# EFFECT OF MICROBIAL INOCULANTS ON
## *STYLOSANTHES GUIANENSIS* cv SCHOFIELD
## FOR HERBAGE PRODUCTION

BY

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**THESIS**
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**1993**

## DECLARATION

I hereby declare that this thesis entitled "Effect of microbial inoculants on *Stylosanthes guianensis* cv Schofield for herbage production" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree diploma associateship fellowship or other similar title at any other University or society

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# CERTIFICATE

Certified that this thesis entitled, "Effect of microbial inoculants on *Stylosanthes guianensis* cv Schofield for herbage production" is a record of research work done independently by Miss SREEDURGA N under my guidance and supervision and that it has not previously formed the basis for the award of any degree fellowship or associateship to her

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I am greatly obliged to Sri P Chandrasekharan Professor and Head Department of Agronomy for going through the thesis and offering valuable suggestions

I owe a debt of gratitude to Dr G Raghavan Pillai Professor Department of Agronomy for his tireless and sincere help guidance and valuable suggestions during the course of my study and helpful criticism of the thesis

I am deeply indebted to Dr P Sivaprasad Associate Professor Department of plant pathology for the keen interest and immense help received from him throughout the study and critical evaluation of the script

I sincerely thank Dr G K Balachandran Nair Professor Department of Agronomy for suggesting the problem and giving guidance during the initial stages of the project

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N Sreedurga

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| mm | millimetre |
| cm | centimetre |
| m | metre |
| g | gram |
| kg | kilogram |
| t | tonne |
| ml | millilitre |
| ppm | parts per million |
| ha | hectare |
| $^{o}C$ | degree celsius |
| cv | cultivar |
| MAT | Month after transplanting |
| NCA | National Commission on Agriculture |
| VAM | Vesicular arbuscular mycorrhiza |
| Fig | Figure |
| KAU | Kerala Agricultural University |

# INTRODUCTION

# INTRODUCTION

The livestock population in India is the largest in the world but the production of milk and milk products is the lowest The daily percapita consumption of milk in India is about 114g while that recommended by medical authorities is 280g The introduction of crossbred animals with high productivity is a vital step in bridging this gap But to maintain the productivity of these exotic animals adequate quantity of good quality forage is a must

Kerala with a cattle population of 3 42 million produces 14 25 lakh tonnes of milk per year Milk production in Kerala mainly depends on highly priced concentrates and inferior quality roughages like straw weeds and crop wastes According to NCA (1977) about 65 70 percent of the production cost is accounted by way of feeding charges The requirement of dry fodder in the state is 67 6 lakh tonnes But the present availability is only 40 lakh tonnes of which cultivated fodder contributes only 0 4 lakh tonnes Thus 27 6 lakh tonnes of dry fodder is additionally required to meet the targeted milk production by 2000 A D

The scarcity of fertile farm land and the existing heavy pressure on land makes it almost impossible to attain self sufficiency in cultivated fodder The escalating prices of concentrates necessitates an increased availability of good quality grasses and legumes for economic milk production Hence the viable alternatives are to include fodder crops in different cropping systems and to increase their productivity so that 25 70 percent of the roughage requirements of cattle could be met from cultivated fodder

Legume fodder crops are better in quality and more nutritive They have high crude protein content (10 20%) compared to grasses In addition green legume crops provide carotene and other essential nutrients like calcium and phosphorus *Stylosanthes guianensis* dewit is a perennial leguminous fodder crop introduced to India from Australia The cultivation of this crop is gaining popularity in Kerala due to its suitability under open and partially shaded conditions Observational trials by KLD board have shown that the crop is well suited under sole and mixed cropping with a number of grasses Studies conducted at Vellayani by Chandini (1980) Lekha Sreekantan (1981) and Balachandran Nair (1989) have shown that this crop yielded on an average 35 tonnes of green fodder ha $^{1}$ under rainfed conditions in coconut gardens

The key limiting factor for legume fodder production is their high fertilizer requirement especially phosphorus The fertilizer requirement for *Stylosanthes* as given in the package of practices is N P K @ 20 80 30 kg ha $^{1}$ under Kerala conditions With the exorbitant rise in the price of chemical fertilizers it becomes highly demanding for farmers to use fertilizers even for food crops Hence though the fertilizers cannot be substituted the use of costly fertilizers to forages could be reduced to the maximum extent possible by exploring new avenues

In this context biofertilizers (microbial inoculants) seem to be a welcome boon to farmers With the rapid depletion of fossil fuels which are the source of energy for manufacture of fertilizers efforts should be oriented towards increasing the use of biofertilizers *Rhizobium* is a nitrogen fixing micro organism and in symbiotic association with the roots of legume crops In addition to economising nitrogen fertilizers Rhizobium inoculation serve to enrich soil fertility by augmenting nitrogen fixation

Phosphorus is a vital element in almost all biological systems (Westheimer 1987) and is required in large quantities Legumes have especially high phosphorus requirement but have less extensive root systems and hence are poor foragers of soil phosphorus In Kerala with predominantly acid soils phosphorus fixation as iron and aluminium phosphates is a major problem Vesicular arbuscular mycorrhiza (VAM) which is a fungus in symbiotic association with roots of crops have the ability to harvest even the unavailable or sparingly soluble forms of soil phosphorus and absorb it more readily than roots (Young et al 1986) In addition to phosphorus VAM fungi are known to increase the availability of micronutrients Hence it could be beneficial if the potentiality of these organisms to enhance the acquisition of nutrients and hence increase the productivity could be exploited to our advantage

Legumes have been shown to benefit from the dual action of obligate endophytic fungi (VAM) and Rhizobium in the root nodules Dual inoculation is reported to produce even better results compared to inoculation with either Rhizobium or mycorrhiza alone The synergestic effect due to co inoculation in Stylosanthes and it role in increasing productivity and reducing fertilizers has to be investigated and hence the present study was undertaken with the following objectives

1 To compare the effects of different inoculants (Rhizobium VAM and dual inoculation with Rhizobium and VAM) in increasing the fodder productivity of Stylosanthes

2 To find out if there is any reduction in fertilizer requirement due to inoculation treatments

3 To compare the economics of the above different treatments

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

The present investigation was undertaken with the object of increasing the herbage production of *Stylosanthes guianensis* by microbial inoculation and to save fertilizer without affecting the productivity, so that the production cost could be reduced considerably

The relevant literature on the fodder production of *Stylosanthes guianensis* due to the influence of microbial inoculation especially with respect to *Rhizobium* and mycorrhizal application along with chemical fertilizers are reviewed hereunder Other related crops are also taken into consideration wherever found needful

## 2.1 Effect of Inoculation on growth, yield and quality

### 2 1 1 1 Effect of *Rhizobium* on growth and yield

Karyagin (1980) found that *Rhizobium* strains increased the plant height, root weight fresh fodder, hay and seed yield in soybeans

Jonsson (1982) reported that *Rhizobium* inoculation increased dry matter production in lucerne

Nitrogen application @ 15kg $ha^{1}$ or *Rhizobium* inoculation resulted in significant increases in number of leaves, branches and dry matter $plant^{1}$ of greengram (Srivastava and Sharma, 1982) Similar increase in green fodder yield of lucerne due to inoculation with *Rhizobium* has been reported in a field trial by Gupta et al (1980)

*Medicago sativa* inoculated with *Rhizobium meliloti* strains gave higher fresh fodder yields compared to uninoculated control (Kots 1989)

Dry matter yield of *Medicago sativa* was found to be higher with *Rhizobium* inoculation than without but were not statistically significant (Curcita et al 1989)

2 1 1 2 Effect of *Rhizobium* on nodulation

Oke (1967) reported that *Cajanus* and *Stylosanthes guianensis* formed nodules in 3 weeks

Inoculation of berseem seed with ten strains of *Rhizobium trifolii* was seen to markedly increase the nodulation nitrogen fixation plant nitrogen content and fodder yield (Poi and Kabi 1979)

In pot trials with lucerne Choi et al (1978) found that inoculation with *Rhizobium* increased nodulation to 84 percent while uninoculated control registered 35 percentage nodulation

Inoculation with *Rhizobium* significantly increased number of nodules of lucerne upto 12 5 kg N $ha^{-1}$ (Gupta et al 1980)

Kim et al (1980) observed that *Rhizobium* inoculation increased the nodule number $plant^{-1}$ but had little effect on the nodule dry weight $plant^{-1}$ Nitrogen fixation was significantly increased by nodulation Beneficial effect of inoculation in increasing nodulation nodule dry weight $plant^{-1}$ and nitrogen content has been reported by Prasad and Ram (1988) and Vaishya et al (1983 ) in *Vigna radiata*

### 2 1 1 3 Effect of *Rhizobium* on nutrient uptake

#### 2 1 1 3 1 Nitrogen

A strong correlation was found between crown nodulation and nitrogen content of shoots in *Medicago sativa* (Pijnenborg et al 1981)

No significant difference in plant nitrogen content was noted by Tang et al (1982) on inoculation with *Rhizobium* in *Macroptylum atropurpureum*

*Rhizobium* inoculation significantly increased nitrogen uptake in green gram (Madhava Reddy 1986)

#### 2 1 1 3 2 Phosphorus

Inoculation with *Rhizobium* increased the phosphorus contents of shoots and seeds of mungbean (Yousef et al 1989) Similar increase in phosphorus content was reported by Raju and Verma (1984) in both straw and grain of mungbean due to inoculation

#### 2 1 1 3 3 Other nutrients

Prasad and Ram (1988) observed that *Rhizobium* inoculation increased calcium uptake and concentration in greengram

In mung potassium concentration significantly increased in straw due to *Rhizobium* treatment (Raju and Verma 1984)

### 2 1 1 4 Effect of *Rhizobium* on crude protein content

Karyagin (1980) reported increase in crude protein in hay of soybeans due to *Rhizobium* inoculation Similar increase has been noted in lucerne by Jonsson (1982) due to *Rhizobium* inoculation

In field trials with Medicago sativa it was observed that inoculation with Rhizobium strains increased the dry matter yield at first and second cuts and crude protein content in third and fourth cuts (Ponte et al 1988) Sudhakar et al (1989) found that inoculation or phosphorus application alone increased the protein content in blackgram compared to control but their combinations increased the contents still further

2 1 2 1 **Effect of Vesicular arbuscular mycorrhiza on growth and yield**

Powell and Daniel (1979) reported that when clover seedlings were inoculated with indigenous or $E_3$ strain of mycorrhizal fungi and transplanted into undisturbed cores of eight hill country soils an increased shoot growth from 16 117 percentage was observed It was also noticed that the growth benefit by inoculating mycorrhizal fungus tended to decrease after initial shoot harvest and then was maintained at lower level over subsequent harvests

