of soil sample were weighed into a clean plastic bottle and 100 ml of 1 M ammonium acetate (NH₄OAc) solution (pH 7) added. The contents were shaken for 30 minutes on a mechanical-electric shaker and filtered through No. 42 Whatman paper. This soil extract was then used for Ca and Mg determination.

The soil extract was diluted 10 times for Ca determination by pipetting 5 ml of the solution into a 50 ml volumetric flask, 1 ml of 26.8% lanthanum chloride solution added and diluted to the mark with 1 M NH₄OAc extraction solution. Standard solutions of calcium were prepared by pipetting 0, 1.25, 2.5, 5, 7.5 and 10 ml of calcium standard stock solution into 500 ml volumetric flasks. To each flask, 10 ml of 26.8 % lanthanum chloride solution and 100 ml of the 1 M NH₄OAc were added and made to the mark with distilled water. The sample solutions were sprayed into the atomic absorption spectrophotometer flame. The standard solutions were first measured to calibrate the instrument and to obtain the standard curve which was used to obtain the concentration of the element and expressed in cmol/kg of soil as:

$$\text{Ca (cmol/kg)} = \frac{\text{Graph reading}}{20} \times 10$$

For Mg determination, the soil extract was diluted 17 times by pipetting 3 ml of the soil extract solution into a 50 ml volumetric flask, 5 ml of 5000 ppm Sr as SrCl_2 added and filled to the mark with the 1 M NH_4OAc extracting solution. Standard solutions were prepared by pipetting 0, 2, 5, 10, 15 and 20 ml of magnesium standard stock solution (200 ppm Mg) into 1 litre volumetric flasks. 100 ml of 5000 ppm SrCl_2 and 100 ml of 1 M NH_4OAc extracting solution was added to each flask and made to the mark with distilled water. These solutions contained 0, 0.4, 1, 2, 3 and 4 ppm Mg respectively. The sample solutions and the standard solutions were sprayed into the flame of the atomic absorption spectrophotometer. A standard curve was obtained from the standard solution readings and the concentration of magnesium in the samples calculated and expressed in cmol/kg as follows:

$$\text{Mg (cmol/kg)} = \frac{\text{Graph reading}}{12} \times 17$$

3.3.9 Determination of exchangeable potassium and sodium

Potassium and sodium were determined using a flame photometer as described by Okalebo *et al.* (1993). To extract the cations from the soil, 5 g of each soil sample was weighed and placed into plastic bottles and 100 ml of 1 M ammonium acetate solution. The mixture was shaken for 30 minutes and then filtered through Whatman No. 42 paper. 5 ml of each soil extract solution was pipetted into 50 ml volumetric flasks, 1 ml of 26.8 % lanthanum chloride added and the contents diluted (ten times) to the mark with 1 M NH_4OAc extracting solution. 0, 2.5, 5, 10, 15 and 20 ml of K and Na standard stock solutions were pipetted into 500 ml volumetric flasks and to each flask, 10 ml of 26.8 % lanthanum chloride solution and 100 ml of the 1 M NH_4OAc extracting solution added. These standard solutions contained 0, 1.25, 2.5, 5, 7.5, and 10 ppm K and Na respectively. The sample solutions were analysed in a flame photometer. The standard solutions were first measured to calibrate the instrument and to obtain the standard curve, which was used to calculate the concentrations of the elements as follows:

$$\text{cmol/kg of K (Na)} = \frac{\text{Graph reading}}{39 (23)} \times 10$$

Where value in parenthesis is the equivalent weight of Na.

3.4 Plant analysis

Total N and P in plant tissue were determined following wet oxidation procedure based on Kjeldahl digestion method of Okalebo *et al.* (1993). Plant samples (0.3mm) weighing 0.3 grams each were placed into labelled digestion tubes and 4.4 ml of the digestion mixture added to each tube. Two reagent blanks were also prepared. The digestion was done for two hours at 360°C until the solutions turned colourless. The digests were allowed to cool after which 25 ml of distilled water added to enhance sedimentation. Each solution was made up to the 50-ml mark with distilled water. The mixtures were then shaken thoroughly and allowed to settle and obtain clear solutions for subsequent N and P determination.

For N determination, an aliquot of 5 ml of sample solution was transferred to Markham nitrogen still and 10 ml of 40% NaOH added. This was immediately steam distilled into 5 ml of 1 % boric acid containing 4 drops of the mixed indicator. Distillation was continued for two minutes from the time the indicator turned green, after which the distillate was removed and titrated with N/70 HCl. The end point was reached when the green colour turned to clear pink and the micro-burette reading recorded. A reagent blank value was determined by distilling and titrating the reagent blanks as above. The quantities of N/70 required for the blank were subtracted from the micro-burette readings of the samples giving a corrected volume of N/70 HCl.

$$\% \text{ N} = \frac{C \times 0.2}{W}$$

Where; C = Corrected ml of N/70 HCl.

W = Weight of sample.

For P determination, 5 ml of digest solutions were put into 50-ml volumetric flasks to which 20 ml of distilled water and 10 ml of ascorbic acid reducing solution was added. Two blanks were also similarly prepared. The 50-ml flasks were filled to the mark using distilled water and thoroughly mixed by shaking. Standard P solutions containing 0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ppm P were prepared. Each of the 50-ml standard solution also contained 10 ml of the ascorbic acid reducing solution. The sample solutions, blanks and standards were allowed to stand for 1 hour to permit full colour development. The absorbances were measured at 880 nm wavelength in a colorimeter. A graph of absorbance against standard concentrations was plotted. Solution concentrations for each sample and for the two blanks were determined from the graph. The mean blank value was subtracted from the sample value to give the corrected concentration (C).

$$\% P = \frac{C \times 0.025}{W}$$

Where; C = Corrected concentration of P (ppm).

W= Weight of sample.

3.5 Greenhouse experiment

3.5.1 Sterilization

The greenhouse experiment for determining the rhizobial populations was conducted in the Department of Soil Science at Egerton University. The greenhouse was thoroughly cleaned and thereafter sterilized by spraying with 95 % alcohol. River sand was collected from River Pekera - Baringo District and sieved through a 2mm sieve to remove gravel. The sand was washed thoroughly on running tap water and then sterilized by autoclaving at 15 pounds pressure and 121°C for 2 hours. Plastic growth pots (measuring about 26-cm diameter) were washed in the laboratory with hot water and then sterilized in 10 % sodium hypochlorite solution and rinsed with distilled water. Five hundred grams of the sterilized sand was placed in the sterilized plastic growth pots and then placed in the greenhouse with a day-night

temperature regime of 30-18°C. Undamaged healthy seeds were selected for uniformity by hand sorting and were surface sterilized for 2 minutes in 2 % sodium hypochlorite and then washed thoroughly with sterile, distilled water to remove all traces of the sodium hypochlorite.

3.5.2 Planting and inoculation

Three bean seeds were sown in each pot and thinning was done 10 days after planting leaving only one plant per pot. The plants were supplied with sterile nutrient N-free solution (Somasegaran and Hoben 1994) (Appendix 3) and distilled water added every 3 days. The pots were randomly inoculated with soil 14 days after planting according to Brockwell (1982). A ten grams soil sample was suspended in a 90-ml volume of sterile distilled water and shaken at 100 cycles per minute for ten minutes on a mechanical electric shaker. One ml of this suspension was aseptically pipetted into 4 ml of sterile distilled water in a test tube and shaken for 2 minutes. From this, four 1-ml aliquots were used to inoculate four test plants, while the fifth ml was used to make the next five-fold dilution series the same way, but without shaking. This was serially repeated to give six successive dilutions. The test plants were allowed to grow for four weeks from the time of inoculation and then examined for the presence of nodules. The plants were harvested by uprooting and sand washed off the roots under tap water. Nodulation was then scored as either positive (+) or negative (-) depending on the presence or absence of nodules, respectively. Nodulation was scored as positive when any one seedling bore at least a single nodule.

3.5.3 Estimation of rhizobial population

The rhizobial population in the soil was estimated using the plant infection technique following the procedure of Brockwell, (1982). From the proportion of the plants forming nodules at each dilution level in the greenhouse, the most probable number (MPN) of

Rhizobia in the original soil sample were estimated. The pattern of occurrence of nodulated and non-nodulated test plants in the plant infection test, during the transition across successive dilution levels from all nodulated to non-nodulated (from low dilution to high dilution), was recorded and used as an ordered code. By reference to tables (Appendix 4), the code (Characteristic number) was used to calculate the MPN of *Rhizobia* in the sample under examination. This was done by assigning the results using the Brockwell (1980) Most Probable Number (MPN) of *Rhizobia* calculated from the distribution of positive (nodulated) test plants in the plant infection test based on a tenfold dilution series table (Appendix 4).

$$\text{The No. of Rhizobia} = \frac{E \times V}{W}$$

Where; E = Estimated number per ml of initial suspension from table.