A four fold increase in growth of lucerne was noticed by Owusu Bennoah and Mosse (1979) due to inoculation with Glomus caledonium Saterlee et al (1983) reported an increased top growth of lucerne with mycorrhizal inoculation in pot trials on a clay loam soil

A significant increase in shoot length root length shoot weight and root weight of cowpea greengram and blackgram was observed due to inoculation with VAM (Ramaraj and Shanmugham 1986)

Champawat (1989) reported increased shoot and root dry weight in chick pea with VAM inoculation in unsterilized soil

Inoculation of soybeans with <u>Glomus fasciculatum</u> or indigenous VAM fungi increased the dry matter accumulation in plants (Singh 1990)

**2 1 2 2 Effect of VAM on nodulation**

A strong stimulation of nodulation was noted by Crush (1974) in <u>Centrosema pubescens</u>, <u>Stylosanthes guianensis</u> and <u>Trifolium repens</u> due to mycorrhizal association

La Torraca (1979) reported an increase in nodulation of cowpea due to inoculation with VAM In Lentil (<u>Lens esculenta</u>) Bala and Singh (1983) observed improved nodulation and nitrogen fixation with VA mycorrhizal inoculation

Green et al (1983) found that inoculation of subterranean clover with <u>Glomus fasciculatum</u> <u>Glomus mosseae</u> or both had 2 1 4 and 1 9 times as many nodules as in uninoculated control plants

Soil inoculation with <u>Glomus fasciculatum</u> alone improved the number and dry weight of nodules and nitrogen concentration in shoots of <u>Cicer arietinum</u> equivalent to the effects of seed inoculation with <u>Rhizobium</u> (Subba Rao et al 1986)

**2 1 2 3 Effect of VAM on nutrient uptake**

**2 1 3 3 1 Nitrogen**

A positive correlation between VA mycorrhizal infection and the amount of phosphorus and nitrogen in the tissues of cowpea tomato and maize was reported by Sanni (1976)

Smith and Daft (1977) recorded a higher percentage of nitrogen concentration in shoots of mycorrhizal Medicago sativa at harvest But in sweet gum seedlings inoculation with VAM did not increase the nitrogen concentration in stem or leaves (Schultz et al 1979)

Barea and Azcon Aguilar (1983) suggested that VAM may be of special significance in legumes as the symbiotic nitrogen fixation is influenced by the phosphorus status of the host

Increase in the nitrogen content in the dry matter of lucerne was reported by Nielson (1990) and increase in nitrogen uptake of shoot and root of groundnut plants inoculated with VAM fungi was observed by Champawat (1990)

**2 1 2 3 2 Phosphorus**

Phosphorus has vital function in all biological systems because it is a major plant nutrient required in relatively large amounts (Hayman 1975 Tinker 1980) Mycorrhizal plants not only are large but also usually have an increased concentration and/or content of phosphorus compared to nonmycorrhizal plants (Barea 1991)

Smith and Daft (1977) recorded no significant differences in the phosphate content of mycorrhizal and non mycorrhizal clover plants

Inoculation with VAM did not increase phosphorus concentration in stems leaves or roots of sweet gum (Schultz et al 1979)

Saterlee et al (1983) observed an increase in herbage phosphorus content with mycorrhizal inoculation and phosphorus fertilization in lucerne cv Mesilla Dry matter production and herbage phosphorus content were enhanced by phosphorus application

Stribley et al (1984) reported that shoots of plants infected with VAM contained higher internal concentrations of phosphorus than those of uninfected plants of equal size, over wide ranges of external phosphorus supply and of host plants In Chick pea, soil application of Glomus fasciculatum alone increased the phosphorus concentration in the shoots (Subba Rao et al , 1986)

Le Tacon (1985) generalised that VAM increased the translocation of least soluble elements like phosphorus, zinc and copper

Sattar and Gaur (1989) obtained the highest mean grain yield hay yield and mean phosphorus uptake in Lentil cv L-5 9 inoculated with Glomus fasciculatum

Evaluation of the influence of phosphorus concentration on the symbiotic interaction between Leucaena leucocephala and VAM fungus, Glomus fasciculatum showed that mycorrhizal inoculation significantly increased phosphorus uptake (Habte and Manjunath,1987)

VAM inoculation significantly increased the available phosphorus content of the soil, uptake of phosphorus and other nutrients in green gram (Shanti et al , 1986) Similar increase in phosphorus content in dry matter was reported in lucerne by Nielson (1990) and Champawat (1990) in groundnut, due to VAM inoculation

### 2 1 2 3 3 VAM and acquisition of other nutrients

Studies to ascertain the direct role of VAM in plant uptake of nutrients other than phosphate and nitrogen are very few However the percentage content and/or concentration of major nutrients and trace elements in the shoots are reported and reviewed hereunder

#### 2 1 2 3 3 1 Potassium

Inoculation with VAM did not increase the potassium content in stem leaves or roots or sweet gum plants (Schultz et al, 1979) Harley and Smith (1983) also indicated that there was no conclusive support for the role of VAM in potassium uptake inspite of the fact that the diffusion rate of these ions is rather low in soil solution (Chapin 1970) The potassium content in the dry matter of lucerne was not affected due to inoculation with VAM (Nielson 1990)

Smith et al (1981) reported that mycorrhizal infection can improve the potassium nutrition of <u>Trifolium</u> <u>subterraneum</u> when internal potassium concentrations are generally low It was also suggested that increased potassium uptake may be an indirect result of improved phosphorus nutrition

#### 2 1 2 3 3 2 Calcium and magnesium

In sweetgum plants inoculation with VAM resulted in high concentrations of calcium in leaves but not stems Inoculation did not increase the magnesium concentration of the plant (Schultz et al 1979)

Comments on related papers by Smith and Gianinazzi Pearson (1988) suggested an association of $Ca^{2+}$ distribution in plants with the synthesis and breakdown of polyphosphate granules since the cation is a secondary constituent of these granules

Nielson (1990) reported that VAM inoculation significantly increased the calcium and magnesium content of lucerne

### 2 1 2 3 3 3 Micronutrients

Gray and Gerdemann (1967) and Bowen and Mosse (1969) found that endomycorrhizal association in many plants greatly increased the uptake of phosphorus and zinc from a nutrient solution

Rosse and Harper (1970) reported that mycorrhizal soybean plants accumulated great amounts of nitrogen phosphorus calcium copper and manganese in their foliage than non mycorrhizal plants

VAM increased zinc uptake in subterranean clover but not calcium uptake (Pairunan et al 1980)

Bagyaraj and Manjunath (1980) reported that phosphorus and zinc contents in cotton cowpea and ragi plants inoculated with Glomus fasciculatum were higher than that of uninoculated plants

Higher copper and zinc concentrations were reported in VAM inoculated soybean plants but iron and manganese concentrations were lower than in equivalent phosphorus fertilized plants (Pacovsky 1986)

In growth chamber studies soybeans were either inoculated with mycorrhizal fungus Acaulospora leaevis or untreated and given 0 300 kg $P_2O_5ha^{-1}$ Zinc concentration in leaves of noninoculated plants decreased significantly with increasing rates of applied phosphorus whereas foliar zinc concentration fell only slightly in inoculated plants, because mycorrhizal association increased zinc uptake Uptake of manganese iron and copper was not affected by inoculation (Wang et al 1988)

Arines et al (1989) reported that in red clover (Trifolium pratense) VAM inoculation decreased the magnesuim concentration in shoots and roots of plants

**2 1 3 1 Effect of dual inoculation with VAM and Rhizobium on growth and yield**

Legumes have the unique ability to form symbiosis with both VAM fungi and root nodule bacteria This tripartite symbiosis helps the plant with two vital elements nitrogen and phosphorus (Hayman 1982)

Daft and Giahmi (1974) found that infection of Phaseolus with Endogone and Rhizobium in comparison to Rhizobium alone significantly increased the growth

Dual inoculation with the symbionts Glomus and Rhizobium significantly increased the dry weight of shoots over single inoculation with Glomus or Rhizobium (Bagyaraj et al 1979) Barea et al (1980) also reported increase in dry weight of lucerne by dual inoculation Sivaprasad et al (1983) reported that inoculation with Rhizobium and mycorrhiza increased the growth of Leucaena leucocephala

Glycine max plants were grown in a green house sand medium low in available nitrogen and phosphorus and inoculated either with VAM fungus alone or a strain of Rhizobium japonicum alone or both endophytes together or left uninoculated to serve as control Nodulated plants contained 4 5 times the phytomass of noninoculated controls and plants with VAM + Rhizobium were 18 percent greater in dry weight than nodulated nonmycorrhizal plants due to positive VAM Rhizobium interaction (Pacovsky et al 1986)

Rinj et al (1987) reported that dual inoculation with Rhizobium and Glomus fasciculatum significantly increased the growth in Leucaena leucocephala when compared to single inoculation with either organism

Seedlings of Acacia auriculiformis grown in soil inoculated with Rhizobium recorded the greatest shoot and root dry weights compared to uninoculated control VAM inoculation alone and VAM + Rhizobium inoculation (Chang et al 1986)

**2 1 3 2 Effect of dual inoculation on nodulation**

An increase in the number and weight of root nodules was reported by Daft and Giahmi (1974) due to inoculation with Endogone and Rhizobium on Phaseolus compared to Rhizobium alone

Bagyaraj et al (1979) reported an increase in the number dry weight and nitrogen content of root nodules in plants inoculated with Rhizobium and Glomus sp compared to those plants inoculated only with Rhizobium Similar results were got in pot experiments using unsterilized soil in Stylosanthes (Mosse 1977)

Dual inoculation of soybean (Glycine max) with VA endophytes and Rhizobium japonicum significantly increased the number and weight of nodules in natural field soil compared to Rhizobium alone (Varma 1979)

Crush (1982) concluded from his experiments with Trifolium repens that inoculation with Glomus tenuis in the presence of Rhizobium trifolii had no effect on nodule initiation on a wide range of soil phosphate levels

Pandhar et al (1986) obtained a higher level of nodulation in mungbean when inoculated with Rhizobium and VA mycorrhiza Higher nodule weight and hence higher nitrogen fixation and specific nodule activity were reported in soybeans due to Glomus fasciculatum Rhizobium japonicum interactions (Pacovsky et al 1986)