V = volume of initial suspension.

W= Weight of soil sample used (g).

3.6 Statistical analysis

The data on soil pH, exchangeable aluminium, available P, calcium and magnesium, number and weight of nodules, rhizobial populations, N and P plant contents, number of pods and seeds per plant and grain yield were subjected to analysis of variance using the computer program MSTAT-C (1990). LSD and DMRT mean separation procedure at 0.05 level of probability was used to compare the means.

CHAPTER FOUR

4.0

RESULTS AND DISCUSIONS

4.1 Soil chemical properties

The soil pH determined at the time the field trial was laid out was 4.4. This pH is below the critical value for bean production, which has been given as 5.5 (Franco and Munns, 1982). As such it is expected that bean production in this soil may be constrained probably through direct or indirect effects of acidity such as Al toxicity and phosphorus and calcium deficiency. The value of C.E.C. obtained in this study (17.0 cmol/kg of soil) was above the medium cation exchange capacity of soil (15 cmol/kg of soil) common in tropical soils (Cottenie, 1980). Aluminium concentration of 0.7 cmol/kg (16.0 %) of soil was high and could be toxic for the bean crop. The critical % aluminium saturation level for beans is 0-25 % (Anon., 1986). This high level of aluminium could be attributed to the low pH, which causes the release of soluble aluminium ions (Syed-Omar *et al.*, 1991)

The soil available phosphorus (7.2 ppm) was low based on the interpretation of Olsen and Sommers (1982) in which less than 15 ppm P is considered to be low and thus a high phosphorus response expected. Low phosphorus could be attributed to the presence of aluminium, which could have bound most of the available phosphorus into unavailable forms (Hue *et al.*, 1991).

The total N content (0.09 %) in the experimental site was low according to Tekalign *et al.* (1991) who rated 0.05-0.12 % total nitrogen as being low. Thus the soil is not suited to support optimum growth of beans. The low nitrogen content of the soil may have been due to reduced rate of microbial activity in the soil caused by low soil pH, which in turn reduces the amount of organic matter. Organic carbon test value obtained (1.2 %) was thus also low according to the rating of Tekalign *et al.* (1991).

4.2 Effect of lime on soil chemical properties, bean nodulation, N and P uptake, yield and rhizobial population

4.2.1 Soil chemical properties

The results of the effect of lime on soil pH, exchangeable aluminium, available P, Ca and Mg are presented in Tables 4.1 and 4.2 for the 1999 short rains and 2000 long rains cropping seasons respectively.

Table 4.1: Effect of lime on soil chemical properties after the 1999 short rains cropping season

Lime level	Soil Properties				
	pH	Exch. Alcmol/kg.....	Ca	Mg	Avail. P (ppm)
0 t/ha	4.4	0.6	4.4	2.5	8.2
6 t/ha	4.9	0.4	5.8	2.8	9.1
Mean	4.7	0.5	5.1	2.7	8.7
LSD (P≤0.05)	0.15	0.05	1.05	0.47	0.82
C.V (%)	4.5	23.4	20.6	22.2	13.7

Table 4.2: Effect of lime on soil chemical properties after the 2000 long rains cropping season

Lime level	Soil Properties				
	pH	Exch. Alcmol/kg	Ca	Mg	Avail. P (ppm)
0 t/ha	4.5	0.6	4.6	2.5	8.9
6 t/ha	5.2	0.2	6.8	2.9	10.3
Mean	4.8	0.4	5.7	2.7	9.6
LSD (P≤0.05)	0.15	0.04	1.29	0.34	0.98
C.V (%)	4.4	20.4	20.6	13.1	15.1

Lime significantly ($p \leq 0.05$) increased soil pH from 4.4 (before planting) to an average of 4.9 and 5.2 during the 1999 short rain and 2000 long rain seasons respectively. The soil pH of the limed plots in both seasons were significantly higher from that of the unlimed plots. The increase was by 11.4 % in the 1999 short rains cropping season and 15.6 % in the 2000 long rains cropping season. Lime significantly ($p \leq 0.05$) decreased soil exchangeable aluminium. Lime reduced the aluminium concentration in the first season and second season by 33.3 % and 66.7 % respectively. Lime also significantly increased available soil P by 11.0 % and 15.7 % in the 1999 short rains and 2000 long rains cropping seasons respectively. Calcium concentration increased significantly ($p \leq 0.05$) in the first season by 31.8 % and in the second season by 47.8 %. Lime had no significant effect on the concentration of magnesium in the 1999 short rains cropping season but it significantly increased in the 2000 long rains cropping season by 16.0 %. Lime however had no significant effect on soil total N in both growing seasons.

The increase in pH and decrease in aluminium concentration can be attributed to the release of hydroxyl ions (OH) liberated from the reaction of lime and water and the precipitation of aluminium ions as aluminium hydroxide through reaction with the hydroxyl ions (Syed-Omar *et al.*, 1991). The increase in available soil P due to liming could also be attributed to the increase in soil pH hence low P fixation. Calcium applied through the liming material could have been converted into exchangeable calcium hence the significant increase in Ca concentration. The effect of lime on various soil properties resulted in a reduction in some of the properties that are considered to limit bean nodulation and yield (exchangeable acidity and concentration of exchangeable Al^{3+}) and an increase in favourable properties (concentration of exchangeable Ca^{2+} and Mg^{2+} , available P and pH). Laudelout (1993) and Ingerslev (1997) have observed similar results in acid soils.

4.2.2 Bean nodulation, N and P plant contents, yield and rhizobial population

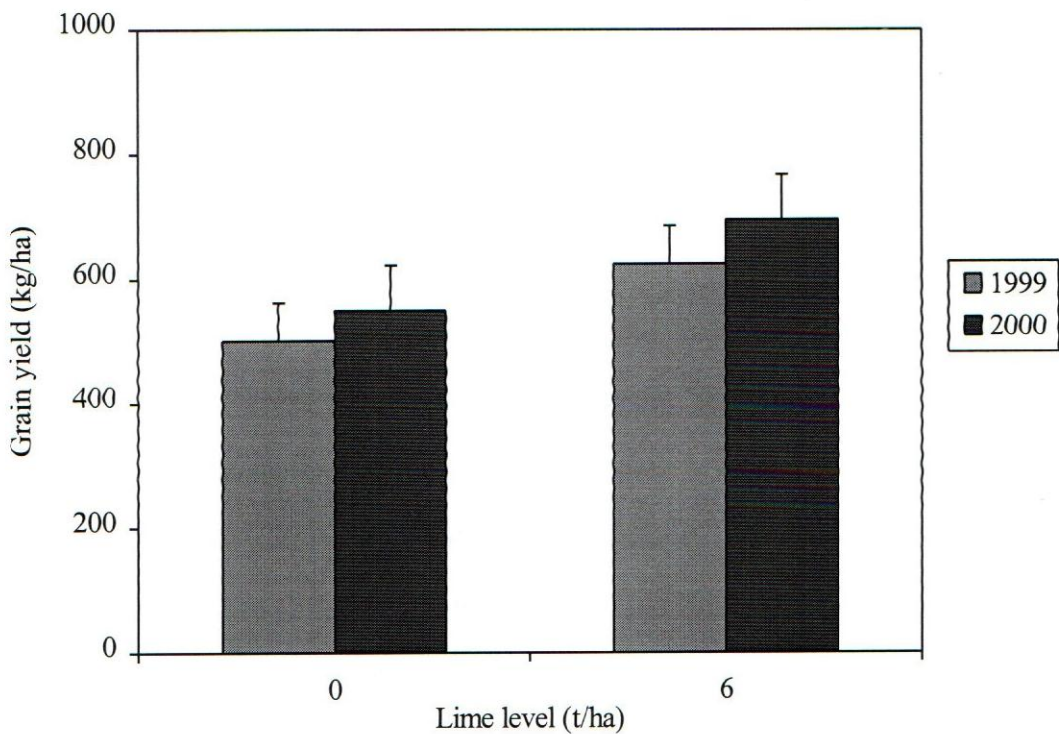
Tables 4.3 and 4.4 present the results of lime effect on bean nodule numbers and weights, N and P plant contents and rhizobial populations in the 1999 short rains and 2000 long rains cropping seasons respectively. The effect of lime on grain yield in both seasons is shown in Figure 2.