Rinj et al (1987) observed that there was significant increase in nodulation and nitrogen fixation of Leucaena leucocephala by dual inoculation with Rhizobium and Glomus fasciculatum when compared to single inoculation with either organism

Rawat et al (1991) reported that the nodule number was significantly increased by Rhizobium and mycorrhiza treatment Nodule weight did not differ significantly with the application of Rhizobium and mycorrhizal strains Rhizobium strains were significantly superior with regard to shoot weight Mycorrhizal strains and their combinations significantly increased shoot weight over non mycorrhizal strains

**2 1 3 3 Effect of dual inoculation on nutrient uptake**

**2 1 3 3 1 Nitrogen**

An increase in nitrogen uptake due to dual inoculation was obtained in pot culture experiments with lucerne Inoculation with either Glomus mosseae or Rhizobium meliloti alone influenced nutrient uptake only slightly (Barea et al 1980)

Chang et al (1986) reported higher uptake of nitrogen and phosphorus due to dual inoculation with Rhizobium and Glomus fasciculatum in Acacia sp compared to uninoculated control and Rhizobium or VAM alone

Karunaratne et al (1987) also observed increased tissue nitrogen content of soybean due to dual inoculation with Glomus mosseae and Rhizobium japonicum

Increase in nitrogen uptake was found in plants inoculated with Rhizobium trifolii and mycorrhiza compared to non mycorrhizal plants by Morton et al (1990) Shoot nitrogen concentration was lower in mycorrhizal than in non mycorrhizal plants

2 1 3 3 2 **Phosphorus**

Daft and Giahmi (1974) reported an increase in phosphorus content and protein content of plants due to inoculation with Endogone and Rhizobium compared to Rhizobium alone in Phaseolus

Asimi et al (1980) while studying the influence of soil phosphorus levels on the interactions between mycorrhiza and Rhizobium in soybean observed an improved uptake of phosphorus by dually inoculated and mycorrhiza alone plants

In pot tests higher phosphorus concentration was observed in mycorrhizal plants by Huang et al (1983) and it was suggested that significant difference in nutrient uptake was because of increase in growth. Similar results were obtained by Jensen et al (1984) in lucerne and in chickpeas by Subba Rao et al (1986).

Morton et al (1990) reported that phosphorus concentration phosphorus uptake and number of nodules were greater with dual inoculation compared to non mycorrhizal plants.

2 1 3 3 3 Other nutrients

High calcium concentration was observed in pot tests due to dual inoculation and significant difference in shoot nutrient uptake was attributed to increased growth (Huang et al 1983). Hepper and Shea (1984) reported that infection of lettuce roots by Glomus caledonium and Glomus mosseae was dependent on the amount of calcium in soil those growing in low calcium soils being poorly infected.

In a field experiment where Cicer arietinum was inoculated with Glomus albidus with or without Rhizobium inoculation with Glomus albidus and Rhizobium increased the number of nodules plant $^{-1}$ nodules dry weight plant $^{-1}$, nodule haemoglobin content and total nodule nitrogen (Rai 1988).

**2 2 Effect of major nutrients on growth and yield**

**2 2 1 Effect of nitrogen on growth**

Mc lean et al (1974) reported that in fieldbeans, application of nitrogen increased plant weight.

Posypanov and Knyaseva (1974) stated that different levels of mineral nitrogen supply had practically no effect on plant growth and development.

Edge et al (1975) reported that leaf area index plant height and size were related to application of nitrogen in snap beans

Moursi et al (1976) found that application of nitrogen increased vegetative growth of lupin as reflected in its plant height and number of branches plant $^{1}$

Agboola (1978) observed that top growth of cowpea was increased with increasing nitrogen levels

In general it could be observed that increase in nitrogen supply increased the vegetative growth

**2 2 1 2 Effect of nitrogen on nodulation**

Medicago sativa plants were inoculated with Rhizobial strains and/or given 0 75 or 150 ppm nitrate nitrogen and it was observed that the percentage of plants nodulated and nodule pinkness decreased with increasing nitrogen rate (Viteri et al 1988)

Nitrate fertilizers decreased nitrogen fixation in while clover (Robin et al 1990)

**2 2 1 3 Effect of nitrogen on forage yield**

In a red clover timothy mixture in a dermapodzolic soil in Moscow region application of 150 kg $NH_4$ $NO_3$ ha $^{1}$ had no effect on the hay yield of red clover in the first year but in the second year red clover yield was decreased from 3 3 to 1 72t ha $^{1}$ (Kharkov and Shekhovtseva 1976)

The green fodder yield of berseem remained unaltered due to different levels of nitrogen and phosphorus tried indicating that the crop can be grown without addition of nitrogen and phosphorus in soils rich in nitrogen and phosphorus (Katti et al 1982)

In *Medicago sativa* dry forage yield was found to increase with increasing nitrogen rate (Zecdan et al 1988)

Increase in dry forage yield of summer cowpea was reported upto 40 kg N ha $^{1}$ (Rai and Patel 1991)

**2 2 2 1 Effect of Phosphorus on growth**

Mariyappan (1978) observed that phosphorus levels upto 120 kg ha $^{1}$ increased the height of *Stylosanthes gracilis*

Sharma and Lavania (1980) found that application of phosphorus as calcium superphosphate at the rate of 62 03 gm $^{2}$ twice increased the shoot length and spread significantly in *Vicia hirsuta* and *Vicia sativa*

Progressive increase in leaf area index of cowpea was reported by Balakumaran (1981) with increasing levels of phosphorus

Geetha Kumari (1981) reported increased plant height with phosphorus application in cowpea

Increasing the level of phosphorus from 40 to 120 kg $P_2O_5$ $ha^{-1}$ significantly increased the plant height spread nodule weight and nodule number of *Stylosanthes gracilis* (Lekha Sreekantan 1981)

Application of phosphorus @ 160 kg ha $^{1}$ recorded the maximum number and length of branches in *Stylosanthes gracilis* Maximum spread and height of *Stylosanthes sp* was achieved at 80 Kg $P_2O_5$ $ha^{-1}$ (Balachandran Nair 1989)

### 2 2 2 2 Effect of phosphorus on nodulation

Phosphorus application is known to increase seedling vigour nodulation and quality of forage legumes (Gates 1974 Khare and Rai 1968 Andrew and Robins 1969)

In an acid soil low in available phosphorus four species of medics showed poor growth and little or no nodulation Nodulation was significantly increased by adding phosphorus to soil (Wagner et al 1978)

Mariyappan (1978) noticed that maximum number of nodules (464 41) and nodule weight (452 49 mg) were recorded by the phosphorus level of 120 kg $P_2O_5$ $ha^{-1}$

Singh and Singh (1980) reported that increasing $P_2O_5$ from 0 to 160 kg $ha^{-1}$ increased both fresh and dry weight of nodules $plant^{-1}$ and percentage of effective nodules and C E C of roots

In a glass house experiment in Australia it was observed that chick peas showed strong positive responses in nodule number with increasing phosphorus rates while its effects were less and more variable in lupins (Jessop et al 1989)

Phosphorus @ 120 kg $ha^{-1}$ was significantly superior and recorded maximum nodule number and nodule weight and was on par with $P_2O_5$ @ 160 kg $ha^{-1}$ (Balachandran Nair 1989)

### 2 2 2 3 Effect of phosphorus on forage yield

Skerman (1977) reported corroborative results and observed that Stylosanthes spp are efficient in extracting phosphorus from the soil

In an experiment with *Stylosanthes guianensis* fertilized with phosphorus at 25 50 100 or 200 kg $ha^{-1}$ as super phosphate in a granitic sandy loam soil of North Queensland Bruce and Teitzel (1978) found that maximum dry matter yield was obtained with 25kg $P_2O_5$ $ha^{-1}$ *Stylosanthes* Similar increase in dry matter was reported in *Stylosanthes gracilis* at 120 Kg $P_2O_5$ $ha^{-1}$ (Mariyappan 1978)

Application of 45 Kg P $ha^{-1}$ significantly increased the dry matter yield of *Stylosanthes hamata* grown in native pasture in North Queensland (Gilbert and Shaw 1980)

Singh (1985) reported increase in plant height and dry matter in leaves stem and root of *Glycine javanica* with increasing phosphorus concentrations

In Nigeria Mohammed Saleem and Von Kaufman (1985) reported that dry matter yield of *Stylosanthes* cv Cook responded to increasing levels of phosphorus upto 80 Kg $P_2O_5$ $ha^{-1}$ The same trend was noticed in cv Schofield and the response was less marked in cv Verano

Balachandran Nair (1989) reported that 120 Kg $P_2O_5$ and lime @ 375 Kg $ha^{-1}$ were conducive for maximum green matter yield in *Stylosanthes spp.*

In *Medicago sativa* response to applied phosphorus was observed with 90 Kg $P_2O_5$ $ha^{-1}$ and further increase to 120 Kg $P_2O_5$ $ha^{-1}$ was not significant (Shah et al 1991)

Application of 50 kg $P_2O_5$ $ha^{-1}$ to four *Medicago sativa* cvs recorded average yields of 33 5 t of fresh fodder 7 95 t of dry matter and 1 72 t of crude protein ha $^{-1}$ compared to 30 1, 7 12 and 1 52 t respectively without phosphorus application No further increase in yield was noticed with 100 kg $P_2O_5$ ha $^{-1}$ (Patel et al 1990)

2 2 3 1 **Effect of potassium on growth**

El leboudi et al (1974) observed an increase in growth in beans when 250 kg K ha $^{-1}$ was applied

Groneman (1974) observed that potash fertilizers had little effect on growth in a three year trial with soybean

Peck and Buren (1975) concluded that snap beans grown with high rates of potassium made excessive vegetative growth

Annamma George (1980) reported an increase in height and number of leaves of blackgram with the application of potassium fertilizers upto 30 kg ha $^{-1}$

2 2 3 2 **Effect of potassium on nodulation**

Increasing potassium application from 0 to 448 kg $K_2O$ ha $^{-1}$ increased the nodule number and nitrogen fixation in alfafa (Collins et al 1986)

Leaf removal of white clover reduced nitrogen fixation which was higher with potassium application in the first harvest but not with subsequent harvests (Robin et al 1990)

High rates of potassium fertilization increased the nodule number and nodule mass in alfafa (Tindall and Bond 1990)

### 2 2 3 3 Effect of potassium on forage yield

In *Stylosanthes hamata* there was no significant response to phosphorus and potassium in respect of plant height green forage yield dry matter and crude protein yield (Rai and Patel 1985)