Table 4.3: Lime effect on nodulation, N and P plant contents and rhizobial populations in the 1999 short rains cropping season

Lime level	No. of nodules/plant	Wt. of nodules/plant (mg)	N (%)	P (%)	Rhizobial population (No./g of soil)
0 t/ha	6.4	2.3	2.7	0.3	73.3
6 t/ha	8.2	2.9	2.9	0.3	92.7
Mean	7.3	2.6	2.8	0.3	83
LSD ($P \leq 0.05$)	1.04	0.23	0.31	0.09	21.48
C.V (%)	20.5	12.5	13.4	12.8	31.1

Table 4.4: Lime effect on nodulation, N and P plant contents and rhizobial populations in the 2000 long rains cropping season

Lime level	No. of nodules/plant	Wt. of nodules/plant (mg)	N (%)	P (%)	Rhizobial population (No./g of soil)
0 t/ha	9.3	3.0	2.9	0.3	141.3
6 t/ha	15.0	5.8	3.2	0.4	220.4
Mean	12.2	4.4	3.1	0.4	180.9
LSD ($P \leq 0.05$)	5.08	2.13	0.15	0.02	18.72
C.V. (%)	33.7	24.8	7.2	11.3	33.9



LSD (1999) = 78.14 LSD (2000) = 51.97

Figure 2: Lime effect on grain yield in the 1999 short rains and 2000 long rains cropping seasons

Lime significantly ($p \leq 0.05$) increased nodulation in terms of nodule number and nodule weight and tissue P content in both the first and second growing seasons. Lime increased the tissue P content by 11.5% over the unlimed plots in both growing seasons. Lime had no significant ($p \leq 0.05$) effect on rhizobial populations and plant N content in the first season but it significantly ($p \leq 0.05$) increased the populations and plant N content in the second season by 56 % and 10.3 % respectively. Lime significantly ($p \leq 0.05$) increased grain yield by 24.5% and 26.4% in the first and second seasons respectively. Lime however had no significant ($p \leq 0.05$) effect on number of pods per plant and number of seeds per pod in the two planting seasons.

The increase in nodulation due to lime application was probably as a result of the increase in the soil pH thus suppressing exchangeable aluminium. Hence, better nodulation was enhanced probably because root infection was favoured by improved rhizosphere pH and calcium contents. This provided better soil environment that would have stimulated microbial activity hence improved root infection and nodulation. These improved soil conditions were more pronounced in the second season given that the lime had permeated well into the soil and also taking a longer period to neutralize acidity. Ssali and Mureria (1982) obtained similar results, whereby liming caused a linear increase in nodule number and nodule weight of beans. The significant increase in rhizobial populations only in the second season could be because the lime had permeated well in the soil and had adequate time to react and thus improved the soil pH that was favourable for rhizobial survival.

The increase in plant P due to liming shows a stimulatory role of lime on P uptake by beans. This could be attributed to the fact that liming enhanced the utilization of soil phosphates by beans through the amelioration of aluminium toxicity and decreased P fixation thereby availing more P to the plants. Chien *et al.* (1995) observed that liming acid *Ultisols* to raise soil pH from 4.4 to 5.0 increased the agronomic effectiveness of Triple Super Phosphate in soybean production. Robson and Abbott (1989) also reported an increased mineralization of organic P sources (phytins and nucleic acids) when an acid soil is limed to pH of about 5.5.

The significant effect of lime on plant tissue nitrogen only in the second season can be explained by the fact that lime had permeated well in the soil and could have provided ample conditions for N uptake by the beans. Lime increased the soil pH and as a result increased the number of microorganisms involved in N mineralization hence, an increased N supply to plants (Russell, 1988; Robson and Abbott, 1989). The resultant increase in pH and available P as well as a reduction in aluminium concentration due to liming may have resulted in

improved root growth, thus enhancing nutrient uptake for improved yields. In investigating the effect of lime on growth, yield and quality of French beans (*Phaseolus vulgaris*), Shukla *et al.* (1984), found that application of lime at 12 t/ha improved plant height, pod length, seed yield and 100–seed weight of the crop grown for three successive seasons.

4.3 Phosphorus effect on available P, bean nodulation, N and P plant contents, yield and rhizobial population

4.3.1 Available P

Fertilizer P application significantly ($P \leq 0.05$) increased available soil P in both growing seasons (Appendix 6a,b). Available phosphorus was 7.4 ppm and 7.6 ppm where no P was applied in the 1999 short rain and 2000 long rain cropping seasons respectively. Application of 60 kg P_2O_5 /ha and 120 kg P_2O_5 /ha increased the available P to 8.5 ppm and 10.1 ppm in the 1999 short rain season and 9.5 ppm and 11.1 ppm in the 2000 long rain cropping seasons, for the two respective rates of P_2O_5 . The application of P at the rate of 120 kg P_2O_5 /ha resulted in a significantly higher available P than at 60 kg P_2O_5 /ha, which was in turn significantly higher than when no P was applied.

4.3.2 Nodulation, N and P plant content and rhizobial population

Tables 4.5 and 4.6 show the effect of P application on the number and weight of nodules, N and P plant contents and rhizobial populations for the 1999 short rains and 2000 long rains cropping seasons respectively.

Table 4.5: Effect of phosphorus on nodulation, N and P plant content and rhizobial population in the 1999 short rains cropping season

P level (kg P ₂ O ₅ /ha)	No. of nodules/plant	Wt. of nodules/plant (mg)	N(%)	P (%)	Rhizobial population (No/g of soil)
0	2.0	1.6	2.5	0.3	69.3
60	9.8	3.0	2.9	0.3	103.7
120	10.3	3.2	2.9	0.4	76.1
Mean	7.4	2.6	2.8	0.3	83.0
LSD (P≤0.05)	1.27	0.28	0.31	0.04	35.43
C.V (%)	20.49	12.52	13.36	12.77	31.12

Table 4.6: Effect of phosphorus on nodulation, N and P plant content and rhizobial population in the 2000 long rains cropping season

P level (kg P ₂ O ₅ /ha)	No. of nodules/plant	Wt. of nodules/plant (mg)	N(%)	P (%)	Rhizobial population (No/g of soil)
0	4.7	1.7	2.7	0.3	151.2
60	18.8	6.6	3.1	0.3	244.2
120	12.9	4.9	3.2	0.4	147.3
Mean	12.1	4.4	3.0	0.3	181.2
LSD (P≤0.05)	6.23	2.60	0.18	0.03	22.93
C.V (%)	33.71	24.76	7.17	11.26	33.86

Phosphorus application significantly ($p \leq 0.05$) increased the number and weight of nodules per plant recorded at 50% flowering in both cropping seasons. In both seasons, there were no significant differences in nodule numbers and nodule weights between plots that received 60 kg P₂O₅/ha and 120 kg P₂O₅/ha. However, the application of P at either 60 kg P₂O₅/ha or 120 kg P₂O₅/ha resulted in significantly higher nodule numbers and weights than at 0 kg P₂O₅/ha. The application of phosphorus fertilizer did not significantly influence the rhizobial populations in the first season. However, the effect of P application in the second season was

inconsistent. The lower rate of 60 kg P₂O₅/ha seemed to significantly increase the rhizobial population whereas at high application rate of 120 kg P₂O₅/ha, the rhizobial population was not significantly different from the control. This therefore indicates that a high P level is detrimental to rhizobial populations while P at a lower rate enhances favourable multiplication of rhizobial populations. P application significantly ($p \leq 0.05$) increased P and N plant content in both seasons. Whereas P at 60 kg P₂O₅/ha was not significantly different from the control in both seasons, P at 120 kg P₂O₅/ha significantly increased P uptake by 33 % in both seasons. Fertilizer P application however affected N uptake differently. The application of 60 kg P₂O₅/ha resulted in a significantly higher N uptake than the control by 16 % and 14.8 % in the first and second seasons respectively. The higher rate of 120 kg P₂O₅/ha also significantly increased the N uptake by 16 % and 18.5 % in the first and second seasons respectively but the effect was not significantly different from that of 60 kg P₂O₅/ha.