In soils with high potassium and medium phosphorus application of 80 Kg $P_2O_5$ and 40 Kg $K_2O$ ha $^{-1}$ increased average fresh fodder yield of alfafa by 22 and 4 percent and dry matter yields by 24 and 4 percent respectively Further increase in phosphorus and potassium rates were not effective (Collins et al 1986)

## 2 3 Effect of major nutrients on uptake

### 2 3 1 Nitrogen

Enikov and Velchev (1976) observed that nitrogen application increased the nitrogen content and decreased phosphorus and potassium content in chickpea

El Bakry et al (1980) reported that nitrogen application in beans increased nitrogen in plant parts

### 2 3 2 Phosphorus

From trials on red loam soil Sasidhar and George (1972) reported that increasing rates of $P_2O_5$ application increased the nitrogen content in lablab

Falade (1973) reported that phosphorus had no effect on the nitrogen content in *Stylosanthes gracilis*

While increasing rates of $P_2O_5$ form 0 to 180 kg ha[1] applied to three berseem cultivars Dhar (1978) found that phosphorus content of the herbage also increased with incremental doses of phosphorus

On increasing the phosphorus level significant increase in the potassium content of Stylosanthes gracilis was obtained by Mariyappan (1978)

Lekha Sreekantan (1981) obtained significant increase in nitrogen content in the dry matter with increase in the dose of phosphorus applied in the soil

Lekha Sreekantan (1981) and Balachandran Nair (1989) also observed an increase in $P_2O_5$ content of herbage with increase in phosphorus applied

2 3 3 Potassium

Stewart and Reed (1969) found that application of potassium to peas decreased the calcium and magnesium contents in the forage

Yuan et al (1970) obtained increased potassium content in leaf when soil application of potassium was done in soybean

Johnson and Evans (1975) obtained higher potassium content in leaf when potassium was applied in southern peas

## 2 4 Effect of major nutrients on the crude protein content

### 2 4 1 Nitrogen

Solomko and Rudin (1977) reported that in lucerne addition of 60 Kg N ha $^{1}$ along with phosphorus and potassium increased crude protein yield

Lee et al (1990) reported an increase in dry matter yield and crude protein content with addition of nitrogen in Medicago sativa

### 2 4 2 Phosphorus

A significant increase in protein content in Stylosanthes gracilis was recorded by Mariyappan (1978) when phosphorus was applied at the rate of 120 kg ha $^{1}$

From an experiment on blackgram Annamma George (1980) found that protein yield of bhusa increased by increasing the level of phosphorus from 30 to 45 kg $P_2O_5$ ha $^{1}$ but a further increase in $P_2O_5$ reduced the protein yield

Lekha Sreekantan (1991) observed that increasing the dose of phosphorus applied in the soil significantly increased the crude protein content in Stylosanthes gracilis

Arya and Kabra (1988) reported that application of varying phosphorus levels upto 75 Kg ha $^{1}$ did not significantly affect the protein percentage both in grain and straw of summer mung

## 2 4 3 Potassium

Johnson and Evans (1975) reported higher crude protein content in leaf when potassium was applied in southern peas

Rai and Patel (1985) found that there was no significant response to potassium in terms of crude protein yield in Stylosanthes hamata However application of 30 Kg $K_2O$ $ha^{-1}$ increased crude protein yield by 3 2 percent

Patel et al (1985) reported that application of 50 Kg $K_2O$ $ha^{-1}$ to alfafa cv Anand 2 gave highest yield of 8 40 t of dry matter and 1 76 t of crude protein

## 2 5 Mycorrhizal colonization in the root

Uninoculated plants of lucerne reached 45 percent infection from indigenous endophytes and inoculated plants reached 70 percentage Inoculation responses were not related to infection level Lucerne responded most from inoculation with most available phosphorus (Owusu Bennoah and Mosse 1979)

Chambers et al (1980) reported that addition of combined nitrogen decreased mycorrhizal development in young clover roots $NH_4^+$ being more effective than $NO_3^-$ ions

The percentage mycorrhizal infection decreased with increasing phosphorus levels in grasslands huia white clover soils (Lim and Cole 1984) whereas in a phosphorus deficient nonsterile soil application of phosphorus @ 22kg P $ha^{-1}$ increased nodulation and number of endomycorrhizal spores in the rootzone of both pigeonpea and cowpea (Manjunath and Bagyaraj 1984)

Saif (1986) recorded stimulated mycorrhizal infection with low levels of phosphorus while high levels tended to reduce infection in tropical forage legumes NPK fertilization slightly reduced spore numbers without affecting infection Potassium fertilization increased infection on legumes but had no effect on grasses

In pot experiments Habte and Manjunath (1987) observed that level of mycorrhizal infection increased in *Leucaena* roots as the concentration of phosphorus was raised from 0 002 to 0 153mgml $^{-1}$ Higher levels of phosphorus decreased mycorrhizal infection but the level was never less than 50 percent

Lipmann et al (1988) suggested that root colonization percentages of more than 20 percent can be characterized as high for field sites The level of available phosphorus content of soils influences root colonization percentages Most of the low colonization percentages found in soils cannot be related to high phosphorus fertility Good development of VAM fungi was also realised when plants were sufficiently fertilized

## 2 6 Interaction between inoculants and fertilizers

### 2 6 1 *Rhizobium* Fertilizer interaction

Subba Rao (1975) reported significant increase in number of nodules over uninoculated control at all nitrogen levels except at 37 5 kgha $^{-1}$ In uninoculated treatment there was no increase in number of nodules even with addition of 12 5 kg N ha $^{-1}$

Vargas and Suhet (1981) reported increased total nitrogen dry weight and nodulation in <u>Centrosema pubescens</u> by inocualtion with <u>Rhizobium</u> strains compared to combination of inoculation and 75 kg N ha $^{1}$

Maximum yield and nodulation of soybean without <u>Rhizobium</u> inoculation in field tests where soybeans had been grown previously was obtained with 40 kg N ha $^{1}$ Inoculation did not improve nodulation and crop yield (Shahidullah and Hussain 1980)

Soybeans inoculated with <u>Rhizobium japonicum</u> and given low rates of nitrogen and medium to high rates of phosphorus exhibited increased nodule number dry weight and leg haemoglobin content (Dadson and Acquaah 1984)

Raju and Verma (1984) obtained significant increase in nodulation of mung due to <u>Rhizobium</u> alone or <u>Rhizobium</u>+15kgNha $^{1}$ Maximum dry weight plant $^{1}$ protein yield and nitrogen uptake were got with <u>Rhizobium</u> + 15kg N ha $^{1}$

<u>Medicago sativa</u> cv Apollo grown in low nitrogen soil and given 0 224 kg nitrogen as $NH_4$ $NO_3$ responded to applied nitrogen only in uninoculated controls All nitrogen later decreased nodulation (Eardly 1985)

Phosphorus application and inoculation increased dry matter and nitrogen yield in <u>Centrosema pubescens</u> Inoculation increased nitrogen fixation at all phosphorus levels Phosphorus did not significantly increased nitrogen fixation in the absence of inoculation (Impithuksa and Rungratanakasin 1986)

Highest top dry matter and total nitrogen in two soils (infertile and medium fertile soils) were obtained with inoculation and phosphorus treatment (200 kg $P_2O_5$ ha $^{-1}$) The percentage increase was 116 percent in infertile soil and 46 percent in more fertile soils (Garza et al 1987)

Viteri et al (1988) observed increased plant weights by inoculation with Rhizobium strains and increasing nitrogen rates ie with 0 75 and 150 ppm nitrogen the plant dry weight was 11 6 26 1 and 29 9 mg and 6 8 29 1 and 39 2 mg without inoculation

In a trial with *Vigna radiata* cv $B_1$ Basu et al (1989) observed that seed inoculation with Rhizobium strains increased nodulation and shoot dry weight Application of 20 30 or 40 kg Nha $^{-1}$ gave 0 91 0 98 and 0 90 t ha $^{-1}$, compared to 0 70 t without nitrogen

Homachen et al (1989) observed no significant interaction between inoculation treatment and other factors in *Stylosanthes humilis* Top dry weight with 100 kg N ha $^{-1}$ (21 9g plant $^{-1}$ ) was significantly greater than Rhizobium strain CB 2248 which in turn was significantly greater than uninoculated control plants (13 7kg plot $^{-1}$)

**2 6 2 Vesicular arbuscular mycorrhiza (VAM) Fertilizer interaction**

Mosse et al (1976 found that the combination of rock phosphate and VA mycorrhiza acted significantly in increasing the plant dry weight in several crop plants

Mosse (1977) reported that in some cerrado soils, rock phosphate application particularly when coupled with mycorrhizal inoculation served as a better source of phosphorus than the more soluble forms of phosphates

Waidyanatha et al (1979) found that in mycorrhizal Pueraria and Stylosanthes the application of rock phosphate greatly stimulated nodulation and nodule activity

Non mycorrhizal clover plants produced more dry matter than mycorrhizal plants supplied with superphosphate Plants supplied with rock phosphate had the same dry weight at a given phosphorus concentration in the tops (Pairunan et al 1980)

Nielson and Jensen (1983) reported increased uptake of fertilizer phosphorus with mycorrhizal inoculation in lucerne

Paulino et al (1986) showed increased uptake of phosphorus by mycorrhizal Centrosema and Macroptylum plants over uninoculated plants when tested with rock phosphate and soluble forms of phosphorus Nodulation nitrogen fixation and rock phosphate utilization were increased by mycorrhizal inoculation

Santhi et al (1988) reported that in green gram among the different sources of phosphorus tried rock phosphate was more efficiently utilised when applied with VA mycorrhiza VAM inoculation with 50 per cent rock phosphate was as a good as full dose of phosphorus alone

### 2 6 3 Dual inoculation Fertilizer interaction

The responses of pigeonpea and cowpea to dual inoculation with *Glomus fasciculatum* and/or rock phosphate was studied in phosphorus deficient non sterile soil Plants inoculated with both mycorrhiza and *Rhizobium* and supplemented with phosphorus recorded the highest shoot dry weights nitrogen and phosphorus contents indicating the need for addition of small amounts of phosphorus to derive maximum benefit from dual inoculation with *Rhizobium* and VAM (Manjunath and Bagyaraj 1984)

Dual inoculation with *Glomus fasciculatum* and *Rhizobium* spp registered the highest phosphorus status in the plants at $N_{20}P_{50}$ level of fertilizer application in chickpeas (Subba Rao et al 1986)