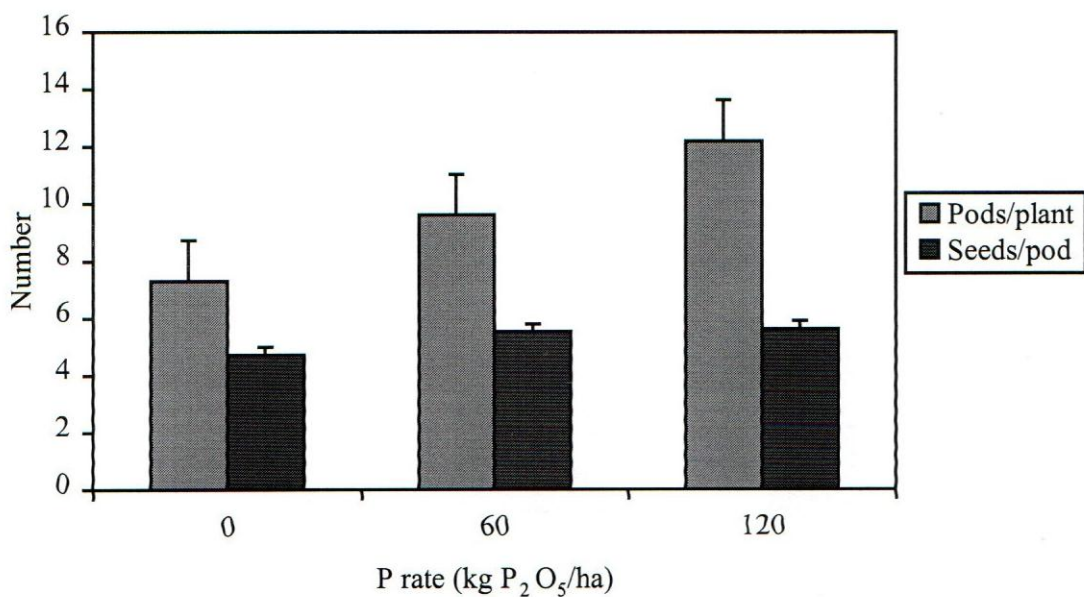
The fact that nodulation did not significantly ($p \leq 0.05$) increase without supplemental phosphorus shows that phosphorus was an absolute requirement for nodule growth and development. The P fertilizer was effective probably by alleviating the adverse effects of aluminium toxicity due to precipitation of Al-phosphates and other non-toxic soluble Al-P complexes. Phosphorus fertilizer might have also improved nodule metabolism hence increased nodule numbers and weights (Ssali and Keya, 1983; Bonetti *et al.*, 1984; Floor, 1985; Pereira and Bliss, 1989). The increase in number and weight of nodules with increasing rates of P observed in the first season is in agreement with the findings of Tsai *et al.* (1993) in common bean and Cruz *et al.* (1997) in yam bean in acid infertile soils. In the second season however, P application at 120 kg P₂O₅/ha gave lower numbers and weights of nodules than at 60 kg P₂O₅/ha. This indicates that P at 60 kg P₂O₅/ha gave a maximum response to nodulation.

The increased tissue N and P contents could be a factor of the higher nitrogen and phosphorus uptake by the plants. The significant high N uptake at 60 and 120 kg P₂O₅/ha could be related to the effect of P on nodulation whereby the increased nodulation could have led to high N fixation and uptake. Cruz *et al.* (1997) found that P fertilization increased N uptake of yam bean in an acid soil whereby P fertilization appeared to have a significant effect on total N in the plants. The increase in tissue P contents with P application also agrees well with findings on different types of legumes and soil types (Engels, 1993; Gobara *et al.*, 1993; Cruz *et al.*, 1997).

4.3.3 Grain yield and yield components

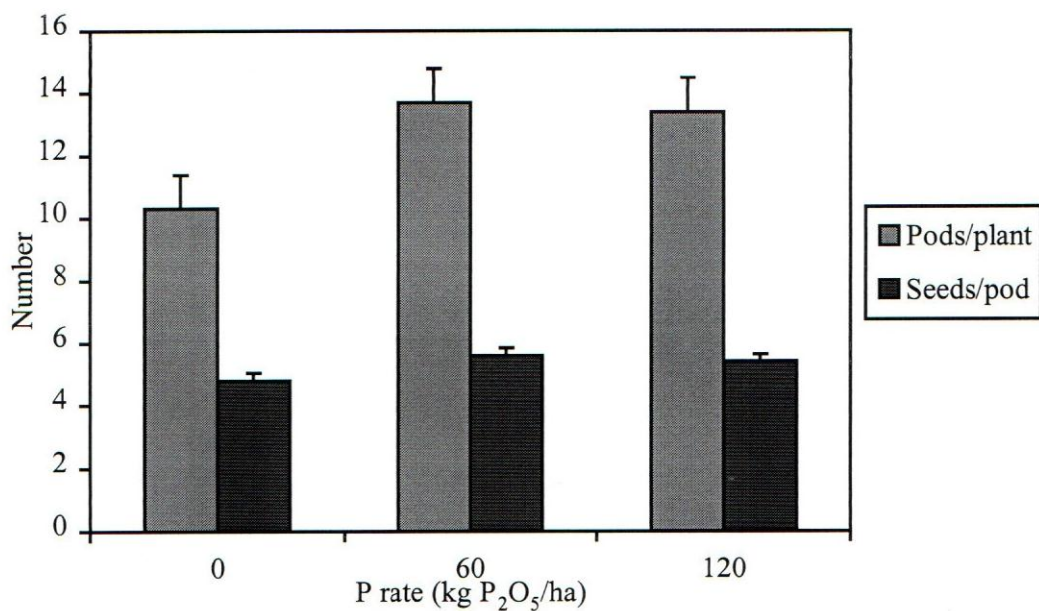
The results for the number of pods per plant and seeds per pod for the 1999 short rain and 2000 long rain cropping seasons are shown in Figures 3 and 4 respectively while those of grain yield are shown in Figure 5.

Phosphorus significantly ($p \leq 0.05$) increased number of pods/plant, seeds/pod and grain yield. Phosphorus application significantly ($p \leq 0.05$) increased pod numbers recorded at 90 days after planting by an average of 48.3% and 32.1 % over where there was no P application and number of seeds per pod at harvesting by 18.7 % and 13.8 % in the first and second planting seasons respectively. P application at 120 kg P₂O₅/ha gave a higher number of pods/plant and number of seeds/pod than P at 60 kg P₂O₅/ha of 15.5 % and 33.3% respectively in the 1999 short rains cropping season. Unlike in the 2000 long rains cropping season, P at 60 kg P₂O₅/ha gave a higher number of seeds/pod by 12.1 % and number of pods/plant by 30.9 % than P at 120 kg P₂O₅/ha. However, both P applications at 120 kg P₂O₅/ha and 60 kg P₂O₅/ha were not significantly different from each other on the number of pods/plant and seeds/pod but were significantly different from P at 0 kg P₂O₅/ha.



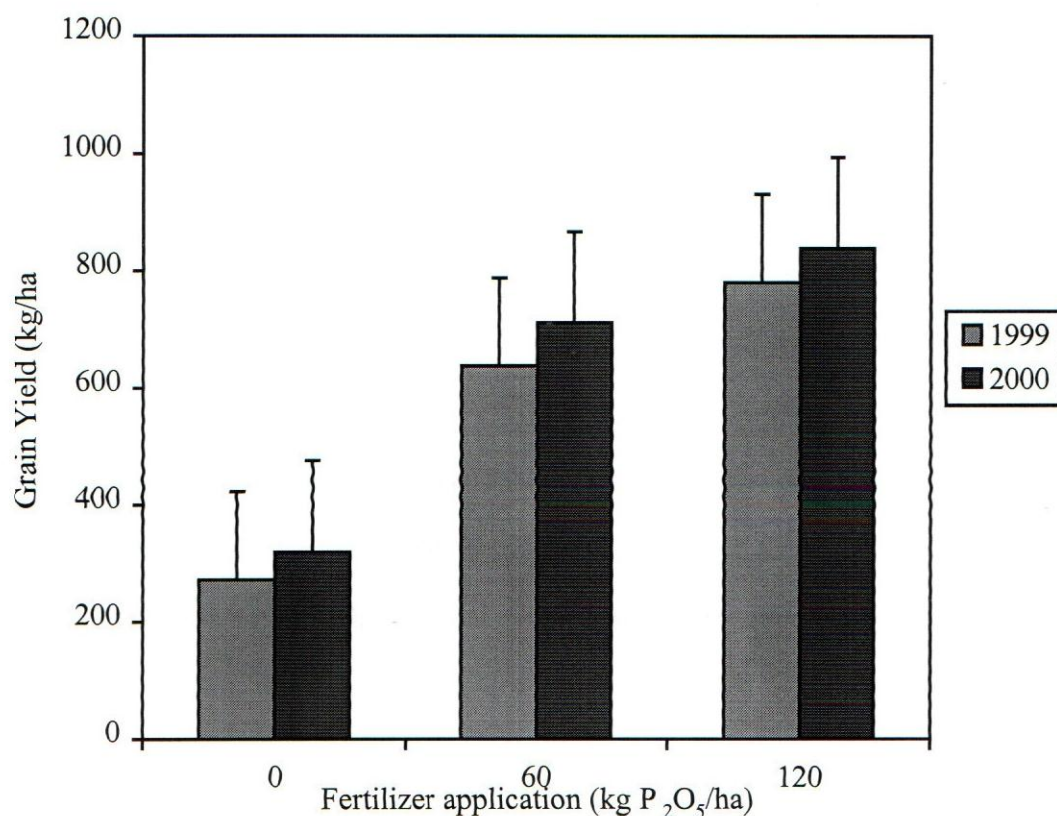
LSD (Pods/plant) = 2.73 LSD (Seeds/pod) = 0.77

Figure 3: Effect of phosphorus on number of pods/plant and seeds/pod in the 1999 short rains cropping season



LSD (Pods/plant) = 1.98 LSD (Seeds/pod) = 0.61

Figure 4: Effect of phosphorus on number of pods/plant and seeds/pod in the 2000 long rains cropping season



LSD (1999) = 95.7 LSD (2000) = 70.99

Figure 5: Effect of phosphorus on grain yield in the 1999 short rains and 2000 long rains cropping seasons

Grain yields were significantly influenced by P application both in the first and second growing seasons. In the first season, P application at 60 kg P₂O₅/ha increased grain yields by 134.2 % while the increase at 120 kg P₂O₅/ha was 186.6 % over the control. Plots that received 120 kg P₂O₅/ha gave significantly different yields from those that received 60 kg P₂O₅/ha. In the second season, grain yield increased by 122.4 % and 162.2 % at 60 kg P₂O₅/ha and 120 kg P₂O₅/ha respectively. The yields at 60 kg P₂O₅/ha and 120 kg P₂O₅/ha in the second season were not significantly different probably because the trial was conducted on the area where P₂O₅ was applied for the preceding crop and the residual effect of P₂O₅ may have affected the yield positively.