In pot trials with *Vigna radiata* grown in a phosphorus deficient soil soil inoculation with mycorrhizal fungus(*Glomus fasciculatum*) or 15 kg N ha $^{-1}$ increased the plant nodule dry weight and yields Inoculation with *Rhizobium* and/or mycorrhizal fungus in combination with phosphorus gave highest yields (Guptaet al 1988)

Meena Kumari and Nair (1991) reported increase in number of nodules shoot dry weight and nitrogen content of cowpea with dual inoculation and added rock phosphate

# MATERIALS AND METHODS

# MATERIALS AND METHODS

The present investigation envisages the possibility of increasing the herbage production of <u>Stylosanthes guianensis</u> by microbial inoculation and thus to save fertilizer without affecting the productivity

The field experiment was conducted during the period from July 1991 to January 1992 The materials used and the methods adopted for the study are detailed hereunder

## 3 1 MATERIALS

### 3 1 1 Experimental site

The experiment was conducted at the Instructional Farm attached to the College of Agriculture Vellayani The farm is located at 8 5$^{o}$ N latitude and 76 9$^{o}$ longitude at an altitude of 29 m above mean sea level

### 3 1 2 Soil

The soil of the experimental area is red sandy clay loam The data on the physico chemical properties of the soil of the experimental site are given below

A) Physical Properties

Mechanical Composition

| Constitute | Content in Soils (%) | Method used |
|---|---|---|
| Coarse sand | 14 70 | International Pipette |
| Fine sand | 33 30 | |
| Silt | 26 50 | Method (Piper 1950) |
| Clay | 25 50 | |
| Textural class | Sandy clay loam | |

B CHEMICAL COMPOSITION

| Constituent | Content in Soil (kg ha$^{-1}$) | Rating | Method used |
|---|---|---|---|
| Total nitrogen | 3920 | | Modified Microkjeldhal method (Jackson 1973) |
| Available nitrogen | 313 6 | Medium | Alkaline Potassuim permanganate method (Subbiah and Asija 1956) |
| Available $P_2O_5$ | 39 5 | Medium | Bray colorimetric method (Jackson 1973) |
| Available $K_2O$ | 68 14 | Low | Ammonium acetate method (Jackson 1973) |
| Available Calcium | 414 24 | | Ammonium acetate method (Jackson 1973) |
| Available Magnesium | 52 9 | | Ammonium acetate method (Jackson 1973) |
| Organic carbon | 0 143 | Low | Walkely and Black rapid titration method (Jackson 1973) |
| pH | 4 8 | Acidic | 1 2 5 soil solution ratio using pH meter |

### 3 1 3 Season

The experiment was conducted during the period from July 1991 to January 1992

### 3 1 4 Weather conditions

The experimental site enjoys a humid tropical climate The meteorological parameters recorded during the experimental period are given in Appendix and depicted in Fig 1 Data on total rainfall minimum temperature maximum temperature and relative humidity during the entire crop season were collected from the meteorological observatory at the College of Agriculture and presented as weekly averages

The mean maximum and minimum temperatures during the cropping season ranged form 28 01 °C to 32 6 °C and 19 53 °C to 24 64 °C respectively The mean relative humidity ranged from 70 43 percent to 87 36 percent The mean rainfall of the cropping period ranged from 0 to 33 37 mm

3 1 5 **Cropping history of the field**

The experimental site was partially shaded coconut garden having palms of 60 70 years age permitting 70 percent of solar radiation to filter through the canopy The land was left fallow prior to the commencement of the trial before which it had been cropped with guinea grass

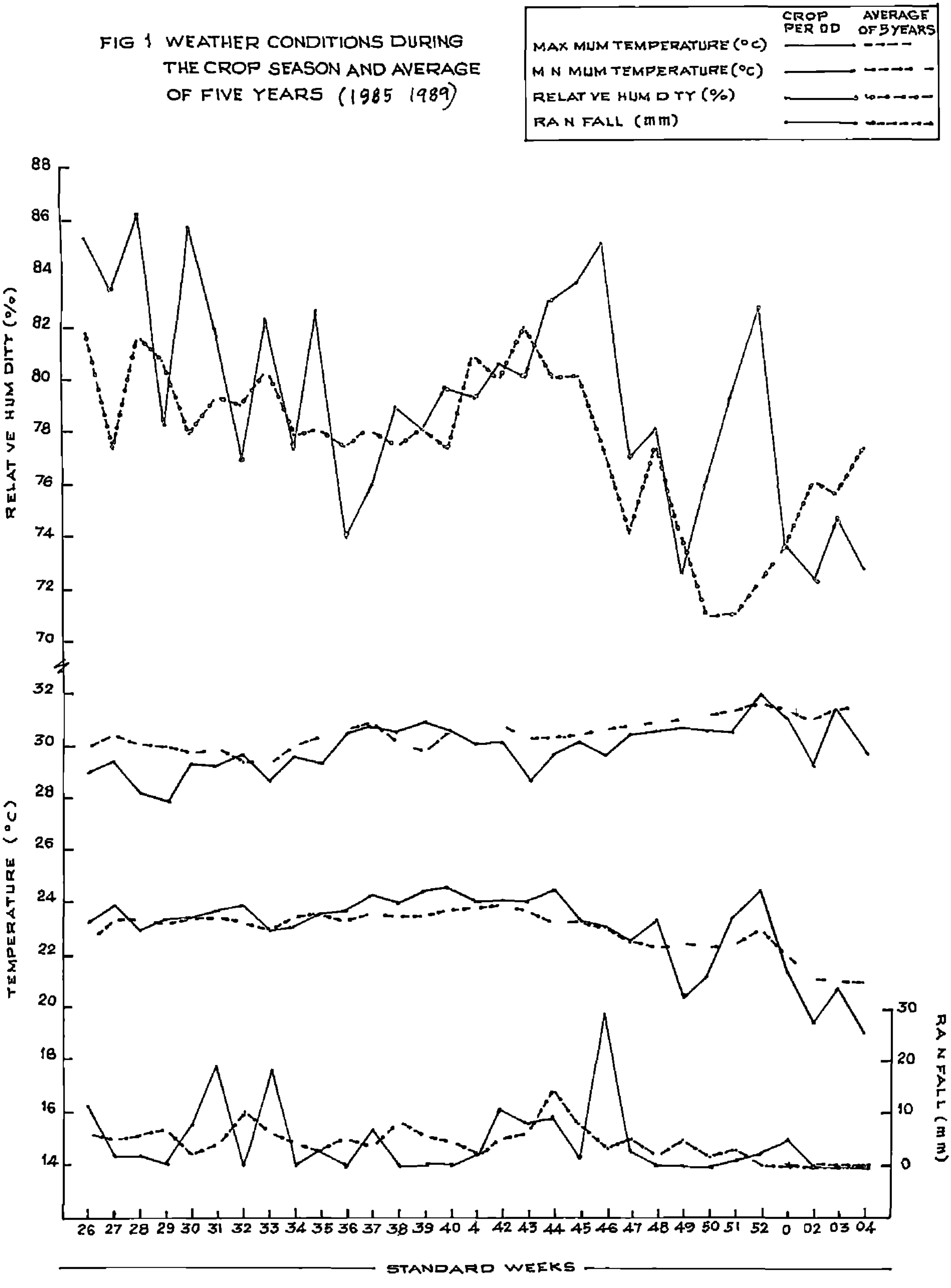
3 1 6 **Variety**

Stylosanthes guianensis cv Schofield or stylo common is a perennial leguminous fodder crop native to Brazil in South America This crop besides enriching the soil fertility increases the cation exchange capacity of the soil and is valued for its high quality fodder

3 1 7 **Source of seed**

The seeds were obtained from the Kerala Livestock Development Board Dhoni

FIG 1 WEATHER CONDITIONS DURING THE CROP SEASON AND AVERAGE OF FIVE YEARS (1985 1989)



3 1 8 Fertilizers

Fertilizers with the following analysis were used for the study

| | |
|---|---|
| Urea | 46 percent N |
| Mussoori Rock Phosphate | 20 percent $P_2O_5$ |
| Muriate of Potash | 60 percent $K_2O$ |

3 1 9 Inoculants

3 1 9 1 Rhizobium culture

The Rhizobium culture for inoculation of Stylosanthes seeds was obtained from the Division of Plant pathology College of Agriculture Vellayani Culture of cowpea type Rhizobium strain STYLO 1 was used for seed inoculation at the rate of 100g acre $^{-1}$

3 1 9 2 Mycorrhizal inoculum

Panicum maximum (Jacq ) infected with mycorrhizal fungi (Glomus fasciculatum) was grown in sterile soil sand mixture for three months This soil sand mixture containing mycorrhizal spores infected root segments chlamydospores and hyphae served as mycorrhizal inoculum About 50 ml of mycorrhizal inoculum containing 250 300 spores was placed two cm below the seed prior to sowing

3 2 METHODS

3 2 1 NURSERY

3 2 1 1 Land preparation

The land was dug twice stubbles were removed clods broken and levelled Four raised nursery beds of 4 5 sq m each were taken

### 3 2 1 2 Lay out

The lay out plan of the nursery plot is given in Fig 2

### 3 2 1 3 Seeds and sowing

A seed rate of 1 5 $kgha^{-1}$ as recommended for partially shaded conditions in the package of practices KAU was adopted Seeds were divided into four equal parts for sowing in the four nursery beds

### 3 2 1 4 Methods of sowing

1 No inoculants ($I_0$)

Seeds were uniformly broadcast in the nursery plot and covered with a thin layer of sand

2 Rhizobium ($I_1$)

Seeds were thoroughly mixed with the Rhizobium culture and broadcast evenly in the nursery plot

3 Vesicular Arbuscular Mycorrhiza (VAM) ($I_2$)

Soil of the nursery plot was inoculated with the mycorrhizal culture prior to sowing Uninoculated seeds were sown broadcast in the nursery plot

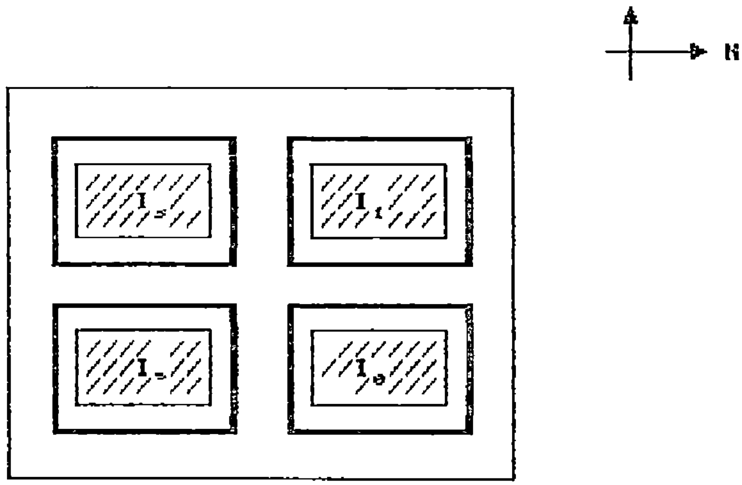
4 VAM + Rhizobium ($I_3$)

The soil of the nursery plot was treated with the mycorrhizal culture and seeds inoculated with Rhizobium were sown broadcast in the nursery plot

### 3 2 2 Main field

### 3 2 2 1 Land Preparation

The main field was dug twice stubbles removed clods broken and field was laid out into blocks are plots




Treatments

$I_0$ No inoculants (Control)

$I_1$ Rhizobium

$I_2$ Vesicular Arbuscular Mycorrhiza (VAM)

$I_3$ – VAM + Rhizobium

Fig 3 LAY OUT PLAN OF THE NURSERY PLOT

3 2 2 2 **Fertilizer application**

Fertilizers were applied to all the plots as per the treatment Nitrogen phosphorus and potassium were applied to the plots in the form of urea mussoori phos and muriate of potash respectively The entire quantity of fertilizers were applied as basal dose one day prior to transplanting

3 2 2 3 **Transplanting**

A light irrigation was given to the nursery beds before pulling out the seedlings 45 day old seedlings were carefully removed from the nursery plots To assure minimum damage to the roots transplanting was done with a ball of earth around the roots Small furrows were taken in the mian field at the recommended spacing of 30x10 cm and the uprooted seedlings were transplanted as per the treatments Irrigation was given immediately after transplanting to enbale the seedlings to withstand transplanting shock

3 2 2 4 **After cultivation**

At seven days after transplanting gap filling was done Regular weeding operations were carried out at 45 days after transplanting and after first cut

3 2 2 5 **Plant Protection**

Control of grasshopper and molecricket was achieved by dusting BHC 5% as a prophylatic measure

### 3 2 2 6 Harvesting

The first harvest of green fodder was taken at three months after transplanting and the second two months thereafter The plants were cut with sickles at a height of 15 cm from the ground The weight of green fodder in kg ha $^{-1}$ was recorded

## 3 3 Technical Programme

### 3 3 1 Design and Lay out

The experiment was laid out as strip plot experiment in RBD with 12 treatment combinations The lay out plan is shown in Fig 3

| | |
|---|---|
| Gross plot size | 4 5 x 4 m |
| Net plot size | 2 7 x 3 6 m |
| Spacing | 30 x 10 cm |
| Treatment combinations | 12 |
| Replications | 4 |
| Total number of plots | 48 |

### 3 3 2 Treatments

a Inoculations 4

1 Control ($I_0$)

2 <u>Rhizobium</u> ($I_1$)

3 Vesicular arbuscular mycorrhiza (VAM) ($I_2$)

4 <u>Rhizobium</u> + VAM ($I_3$)

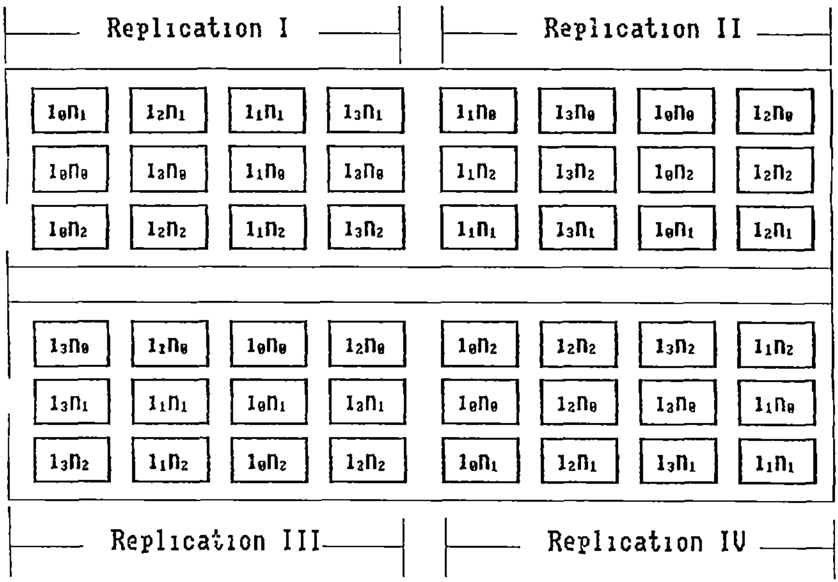
b Nutrient levels 3

1 No nutrients ($N_0$)

2 50 percent of the recommended nutrient dose ($N_1$)

3 100 percent of the recommended nutrient dose ($N_2$)

Recommended nutrient dose N P K @ 20 80 30 kg ha $^{-1}$




A. Inoculants

1 Control ($I_0$)

2. *Rhizobium* ($I_1$)

3 Vesicular Arbuscular mycorrhiza (VAM) ($I_2$)

4. VAM + *Rhizobium* ($I_3$)

B. Nutrient levels

1. No nutrients ($N_0$)

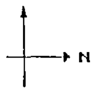
2. 50 percent of the recommended dose ($N_1$)

3. 100 percent of the recommended dose ($N_2$)

Gross plot size : 4.5 x 4 m

Net plot size : 2.7 x 3.6 m

Fig 3 LAYOUT PLAN OF THE EXPERIMENT PLOT



### 3 3 3 Treatment combinations

The treatment combinations are as follows

| | | | |
|---|---|---|---|
| $T_1$ | $i_0 n_0$ | $T_7$ | $i_2 n_0$ |
| $T_2$ | $i_0 n_1$ | $T_8$ | $i_2 n_1$ |
| $T_3$ | $i_0 n_2$ | $T_9$ | $i_2 n_2$ |
| $T_4$ | $i_0 n_0$ | $T_{10}$ | $i_3 n_0$ |
| $T_5$ | $i_1 n_1$ | $T_{11}$ | $i_3 n_1$ |
| $T_6$ | $i_1 n_2$ | $T_{12}$ | $i_3 n_2$ |

### 3 4 Biometric observations

In each plot two rows all around were left as border rows Two rows of plants were ear marked for destructive sampling The next row was also left as border Biometric observations were taken from ten plants selected at random in the net plot and average values were recorded

### 3 4 1 Height of the Plant

Height of the plant was measured from the base of the plant to the growing tip of the tallest branch and expressed in cm

### 3 4 2 Spread of the plant

The spread was measured as the maximum lateral diameter through the main stem of each plant and expressed in cm

### 3 4 3 Number of branches

The total number of branches in each plant was counted and was recorded as mean branch number

### 3 4 4 Length of branches

The length of all branches of each plant was measured and mean length was recorded

### 3 4 5 Leaf stem ratio

From the observational row in each plot ten plants were cut at the base The leaves and stem were seperated and oven dried for three days The dry weight of leaves and stem of individual plants were recorded and the ratio computed by dividing the leaf dry weight by the stem dry weight

### 3 4 6 Nodule number

Ten seedlings were selected at random from each nursery plot at transplanting and the mean nodule number was recorded Five plants from the destructive sampling row in each main plot were carefully uprooted and the number of nodules were counted at each cut

### 3 4 7 Nodule weight

From the same plants uprooted for counting the nodule number the nodules were separated and weighed in a sartorius balance and recorded in mg

### 3 4 8 Mycorrhizal colonization in the root

Five plants were carefully uprooted from the destructive sampling row the roots were washed and the VA mycorrhizal infection in the root samples was observed by staining the root tissue (Phillips and Hayman 1970)

## 3 5 Analytical procedures

### 3 5 1 Plant analysis

The whole plant was analysed for nitrogen phosphorus potassium calcium magnesium and micronutrients Iron manganese zinc and copper The plant samples were dried in an oven at 80°C till constant weights were achieved The samples were then ground to pass through a 0 5 mm mesh in a Wiley mill The required quantity of samples were then weighed out in an electronic balance and analysis was carried out

### 3 5 1 1 Total nitrogen content

Total nitrogen content was estimated by modified microkjeldhal method (Jackson 1973) and the values were expressed as percentages

### 3 5 1 2 Uptake of nitrogen

This was calculated by multiplying the nitrogen content of the plant with the total dry weight of the plant The uptake values were expressed in kgha $^{1}$

### 3 5 1 3 Total phosphorus content

Phosphorus content was estimated colorimetrically (Jackson 1973) by developing colour by vanadomolybdophosphoric yellow method and read in spectronic 2000 Spectrophotometer

### 3 5 1 4 Uptake of phosphorus

Phosphorus uptake was calculated by multiplying the phosphorus content and dry weights of the plants The values were expressed in lgha $^{1}$

### 3 5 1 5 Total potassium, calcium and magnesium contents

Total potassium calcium and magnesium contents in plants were estimated by the flame photometric method in a Perkin Elmer 3030 Atomic Absorption Spectro photometer after wet digestion of the sample with diacid mixture (Perkin Elmer Corporation 1982)

### 3 5 1 6 Uptake of potassium, calcium and magnesium

This was calculated by multiplying the dry weights and respective values for potassium calcium and magnesium contents of the plants Uptake values were expressed in kgha $^{1}$

### 3 5 1 7 Crude protein content

The percentage of crude protein was calculated by multiplying the percentage of nitrogen in the plant by the factor 6 25 (Simpson et al 1965)

### 3 5 1 8 Content and uptake of mircronutrients

The content of micronutrients (Iron Manganese Zinc and Copper) was estimated by the flame photometric method in a Perkin Elmer 3030 Atomic Absorption Spectro Photometer after digestion of the sample using diacid mixture (Perkin Elmer Corporation 1982) Uptake was calculated by multiplying the micronutrient contents with dry weights of the plants and expressed in kg ha $^{1}$

### 3 5 2 Soil Analysis

Soil samples were taken from the experimental area before and after the experiment The air dried soil samples were analysed for total nitrogen available nitrogen available phosphorus available calcium and available magnesium contents Available nitrogen content was estimated by alkaline potassium permanganate method (Subbiah and Asija 1956) available phosphorus content by Bray s colorimetric method (Jackson 1973) and available potassium calcium and magnesium by ammonium acetate method (Jackson 1973)