The responses in grain yield and yield parameters could probably be due to the fact that the available phosphorus in the experimental soil (7.2 ppm) was very low as per Tekalign et al (1991). The high response of the beans to phosphorus fertilization with respect to pods per plant and seeds per pod could be the result of improved nitrogen and phosphorus metabolism as indicated by the increased N and P contents in the plants. Xia (1997) showed that in most cases, the number of seeded pods per plant or number of seeds per plant of faba bean increased with increased nutrient (N, P, K) concentration. Phosphorus promotes cell division, development of meristematic tissues and the efficient absorption and utilization of other plant nutrients (Marschner 1995) and this could have resulted to increased number of pods and seeds. Cruz *et al.* (1997), also showed that P fertilization significantly affected dry matter production of yam bean in an acid soil with up to a 8-fold increase in growth relative to controls and hence high seed yields.

Ayara (1988) found that common bean showed an increased grain yield when P doses were raised from 0 to 55 kg P₂O₅/ha. Response of common beans to phosphorus application with respect to grain yields could be attributed to low available phosphorus status of the experimental soil. This response could also be attributed to the stimulatory effects of phosphorus on physiological growth parameters and yield promoting characteristics such as the number of flowers and the number of pods.

4.4 *Rhizobium* inoculation effect on bean nodulation, N and P plant contents, yield and rhizobial population

Rhizobium inoculation did not significantly ($p \leq 0.05$) influence nodulation, P uptake, yield parameters and grain yield in both growing seasons. *Rhizobium* inoculation however significantly ($p \leq 0.05$) increased tissue N content and rhizobial populations in both growing

seasons (Table 4.7). Inoculation increased the rhizobial populations about five times in the first season and more than two times in the second season.

Table 4.7: Effect of *Rhizobium* inoculation on tissue N content and rhizobial population in the 1999 short rains and 2000 long rains cropping seasons

<i>Rhizobium</i> inoculation	Tissue N content (%)		Rhizobial population (No./g of soil)	
	1999	2000	1999	2000
With inoculation	2.9	3.2	138.8	249.8
Without inoculation	2.6	2.8	27.2	112.0
Mean	2.8	3.0	83	180.9
LSD (P≤0.05)	0.25	0.15	41.95	18.72
C.V (%)	13.36	7.17	31.12	33.86

Nevertheless, nodule number and fresh weight have been found to be positively influenced upon inoculation with commercial strains in beans (Weiser *et al.*, 1985), haricot bean (Gobara *et al.*, 1993) and in yam bean in an acid soil (Cruz *et al.*, 1997). Ssali and Mureria (1982) also observed that two locally isolated *Rhizobium* strains, BA37 and BA65 produced significant responses to nodulation, flowering, plant height, pod formation and seed yield in Canadian wonder, Rose coco and Mwezi moja bean varieties. However, the response was not consistent in all parameters studied.

The non-significant response of nodulation and yield to *Rhizobium* inoculation implies that inoculation was not effective. This could be attributed to the fact that the introduced *Rhizobia* were not competitive enough to produce significant nodulation and yield responses as compared with the native rhizobial populations. Weiser *et al.* (1985), found that inoculation with commercial rhizobial strains compared with uninoculated controls, did not increase seed yield. Chui and Nadar (1984) and Ramos and Roddey (1987) indicated similar results in bean

plants. Ramos and Roddey (1987) attributed this to the low competition of the introduced strain.

Strong correlations occur between the number of *Rhizobia* in the rhizosphere and nodulation in soybean (Herridge *et al.*, 1984; Brockwell *et al.*, 1989; Mathews *et al.* (1993)) and in annual medics (*Medicago* spp.) (Young and Brockwell, 1992). In theory, a single rhizobial cell can initiate nodulation (Marshall *et al.*, 1993) but in practice, a substantially higher number is needed for the expression of full effectiveness. For instance, an inoculum of about 70 cells of *Rhizobium meliloti* was necessary to obtain fully effective nodulation of *Medicago sativa* growing in tubes (150 by 18 mm) of vermiculite (Brockwell *et al.*, 1988). Thus the size of the rhizobial population could be an index of the nodulation potential of the legume.

The increment in plant nitrogen due to *Rhizobium* inoculation could be attributed to increased dinitrogen fixation by the added *Rhizobium* species. Gobara *et al.* (1993) found a significant increment in plant nitrogen content 5 WFS due to inoculation whereas the response of plant dry weight and shoot phosphorus content to inoculation was not marked and did not attain statistical significance.

4.5 Interaction effects on soil chemical properties, nodulation, shoot N and P contents, yield and rhizobial populations

In general, there were no significant ($p \leq 0.05$) interaction effects between lime, phosphorus and *Rhizobium* inoculation on any of the soil parameters measured (soil pH, available P, exchangeable aluminium, calcium and magnesium) and rhizobial populations in both growing seasons. However, there occurred inconsistent significant interaction effects on number and weight of nodules, N and P uptake and grain yield.

4.5.1 Lime by phosphorus interaction

Lime by phosphorus interaction effect was only significant ($p\leq0.05$) on grain yield in both growing seasons (Table 4.8).

Table 4.8: Lime by phosphorus interaction effect on grain yield in the 1999 short rains and 2000 long rains cropping seasons

P level (kg P ₂ O ₅ /ha)	1999			2000		
	Lime level (t/ha)			Lime level (t/ha)		
	0	6	Mean	0	6	Mean
0	192.4	351.0	271.7	227.9	410.3	319.1
60	615.2	657.5	636.4	701.4	718.1	709.8
120	652.6	904.5	778.6	701.9	971.7	836.8
Mean	486.7	637.7	543.7	700.0	694.5	621.9
LSD1 ($p\leq0.05$)= 135.3				C.V1 (%) = 20.11		
LSD2 ($p\leq0.05$) = 100.4				C.V2 (%) = 13.48		

Where; 1 and 2 are values for 1999 short rains and 2000 long rains cropping seasons respectively.

Grain yield increased with increasing levels of P with or without lime. The yields obtained from P application at 60 kg P₂O₅/ha and 120 kg P₂O₅/ha were significantly higher than that obtained from 0 kg P₂O₅/ha in both seasons. P application at 60 kg P₂O₅/ha did not give a significantly different yield from that obtained at 120 kg P₂O₅/ha in plots that received no lime. However, where lime was applied, P at 120 kg P₂O₅/ha gave significantly higher yields than at 60 kg/ha by 37.6 % and 35.3 % in the 1999 short rains and 2000 long rains cropping seasons respectively. This implies that with liming, high P application rates lead to increased bean yields whereas without liming, increasing P rates does not necessarily lead to high bean yields. Thus, without liming, P application at 60 kg P₂O₅/ha could be considered as an optimum level.

Lime application at 6 t/ha resulted in significantly higher yields at 0 kg P₂O₅/ha and 120 kg P₂O₅/ha by 82.4 % and 38.6 % in the 1999 short rains cropping season and 80.0 % and 38.4 % in the 2000 long rains cropping season respectively. P application at 60 kg P₂O₅/ha did not give significant yield differences between plots that received 6 t/ha and 0 t/ha of lime. Nevertheless, bean yields obtained from limed plots were higher than those obtained from unlimed plots. Lime application at 6 t/ha gave significantly higher yields than at 0 t/ha over all P levels by 31.0 % in the 1999 short rains cropping season and by 28.7 % in the 2000 long rains cropping season.

The significant ($p \leq 0.05$) lime by phosphorus interaction effect on grain yield can be attributed to improved soil chemical properties that are favourable for proper bean growth and yield such as increased soil pH due to liming, available P for crops due to low P fixation and direct P application. These factors could have resulted in improved vegetative growth of the bean plants thus enhancing high crop yields.

4.5.2 Lime by *Rhizobium* inoculation interaction

The lime by *Rhizobium* inoculation interaction effect was significant ($p \leq 0.05$) on weight of nodules only in the first season (Table 4.9) and on grain yield in both growing seasons (Table 4.10).