## 3 6 Economics of cultivation

Net Income = Gross income − Cost of cultivation

Benefit cost ratio = Gross income / Cost of cultivation

## 3 7 Statistical analysis

The data generated from the experiment was subjected to analysis of variance technique (ANOVA) as applied to strip plot experiment in RBD as suggested by Cochran and Cox (1962) Analysis excluded data pertaining to initial nodule number nodule weight and mycorrhizal colonization in the root since the observations were made only from a composite sample

# RESULTS

# RESULTS

A field experiment was conducted in the Instructional Farm College of Agriculture Vellayani to study the effect of different microbial inoculants and nutrient levels on the forage production of *Stylosanthes guinanensis* cv Schofield The experimental data collected were statistically analysed to bring out the effect of different levels of nutrients different inoculants and their interactions The results obtained are presented under the following sections

## 4 1 Growth Characters

### 4 1 1 Height of the plants

The influence of different nutrient levels and inoculants on the height of the plants is presented in Table 1

The mean values of plant height showed that due to inoculation plant height did not differ significantly in the first and second months after transplanting But at the time of first cut (90 days after planting) plant height differed significantly Maximum height of 45 66 cm was recorded by the treatment $I_3$ (44 percent more than control) followed by $I_1$ which was on par with $I_2$ In the second cut (60 days after the first cut) the height increase due to inoculation was not significant

With increasing fertilizer dose from $N_0$ to $N_2$ level significant increase in plant height was noticed at the first and second months after transplanting Height was maximum at $N_2$ but it was on par with $N_1$

Table 1 Effect of inoculation and nutrient levels on the height of the plants

| | Height (cm) | | | |
|---|---|---|---|---|
| Treatments | 1 MAT | 2 MAT | First cut | Second cut |
| $I_0$ | 4 68 | 17 71 | 31 60 | 23 41 |
| $I_1$ | 5 22 | 18 60 | 37 66 | 25 72 |
| $I_2$ | 5 01 | 19 81 | 37 45 | 24 28 |
| $I_3$ | 5 93 | 18 45 | 45 56 | 25 78 |
| F (3 9) | NS | NS | 5 303* | NS |
| SEm± | 0 4692 | 2 481 | 4 094 | 2 245 |
| CD(0 05) | | | 10 613 | |
| $N_0$ | 6 10 | 23 35 | 47 48 | 30 55 |
| $N_1$ | 7 05 | 24 70 | 51 44 | 32 78 |
| $N_2$ | 7 70 | 26 53 | 53 35 | 35 75 |
| F (2 6) | 12 260** | 5 618* | NS | 5 931* |
| SEm± | 0 278 | 0 851 | 2 952 | 1 377 |
| CD(0 05) | 0 763 | 2 338 | | 3 783 |

* Significant at 5 percent level

** Significant at 1 percent level

NS Not significant

The increase in plant height with increasing levels of nutrients was not significant in the first cut But at second cut there was significant increase in height with increasing nutrient levels the highest value of 35 75cm being recorded at $N_2$ level which was on par with $N_1$ The interaction effects of inoculation and nutrients levels were not significant at any stages

**4 1 2 Number of branches**

The effect of treatments on the number of branches plant $^{1}$ is presented in Table 2

The effect of inoculation was not significant at the first and second cut But the treatment $I_2$ recorded the highest branch number of 5 81 and 4 74 respectively for the first and second cuts

The fertilizer treatments were found to have significant effect on the number of branches in the first cut the treatments $N_1$ and $N_2$ being on par and significantly superior to control This trend was however not noticed in the second cut

The interaction effects of inoculants and nutrients levels were not significant either at the first cut or second cut

**4 1 3 Length of branches**

The influence of inoculation and nutrient levels on the length of branches is presented in Table 2

In the first cut the maximum length of 36 26cm was recorded by the treatment $I_3$ followed by $I_2$ (32 31cm) In the second cut $I_2$ and $I_3$ treatments recorded similar values and the lowest length of branches was noticed for the treatment $I_0$

The nutrient levels had no significant effect on the length of branches in the first cut The treatment $N_1$ recorded highest value (46 51 cm) at the first cut and at the second cut $N_2$ was significantly superior to $N_1$ with a mean length of 32 94 cm

There were no significant effects due to the interaction between inoculants and different nutrient levels at both the cuts

**4 1 4 Spread of plants**

The effect of inoculants and nutrient levels on the spread of plants is presented in Table 3

The main effect of inoculation showed no significant influence on this character Among the different inoculants tried the treatment $I_3$ registered the maximum spread of 45 00 cm at first cut and 29 63 cm in the second cut

Significant response to increase in nutrient levels was observed on the spread of the plants The maximum spread of the plant was recorded by $N_1$ in both cuts But thereafter a slight decrease in spread was noticed when the nutrient level was increased to $N_2$

The interaction effects between inoculation and nutrients levels were not significant at both cuts

Table 2 Effect of inoculation and nutrient levels on number of branches and length of branches

| Treatment | Number of branches | | Length of branches (cm) | |
|---|---|---|---|---|
| | First cut | Second cut | First cut | Second cut |
| $I_0$ | 5 78 | 4 57 | 30 35 | 20 81 |
| $I_1$ | 5 81 | 4 39 | 32 31 | 22 41 |
| $I_2$ | 5 81 | 4 74 | 31 98 | 23 31 |
| $I_3$ | 5 09 | 4 71 | 36 26 | 23 31 |
| F (3 9) | NS | NS | NS | NS |
| SEm± | 0 935 | 0 605 | 3 717 | 1 319 |
| CD(0 05) | | | | |
| $N_0$ | 6 88 | 5 73 | 39 34 | 25 94 |
| $N_1$ | 7 50 | 6 21 | 46 51 | 30 97 |
| $N_2$ | 8 31 | 6 47 | 45 05 | 32 94 |
| F (2 6) | 5 769 | NS | NS | 30 804 |
| SEm+ | 0 328 | 0 256 | 2 545 | 0 819 |
| CD(0 05) | 0 900 | | | 2 251 |

* Significant at 5 percent level

** Significant at 1 percent level

NS Not significant

Table 3 Effect of inoculation and nutrient levels on the spread of plants

| Treatments | Spread (cm) First cut | Second cut |
|---|---|---|
| $I_0$ | 36 94 | 27 06 |
| $I_1$ | 41 72 | 28 38 |
| $I_2$ | 42 76 | 29 44 |
| $I_3$ | 45 00 | 29 63 |
| F (3 9) | NS | NS |
| SEm+ | 3 4694 | 1 783 |
| CD(0 05) | | |
| $N_0$ | 49 06 | 35 78 |
| $N_1$ | 58 51 | 39 00 |
| $N_2$ | 58 56 | 38 72 |
| F (2 6) | 13 753* | 5 976* |
| SEm± | 1 888 | 0 918 |
| CD(0 05) | 5 187 | 2 524 |

* Significant at 5 percent level

NS Not significant

### 4 1 5 Leaf Stem ratio

The data on the leaf stem ratio of the crop in two cuts is presented in Table 4

Inoculation had no significant effect on leaf stem ratio at the first cut In the second cut the treatment $I_3$ was on par with $I_2$ and $I_1$ but superior to control

Among the different nutrient levels $N_1$ and $N_2$ were on par and significantly superior to $N_0$ in both the cuts

The interaction effects between inoculation and nutrient levels were not significant

### 4 1 6 Number and weight of nodules at transplanting

The mean values of nodule count and nodule weight at transplanting are presented in Table 5

Highest mean value for nodule count was obtained with the treatment $I_1$ followed by the treatment $I_3$ at transplanting A similar trend was noticed in the case of nodule weight also

### 4 1 7 Number of nodules

A significant increase in nodule number was observed in the first cut due to inoculation The highest mean nodule number of 141 6 was recorded in the treatment $I_3$ which was on par with the treatment $I_1$ where the nodule count was 134 3 In the second cut also there was a significant increase in nodule count due to inoculation Here the treatment $I_3$ showed the maximum nodule count of 211 6 which was on par with the treatment $I_1$ which recorded a count of 202 1

Table 4 Effect of inoculation and nutrient levels on the leaf stem ratio

| Treatments | Leaf Stem ratio<br>First cut | <br>Second cut |
|---|---|---|
| $I_0$ | 0 733 | 0 677 |
| $I_1$ | 0 763 | 0 722 |
| $I_2$ | 0 842 | 0 920 |
| $I_3$ | 0 909 | 1 001 |
| F (3 9) | NS | 4 274* |
| SEm± | 0 080 | 0 124 |
| CD(0 05) | | 0 321 |
| $N_0$ | 0 857 | 0 922 |
| $N_1$ | 1 102 | 1 170 |
| $N_2$ | 1 290 | 1 230 |
| F (2 6) | 28 436** | NS |
| SEm± | 0 512 | 0 109 |
| CD(0 05) | 0 141 | |

* Significant at 5 percent level

** Significant at 1 percent level

NS Not significant

Table 5  Mean nodule count and weight at transplanting

| Treatment | Nodule Count | Nodule dry weight (mg) |
|---|---|---|
| $I_0$ | 12 | 0 020 |
| $I_1$ | 26 | 0 050 |
| $I_2$ | 17 | 0 032 |
| $I_3$ | 20 | 0 036 |

Table 6  Effect of inoculation and nutrient levels on number of nodules

| Treatment | First cut | Second cut |
|---|---|---|
| $I_0$ | 85 2 | 127 4 |
| $I_1$ | 134 2 | 202 1 |
| $I_2$ | 102 4 | 157 1 |
| $I_3$ | 141 6 | 211 8 |
| F(3 9) | 12 616** | 12 641** |
| SEm$\pm$ | 1 23 | 18 58 |
| CD(0 05) | 31 975 | 48 178 |
| $N_0$ | 131 4 | 196 2 |
| $N_1$ | 151 4 | 227 3 |
| $N_2$ | 180 7 | 270 5 |
| F(2 6) | NS | NS |
| SEm$\pm$ | 17 99 | 27 09 |
| CD(0 05) | | |

** Significant at 1 per cent level
NS Not Significant

The increase in nodule count in both cuts was not significant as far as fertilizer treatments were concerned The highest nodule number was recorded with the $N_2$ treatment followed by $N_1$ treatment in both the cuts

The interaction effects between inoculation and fertilizer levels were not significant

4 1 8 **Weight of nodules**

The mean weight of nodules due to various treatments are presented in Table 7

Inoculation significantly increased the weight of nodules in the first and second cut In the first cut highest nodule weight of 150 2 mg was observed for the treatment $I_3$ which was on par with $I_1$ recording 144 44 mg There was significant increase in nodule weight in the treatment I3 in the second cut (221 3mg) which was on par with the treatment $I_1$ (212 0 mg) but the treatment $I_2$ and $I_0$ registered low nodule weights which were on par