Table 4.9: Lime by *Rhizobium* inoculation interaction on weight of nodules in the 1999 short rains cropping season

Lime level (t/ha)	Inoculation		
	With inoculation	Without inoculation	Mean
0	2.4	2.2	2.3
6	3.2	2.7	3.0
Mean	2.8	2.5	2.7

LSD ($p \leq 0.05$) = 0.32

C.V (%) = 12.52

Table 4.10: Lime by *Rhizobium* inoculation interaction effect on grain yield in the 1999 short rains and 2000 long rains cropping seasons

Lime level (t/ha)	1999			2000		
	Inoculation	No inoculation	Mean	Inoculation	No inoculation	Mean
0	540.4	457.1	498.8	604.4	494.3	549.4
6	706.7	544.6	625.7	774.5	614.5	694.5
Mean	623.6	500.9	562.2	689.5	554.4	622.0

LSD1 ($p \leq 0.05$) = 110.5

C.V1 (%) = 20.11

LSD2 ($p \leq 0.05$) = 81.98

C.V2 (%) = 13.48

Where; 1 and 2 are values for 1999 short rains and 2000 long rains cropping seasons respectively.

Lime application at 6 t/ha significantly ($p \leq 0.05$) increased the weight of nodules in plots where inoculation was done by 33.0 % and in plots where no inoculation was done by 22.7 %. *Rhizobium* inoculation gave significantly higher nodule weights in limed plots by 18.5 % over unlimed plots. In limed plots however, inoculation did not have any significant effect on the nodule weights. Lime significantly increased the weight of nodules irrespective of inoculation status while inoculation significantly increased nodule weight only when the soil was limed.

Lime significantly ($p \leq 0.05$) increased the grain yield upon inoculation by 30.7 % and 28.1 % in the 1999 short rain and 2000 long rain cropping seasons respectively. Lime also significantly increased the yield without inoculation in the 2000 long rains cropping season by 24.3 % but it did not have any significant effect in the 1999 short rains cropping season without inoculation. Inoculation alone without lime application did not significantly increase the yields in the 1999 short rains cropping season but in the 2000 long rains cropping season, it increased the yields significantly by 22.3 % without liming. Upon liming, inoculation significantly increased bean yields in the 1999 short rains and 2000 long rains cropping seasons by 29.8 % and 26.0 % respectively. This implies that inoculation could be more effective in enhancing high bean yields upon liming. Likewise, liming could be more effective in achieving high bean yields upon inoculation in acid soils.

4.5.3 Phosphorus by *Rhizobium* inoculation interaction

Phosphorus by *Rhizobium* inoculation interaction was significant on number of nodules and weight of nodules in the 1999 short rains cropping season (Table 4.11) and on grain yield in the 2000 long rains cropping season (Table 4.12).

Table 4.11: Phosphorus by *Rhizobium* inoculation interaction effect on nodulation in the 1999 short rains cropping season

P level (kg P ₂ O ₅ /ha)	No. of nodules/plant			Wt. of nodules/plant (mg)		
	Inoculation	No inoculation	Mean	Inoculation	No inoculation	Mean
0	3.3	1.9	2.6	2.0	1.7	1.9
60	9.3	7.4	8.4	3.1	2.9	3.0
120	11.2	9.4	10.3	3.3	3.1	3.2
Mean	7.9	6.2	7.3	2.8	2.6	2.7
LSD1 ($p \leq 0.05$) = 1.79			C.V1 (%) = 20.49			
LSD2 ($p \leq 0.05$) = 0.39			C.V2 (%) = 12.52			

Where; 1= Values for number of nodules and 2= Values for weight of nodules.

Table 4.12: Phosphorus by *Rhizobium* inoculation interaction effect on grain yield in the 2000 long rains cropping season

P level (kg P ₂ O ₅ /ha)	<i>Rhizobium</i> inoculation		
	With inoculation	Without inoculation	Mean
0	394.3	297.9	346.1
60	710.5	709.0	709.8
120	873.8	799.8	836.8
Mean	659.5	602.2	630.9

LSD (p≤0.05) = 100.4
C.V (%) = 13.48

Application of P at 60 kg P₂O₅/ha and 120 kg P₂O₅/ha gave significantly higher number and weight of nodules in both growing seasons over 0 kg P₂O₅/ha. P application at 120 kg P₂O₅/ha gave significantly higher number of nodules than at 60 kg P₂O₅/ha by 20.4 % with inoculation and by 27.0 % without inoculation. However, there were no significant differences between P application at 120 kg P₂O₅/ha and 60 kg P₂O₅/ha on the weight of nodules with or without inoculation. Inoculation resulted to significantly higher nodule numbers at 60 kg P₂O₅/ha by 25.7 % and at 120 kg P₂O₅/ha by 19.1 % but did not significantly influence the weight of nodules at any of the P levels. It was however observed that nodulation of beans with *Rhizobium* in the absence of phosphorus did not have any significant effect. This lack of response might have been caused by poor nodulation and hence low nitrogen fixation activity resulting from phosphorus deficiency in the soil since phosphorus is required for nodule metabolism. Gobara *et al.* (1993) also found a significant *Rhizobium* by phosphorus interaction 11 WFS in nodule numbers and nodule dry weights.

P application at 60 kg P₂O₅/ha and 120 kg P₂O₅/ha gave significantly higher seed yields than at 0 kg P₂O₅/ha with or without inoculation. P application at 120 kg P₂O₅/ha gave a significantly higher seed yield than plots that received 60 kg P₂O₅/ha by 23 % upon

inoculation. There were no significant bean yield differences between P application at 60 kg P₂O₅/ha and 120 kg P₂O₅/ha in plots that were not inoculated with *Rhizobium phaseoli*. *Rhizobium* inoculation did not significantly increase bean yields at any of the P levels.

4.5.4 Lime by phosphorus by *Rhizobium* inoculation interaction

The lime by phosphorous by *Rhizobium* inoculation interaction was only significant on number of nodules in the 2000 long rains cropping season.

Table 4.13: Lime by phosphorus by *Rhizobium* inoculation interaction effect on number of nodules in 2000 long rains cropping season

<i>Rhizobium</i> inoculation	Lime level (t/ha)	Phosphorus levels (kg P ₂ O ₅ /ha)			
		0	60	120	Mean
With inoculation	0	5.3a	14.7ab	7.3ab	9.1
	6	5.0a	12.3ab	20.7b	12.7
	Mean	5.2	13.5	14.0	10.9
Without inoculation	0	4.0 a	14.3ab	10.0ab	9.4
	6	4.3 a	33.7c	13.7ab	17.2
	Mean	4.2	24	11.9	13.3
Lime	0	4.7	14.5	8.7	9.3
	6	4.7	23.0	17.2	15.0
	Mean	4.7	18.8	13.0	12.2

Values followed by the same letter are not significantly (p≤0.05) different.

P application at 60 kg P₂O₅/ha and 120 kg P₂O₅/ha did not have a significantly different effect from the control with or without inoculation when no lime was applied. On liming however, P application at 120 kg P₂O₅/ha gave a significantly higher number of nodules than at 60 kg P₂O₅/ha and 0 kg P₂O₅/ha, which were not significantly different with inoculation. However, when no inoculation was done, P application at 60 kg P₂O₅/ha gave significantly (p≤0.05) higher number of nodules than at 120 kg P₂O₅/ha and 0 kg P₂O₅/ha, which were not

significantly different. Lime did not have any significant ($p \leq 0.05$) effect on the number of nodules at all P levels with or without inoculation but had a significant ($p \leq 0.05$) higher number of nodules at 60 kg P_2O_5 /ha than the control where inoculation was not done.

4.6 CONCLUSIONS AND RECOMMENDATIONS

4.6.1 Conclusions

1. Low soil pH, low available P, N and Ca and high levels of exchangeable aluminium are limiting factors for bean seed production in mollic *Andosols*.
2. Lime alleviated soil acidity stress that hampered bean production.
3. Phosphorus enhanced bean growth and yield.
4. Beans grown in a mollic *Andosol* did not respond to *Rhizobium* inoculation in the absence of phosphorus.

4.6.2 Recommendations

1. Lime and phosphorus should be applied to enhance bean yields in mollic *Andosols*. In addition, the favourable response to inoculation though to a limited extent in some cases stresses the need for the adoption of these treatments either singly or in combination in acid soils.
2. For significant *Rhizobium* inoculation responses to be obtained, acid tolerant *Rhizobium* strains should probably be used for optimum bean production in acid soils.
3. The results of the field work presented in this thesis were carried out in two seasons, therefore, there is need to conduct further experiments covering several seasons, locations and bean genotypes.
4. A controlled glasshouse experiment should be conducted to further check the effect of soil acidity and/or aluminium toxicity on nitrogen fixation and performance of beans since the field experiment might suffer from the compounding effects of many factors.

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APPENDICES

APPENDIX 1: Rainfall data (mm)

Month	1999			2000		
	No. of rain days	Mean (mm)	Total (mm)	No. of rain days	Mean (mm)	Total (mm)
January	3	23.0	68.9	1	8.6	8.6
February	1	8.1	8.1	Nil	Nil	Nil
March	15	27.4	410.4	2	6.2	12.4
April	7	18.8	131.4	5	5.3	27.2
May	7	17.1	119.6	13	22.7	294.6
June	8	14.9	119.0	16	16.9	270.2
July	8	35.4	283.5	20	18.2	365.0
August	16	14.3	227.9	21	18.1	380.3
September	7	9.3	65.0	16	13.6	217.4
October	18	17.2	310.2	17	22.3	379.4
November	12	10.7	127.9	10	18.2	182.4
December	6	15.6	93.5	3	33.1	99.2
Year Total	108	211.8	1966.3	124	183.2	2236.7

Source: Molo National Pyrethrum Research Sub-Centre.

APPENDIX 2: Temperature data (°C)

Month	1999		2000	
	Mean Min. (°C)	Mean Max. (°C)	Mean Min. (°C)	Mean Max. (°C)
January	5.7	22.4	5.3	22.8
February	4.5	23.5	5.0	24.5
March	8.8	21.8	6.7	24.6
April	7.9	21.0	7.8	23.5
May	6.5	20.6	7.5	22.5
June	5.9	20.8	7.0	20.1
July	5.2	18.9	7.8	18.1
August	6.3	18.4	6.4	17.7
September	5.5	21.3	5.9	20.8
October	6.9	20.8	6.7	21.4
November	8.5	19.2	7.8	20.6
December	8.0	20.1	7.3	21.2
Mean	6.6	20.7	6.8	21.5

Source: Molo National Pyrethrum Research Sub-Centre.

APPENDIX 3: N – free Nutrient Solution

Stock Solution	Chemical	g/litre
1	CaCl ₂ .2H ₂ O	294.1
2	KH ₂ PO ₄	136
3	FeC ₆ H ₅ O ₇ .3H ₂ O	6.7
	MgSO ₄ .7H ₂ O	123.3
	K ₂ SO	87.0
	MnSO ₄ .H ₂ O	0.338
4	H ₃ BO ₃	0.247
	ZnSO ₄ .7H ₂ O	0.288
	CuSO ₄ .5H ₂ O	0.100
	CoSO ₄ .7H ₂ O	0.056
	NaMoO ₂ .2H ₂ O	0.048

Preparation:

- Stock solutions were prepared; warm water was used to get the ferric-citrate into solution.
- 10 litres of full-strength plant culture solution was made as follows:
- To 5 litres of water, 5 ml of each stock solution was added and mixed.
- The contents were diluted to 10 litres by adding another 5 litres of water.
- The pH was adjusted to 6.6 – 6.8 with 1 N NaOH.
- For plus N control treatments, KNO₃ (0.05%) was added, giving N concentration of 70 ppm.

APPENDIX 4: Most Probable Number of *Rhizobia* Calculated from the Distribution of Positive (Nodulated) Test Plants in a Plant Infection Test Based on a Fivefold Dilution Series:

Characteristic Number	Estimate	Characteristic Number	Estimate	Characteristic Number	Estimate	Characteristic Number	Estimate
100000	1.1	401000	10.8	440200	71	444030	450
200000	2.6	411000	15.1	441200	98	444130	630
300000	4.6	421000	21.5	442200	141	444230	910
400000	8.0	431000	32.8	443200	218	444330	1 410
010000	1.0	402000	14.1	440300	91	444410	1 430
110000	2.3	412000	19.6	441300	126	444420	2 030
210000	4.0	422000	28.3	442300	182	444430	3 020
310000	6.5	432000	43.6	443300	282	444440	5 050
020000	2.1	403000	18.1	444100	290	444401	1 350
120000	3.5	423000	25.2	444200	410	444411	1 880
220000	5.5	423000	36.4	444300	600	444421	2 690
320000	8.7	433000	56.5	444400	1 010	444431	4 100
030000	3.0	441000	57	444010	270	444402	1 770
130000	4.9	442000	81	444110	380	444412	2 450
230000	7.2	443000	121	444210	540	444422	3 530
330000	11.3	444000	202	444310	820	444432	5 440
410000	11.4	440100	54	444020	350	444403	2 260
420000	16.2	441100	75	444120	490	444413	3 140
430000	24.2	442100	108	444220	710	444423	4 550
440000	40.4	443100	164	444320	1 090	444433	7 060
						444441	7 100
						444442	10 100
						444443	15 100
						444444	25 200
						444445	>35500

Source: Brockwell (1980).

APPENDIX 5 (a): 1999 short rains ANOVA table for soil pH

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.296	0.148	3.2511	0.0579
Factor A	1	0.212	0.212	4.6534	0.0422
Factor B	2	0.275	0.138	3.0270	0.0690
AB	2	0.005	0.002	0.0535	
Factor C	1	0.000	0.000	0.0061	
AC	1	0.004	0.004	0.0977	
BC	2	0.039	0.019	0.4283	
ABC	2	0.076	0.038	0.8388	
Error	22	1.000	0.045		
Total	35	1.908			

APPENDIX 5 (b): 2000 long rains ANOVA table for soil pH

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.285	0.143	3.0682	0.0668
Factor A	1	0.288	0.288	6.1932	0.0209
Factor B	2	0.260	0.130	2.7916	0.0831
AB	2	0.076	0.038	0.8146	
Factor C	1	0.002	0.002	0.0344	
AC	1	0.009	0.009	0.2010	
BC	2	0.149	0.075	1.6029	0.2240
ABC	2	0.108	0.054	1.1623	0.3313
Error	22	1.023	0.046		
Total	35	2.200			

APPENDIX 5 (c): 1999 short rains ANOVA table for soil available P

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	14.047	7.023	5.0015	0.0162
Factor A	1	7.817	7.817	5.5664	0.0276
Factor B	2	44.661	22.331	15.9019	0.0001
AB	2	1.956	0.978	0.6964	
Factor C	1	4.185	4.185	2.9805	0.0983
AC	1	0.214	0.214	0.1523	
BC	2	0.259	0.129	0.0922	
ABC	2	0.432	0.216	0.1537	
Error	22	30.894	1.404		
Total	35	104.464			

APPENDIX 5 (d): 2000 long rains ANOVA table for soil available P

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	5.940	2.970	1.4694	0.2518
Factor A	1	25.008	25.008	12.3726	0.0019
Factor B	2	73.082	36.541	18.0783	0.0000
AB	2	1.769	0.898	0.4443	
Factor C	1	8.089	8.089	4.0021	0.0579
AC	1	0.311	0.311	0.1538	
BC	2	3.674	1.837	0.9089	
ABC	2	2.105	1.052	0.5206	
Error	22	44.468	2.021		
Total	35	164.473			

APPENDIX 5 (e): 1999 short rains ANOVA table for soil exchangeable aluminium

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.053	0.026	0.0493	
Factor A	1	9.996	9.996	18.7418	0.0003
Factor B	2	0.163	0.081	0.1528	
AB	2	1.199	0.600	1.1240	0.3429
Factor C	1	0.033	0.033	0.0619	
AC	1	0.742	0.742	1.3921	0.2507
BC	2	0.011	0.006	0.0106	
ABC	2	0.056	0.028	0.0523	
Error	22	11.734	0.533		
Total	35	23.987			

APPENDIX 5 (f): 2000 long rains ANOVA table for soil exchangeable aluminium

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	2.614	1.307	1.1546	0.3335
Factor A	1	25.857	25.857	22.8462	0.0001
Factor B	2	4.283	2.141	1.8920	0.1745
AB	2	1.063	0.531	0.4695	
Factor C	1	0.714	0.714	0.6309	
AC	1	0.238	0.238	0.2107	
BC	2	0.063	0.032	0.0280	
ABC	2	1.867	0.933	0.8247	
Error	22	24.900	1.132		
Total	35	61.598			

APPENDIX 5(g): 1999 short rains ANOVA table for soil calcium concentration

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	7.473	3.736	0.0250	
Factor A	1	1260.605	1260.605	8.4446	0.0082
Factor B	2	21.222	10.611	0.0711	
AB	2	16.780	8.390	0.0562	
Factor C	1	1.170	1.170	0.0078	
AC	1	79.002	79.002	0.5292	
BC	2	234.427	117.214	0.7852	
ABC	2	111.189	55.594	0.3724	
Error	22	3284.157	149.280		
Total	35	3284.157			

APPENDIX 5 (h): 2000 long rains ANOVA table for soil calcium concentration

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	178.404	89.202	1.4690	0.2519
Factor A	1	1644.843	1644.843	27.0874	0.0000
Factor B	2	29.088	14.544	0.2395	
AB	2	1.345	0.672	0.0111	
Factor C	1	0.548	0.548	0.0090	
AC	1	44.134	44.134	0.7268	
BC	2	73.688	36.844	0.6067	
ABC	2	50.398	25.199	0.4150	
Error	22	1335.917	60.724		
Total	35	3358.364			

APPENDIX 5 (i): 1999 short rains ANOVA table for number of nodules

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	12.056	6.028	2.6911	0.0901
Factor A	1	26.695	26.694	11.9177	0.0023
Factor B	2	524.222	266.111	117.0192	0.0000
AB	2	1.556	0.778	0.3472	
Factor C	1	8.028	8.028	3.5840	0.0716
AC	1	0.028	0.028	0.0124	
BC	2	36.222	18.111	8.0857	0.0023
ABC	2	9.556	4.778	2.1330	0.1423
Error	22	49.278	2.240		
Total	35	667.639			

APPENDIX 5 (j): 2000 long rains ANOVA table for number of nodules

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	8.722	4.361	0.0807	
Factor A	1	289.000	289.000	5.3461	0.0305
Factor B	2	1201.722	600.861	11.1151	0.005
AB	2	144.500	72.250	1.3365	0.2833
Factor C	1	53.778	53.778	0.9948	
AC	1	40.111	40.111	0.7420	
BC	2	294.056	147.028	2.7198	0.0880
ABC	2	382.389	191.194	3.5368	0.0466
Error	22	1189.278	54.058		
Total	35	3603.556			

APPENDIX 5 (k): 1999 short rains ANOVA table for weight of nodules

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.021	0.010	0.0968	
Factor A	1	3.610	3.610	33.9482	0.0000
Factor B	2	17.254	8.627	81.1273	0.0000
AB	2	0.122	0.061	0.5721	
Factor C	1	0.250	0.250	2.3510	0.1395
AC	1	1.068	1.068	10.0413	0.0044
BC	2	1.812	0.906	8.5184	0.0018
ABC	2	0.124	0.062	0.5825	
Error	22	2.339	0.106		
Total	35	26.599			

APPENDIX 5(l): 2000 long rains ANOVA table for weight of nodules

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	13.782	6.891	0.7290	
Factor A	1	74.248	74.247	7.8546	0.0104
Factor B	2	146.655	73.327	7.7573	0.0028
AB	2	48.657	24.329	2.5737	0.0990
Factor C	1	0.840	0.840	0.0889	
AC	1	2.507	2.507	0.2652	
BC	2	33.574	16.287	1.7759	0.1928
ABC	2	23.207	11.604	1.2276	0.3123
Error	22	207.958	9.453		
Total	35	551.427			

APPENDIX 5 (m): 1999 short rains ANOVA table for number of pods per plant

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	27.556	13.778	1.3230	0.2867
Factor A	1	8.028	8.028	0.7709	
Factor B	2	114.389	70.194	6.7403	0.0052
AB	2	14.389	7.194	0.6908	
Factor C	1	0.694	0.694	0.0667	
AC	1	14.694	14.694	1.4110	0.2476
BC	2	39.056	19.528	1.8751	0.1770
ABC	2	9.722	4.861	0.4668	
Error	22	229.111	190.414		
Total	35	483.639			

APPENDIX 5 (n): 2000 long rains ANOVA table for number of pods per plant

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	8.222	4.111	0.7509	
Factor A	1	4.000	4.000	0.7306	
Factor B	2	87.056	43.528	7.9506	0.0025
AB	2	4.167	2.083	0.3805	
Factor C	1	93.444	93.444	17.0683	0.0004
AC	1	58.778	58.778	10.7362	0.0034
BC	2	14.389	7.194	1.3141	0.2890
ABC	2	14.389	7.194	1.3141	0.2890
Error	22	120.444	5.475		
Total	35	404.889			

APPENDIX 5(o): 1999 short rains ANOVA table for number of seeds per pod

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.667	0.333	0.6471	
Factor A	1	0.250	0.250	0.4853	
Factor B	2	6.167	3.083	5.9853	0.0084
AB	2	0.500	0.250	0.4853	
Factor C	1	0.250	0.250	0.4853	
AC	1	0.250	0.250	0.4853	
BC	2	1.167	0.583	1.1324	0.3403
ABC	2	2.167	1.083	2.1029	0.1460
Error	22	11.333	0.515		
Total	35	22.750			

APPENDIX 5 (p): 1999 short rains ANOVA table for Yield (kg/ha)

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	12433.716	6216.858	0.4866	
Factor A	1	135585.970	135585.970	10.6119	0.0036
Factor B	2	1640483.491	820241.745	64.1978	0.0000
AB	2	135608.637	67804.319	5.3068	0.0132
Factor C	1	13974.391	13974.391	1.0937	0.3070
AC	1	144902.045	144902.045	11.3410	0.0028
BC	2	59475.444	29737.722	2.3275	0.1211
ABC	2	3016.455	1508.227	0.1180	
Error	22	281089.546	12776.798		
Total	35	2426569.695			

APPENDIX 5(q): 2000 long rains ANOVA table for Yield (kg/ha)

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	14500.589	7250.294	1.0312	0.3732
Factor A	1	189610.921	189610.921	26.9674	0.0000
Factor B	2	1747123.510	873561.755	124.2424	0.0000
AB	2	129330.419	64665.209	9.1970	0.0013
Factor C	1	5596.537	5596.537	0.7960	
AC	1	164143.795	164143.795	23.3454	0.0001
BC	2	78610.814	39305.407	5.5902	0.0109
ABC	2	6444.915	3222.458	0.4583	
Error	22	154684.435	7031.111		
Total	35	2490045.435			

APPENDIX 5(r): 1999 short rains ANOVA table for plant N content

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.006	0.003	0.0216	
Factor A	1	0.391	0.391	2.8884	0.1033
Factor B	2	1.235	0.617	4.5659	0.0219
AB	2	0.091	0.045	0.3354	
Factor C	1	0.708	0.708	5.2382	0.0321
AC	1	0.020	0.020	0.1484	
BC	2	0.243	0.121	0.8975	
ABC	2	0.043	0.021	0.1586	
Error	22	2.978	0.135		
Total	35	5.711			

APPENDIX 5(s): 2000 long rains ANOVA table for plant N content

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.055	0.027	0.5873	
Factor A	1	0.783	0.783	16.8168	0.0005
Factor B	2	1.601	0.801	17.1885	0.0000
AB	2	0.286	0.143	3.0673	0.0668
Factor C	1	1.583	1.583	33.9976	0.0000
AC	1	0.079	0.079	1.7034	0.2053
BC	2	0.043	0.022	0.4617	
ABC	2	0.110	0.055	1.1781	0.3265
Error	22	1.025	0.047		
Total	35	5.565			

APPENDIX 5(t): 1999 short rains ANOVA table for plant P content

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.000	0.000	0.0941	
Factor A	1	0.003	0.003	1.7383	0.2009
Factor B	2	0.029	0.015	9.4785	0.0011
AB	2	0.000	0.000	0.0887	
Factor C	1	0.001	0.001	0.6531	
AC	1	0.000	0.000	0.0018	
BC	2	0.001	0.000	0.2732	
ABC	2	0.001	0.000	0.1972	
Error	22	0.034	0.002		
Total	35	0.069			

APPENDIX 5(u): 2000 long rains ANOVA table for plant P content

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	0.001	0.000	0.1994	
Factor A	1	0.012	0.012	8.4255	0.0083
Factor B	2	0.050	0.025	17.8600	0.0000
AB	2	0.000	0.000	0.1276	
Factor C	1	0.000	0.000	0.0977	
AC	1	0.000	0.000	0.0977	
BC	2	0.000	0.000	0.0319	
ABC	2	0.002	0.001	0.5823	
Error	22	0.031	0.001		
Total	35	0.095			

APPENDIX 5(v): 1999 short rains ANOVA table for rhizobial populations

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	3002.000	1501.000	0.4075	
Factor A	1	3364.000	3364.000	0.9134	
Factor B	2	7968.167	3984.583	1.0817	0.3564
AB	2	717.167	358.583	0.0974	
Factor C	1	112225.000	112225.000	30.4708	0.0000
AC	1	765.444	765.444	0.2078	
BC	2	4056.500	2028.250	0.5507	
ABC	2	1093.056	546.528	0.1484	
Error	22	81026.667	3683.030		
Total	35	214218.000			

APPENDIX 5(w): 2000 long rains ANOVA table for rhizobial populations

Source	Degree of Freedom	Sum of Squares	Mean Square	F Value	Prob
Replication	2	10462.056	5231.028	0.7135	
Factor A	1	56327.111	56327.111	7.6828	0.011
Factor B	2	72161.556	36080.728	4.9212	0.0171
AB	2	1928.222	964.111	0.1315	
Factor C	1	170844.444	170844.444	23.3024	0.0001
AC	1	13301.778	13301.778	1.8143	0.1917
BC	2	30820.222	15410.111	2.1019	0.1461
ABC	2	7440.222	3720.111	0.5074	
Error	22	161295.944	733.634		
Total	35	524581.556			