Fertilizer levels did not show any significant effect on the nodule weight However the highest nodule weight of 191 8 mg in the first cut and 282 8 mg in the second cut were recorded in the treatment $N_2$ and the lowest by the control

The interaction effects between inoculation and nutrient levels were not significant in both the cuts

Table 7 Effect of inoculation and nutrient levels on the weight of nodules.

| Treatment | Weight of nodules (mg) First cut | Second cut |
|---|---|---|
| $I_0$ | 90 8 | 134 3 |
| $I_1$ | 144 4 | 212 0 |
| $I_2$ | 107 6 | 160 5 |
| $I_3$ | 150 2 | 221 3 |
| F(3 9) | 14 21** | 12 52** |
| SEm± | 12 52 | 19 53 |
| CD(0 05) | 32 453 | 50 103 |
| $N_0$ | 140 6 | 205 4 |
| $N_1$ | 160 4 | 239 8 |
| $N_2$ | 191 8 | 282 8 |
| F(2 6) | NS | NS |
| SEm± | 19 63 | 26 90 |
| CD(0 05) | | |

** Significant at 1 percent level

NS Not significant

4 2 Green matter yield

The mean values of green matter yield at each cut are presented in Table 8

The effect of inoculation at the first cut was significant The treatment $I_3$ registered highest green matter yield of 6466 Kgha $^{1}$ followed by $I_2$ The increase in green matter yield due to the combined application of VAM and Rhizobium ($I_3$) was 51 5 percent over control ($I_0$)

In the second cut there was no significant increase in green matter yield due to inoculation However the treatment $I_3$ recorded 34 2 percent more yield followed by $I_2$ which gave 33 percent more yield than control

Among the fertilizer treatments treatments $N_1$ and $N_2$ were on par and significantly superior to control $N_0$ at both the cuts

The interaction effects were not significant

4 3 Dry matter yield

The results on the effect of treatments on dry matter yield are presented in Table 9

The treatment $I_3$ recorded the highest dry matter yield and was on par with $I_2$ The treatments recorded significantly higher dry matter yield over control ($I_0$) in both cuts

The different nutrient levels also gave significantly higher yields compared to control ($N_0$) Among the treatments $N_1$ and $N_2$ were on par and highly significant over control in both the cuts

The interaction effects between fertilizer levels and inoculation were not significant

Table 8 Effect of inoculation and nutrient levels in the green matter yield

| Treatment | Green matter yield (Kg ha$^{1}$) First cut | Second cut |
|---|---|---|
| $I_0$ | 4267 | 3989 |
| $I_1$ | 5134 | 5117 |
| $I_2$ | 6288 | 5307 |
| $I_3$ | 6466 | 5355 |
| F(3 9) | 5 764** | NS |
| SEm+ | 708 079 | 573 42 |
| CD(0 05) | 1835 52 | |
| $N_0$ | 4945 | 5693 |
| $N_1$ | 8434 | 7006 |
| $N_2$ | 8776 | 7071 |
| F(2 6) | 27 516** | 18 786** |
| SEm$\pm$ | 508 940 | 225 876 |
| CD(0 05) | 1398 47 | 620 941 |

** Significant at 1 percent level

NS Not significant

Table 9 Effect of inoculation and nutrient levels on the dry matter yield

| Treatment | Dry matter yield (Kg ha$^{1}$) First cut | Second cut |
|---|---|---|
| $I_0$ | 791 | 754 |
| $I_1$ | 985 | 1021 |
| $I_2$ | 1231 | 1025 |
| $I_3$ | 1302 | 1035 |
| F(3 9) | 7 779* | 4 111 NS |
| SEm± | 138 222 | 110 81 |
| CD(0 05) | 358 31 | 287 259 |
| $N_0$ | 950 | 1098 |
| $N_1$ | 1626 | 1360 |
| $N_2$ | 1783 | 1377 |
| F(2 6) | 27 720** | 19 134** |
| SEm± | 101 582 | 45 05 |
| CD(0 05) | 279 128 | 123 785 |

* Significant at 5 percent level

** Significant at 1 percent level

NS Not significant

**4 4 Mycorrhizal colonization in the root**

The mean value for the mycorrhizal colonization in the roots of VAM inoculated plants at transplanting was found to be 29 33 percent The mean mycorrhizal colonization at the close of the experiment showed that the highest value of 81 8 per cent was recorded by the treatment combination $i_3n_1$ followed by $i_3n_0$ (75 percent) The least value was recorded for the treatment $i_0n_2$ (17 2 percent)

**4 5 Nutrient uptake**

**4 5 1 Nitrogen uptake**

The mean values of nitrogen uptake by the crop are presented in Table 11

It was seen that inoculation did not have any significant effect on nitrogen uptake by the plant However the treatment $I_1$ showed the highest nitrogen uptake value followed by $I_3$

The different nutrient levels also showed no significant difference in nitrogen uptake However there was progressive increase in uptake from $N_0$ to $N_2$

Interaction between inoculation and different nutrient levels were also found to be not significant

**4 5 2 Phosphorus uptake**

The mean values of phosphorus uptake are presented in Table 11

Inoculation had significant effect on phosphorus uptake The treatment $I_2$ recorded the highest phosphorus uptake value of 17 31 kg ha $^{1}$ which was on par with $I_3$ (16 83 kg ha $^{1}$)

Table 10 Mean mycorrhizal colonization in the root at the end of the experiment

| Treatment | % Mycorrhizal colonization |
|---|---|
| $i_0n_0$ | 21 7 |
| $i_0n_1$ | 24 0 |
| $i_0n_2$ | 17 2 |
| $i_1n_0$ | 27 7 |
| $i_1n_1$ | 23 3 |
| $i_1n_2$ | 20 0 |
| $i_2n_0$ | 35 7 |
| $i_2n_1$ | 62 5 |
| $i_2n_2$ | 65 6 |
| $i_3n_0$ | 75 0 |
| $i_3n_1$ | 81 8 |
| $i_3n_2$ | 68 7 |
| Control | 29 33 |

Control Percentage mycorrhizal colonization in the roots at transplanting

Progressive increase in phosphorus uptake was noted due to increasing fertilizer levels the highest value being for the $N_2$ level (21 63 kg ha $^{1}$) However the differences were not significant

Interaction between inoculation and nutrient levels had no significant influence on phosphorus uptake

4 5 3 **Potassium uptake**

Table 11 gives the mean value of potassium uptake by the crop Potassium uptake was significantly influenced by inoculation and the highest value of 10 26 kg ha $^{1}$ was recorded by the treatment $I_3$ but it was on par with $I_2$

There was significant increase in potassium uptake due to different nutrient levels Potassium uptake value was found to be the highest with $N_2$ which was on par with the treatment $N_1$

The interaction effects between inoculation and fertilizer levels were found to be not significant

4 5 4 **Calcium uptake**

The mean values for calcium uptake are presented in Table 11

No significant effect was observed due to inoculation on the calcium uptake by the plant However the treatment $I_3$ recorded the highest calcium uptake value and the treatment $I_0$ recorded the lowest uptake value

Higher levels of nutrients significantly increased the uptake of calcium by plants The treatment $N_2$ recorded the highest calcium uptake of 66 61 kg ha $^{1}$ which was on par with $N_1$ (66 14 kg ha $^{1}$)

Table 11 Effect of inoculation and nutrient levels on the uptake of major nutrients (kg ha$^{1}$)

| Treatment | Nitrogen uptake | Phosphorus uptake | Potassium uptake | Calcium uptake | Magnesium uptake |
|---|---|---|---|---|---|
| $I_0$ | 38 54 | 9 19 | 12 72 | 33 27 | 10 85 |
| $I_1$ | 52 21 | 15 51 | 16 48 | 47 74 | 14 51 |
| $I_2$ | 48 57 | 17 31 | 16 51 | 43 63 | 15 79 |
| $I_3$ | 52 07 | 16 83 | 18 26 | 56 87 | 15 73 |
| F(3 9) | NS | 14 618** | 4 121* | NS | NS |
| SEm± | 6 39 | 1 62 | 1 89 | 11 73 | 3 03 |
| CD(0 05) | | 4 191 | 4 909 | | |
| $N_0$ | 57 43 | 4 28 | 17 19 | 48 76 | 17 52 |
| $N_1$ | 66 83 | 19 18 | 22 42 | 66 14 | 19 92 |
| $N_2$ | 67 14 | 21 63 | 24 37 | 66 61 | 19 44 |
| F(2 6) | NS | NS | 18 50* | 7 706* | NS |
| SEm± | 3 09 | 2 10 | 1 09 | 4 62 | 1 18 |
| CD(0 05) | | | 2 988 | 12 684 | |

* Significant at 5 percent level

** Significant at 1 percent level

NS Not significant

The interaction effects between inoculation and nutrient levels on calcium uptake were not significant

4 5 5 **Magnesium uptake**

The mean magnesium uptake values are presented in Table 11

Inoculation did not significantly influence magnesium uptake However $I_2$ recorded the highest value for magnesium uptake (15 79 kg ha $^{1}$) Uptake by plants in the $I_0$ treatment was the lowest

Different levels of nutrients did not significantly increase the magnesium uptake by the plants However the treatment $N_2$ recorded the highest uptake value of 19 94 kg ha $^{1}$

Interaction effects between inoculation and nutrient levels had no significant influence on magnesium uptake

4 5 6 **Micronutrient uptake**

The mean uptake values of micronutrients (Iron Manganese Zinc and Copper) are given in Table 12

The effect of inoculation on micronutrient uptake was not significant However all the micronutrients studied showed an increased uptake rate upto $I_2$ Iron and copper showed a slight decrease in uptake with the treatment $I_3$

As far as nutrient levels were concerned the different levels had no significant influence on micronutrient uptake

Table 12 Micronutrient uptake as influenced by inoculation and different nutrient levels (kg $ha^{-1}$)

| Treatement | Iron | Manganese | Zinc | Copper |
|---|---|---|---|---|
| $I_0$ | 4 06 | 2 11 | 1 49 | 5 73 |
| $I_1$ | 4 14 | 2 15 | 1 49 | 6 07 |
| $I_2$ | 5 86 | 2 11 | 1 64 | 6 38 |
| $I_3$ | 5 30 | 2 36 | 1 97 | 6 16 |
| F(3 9) | NS | NS | NS | NS |
| SEm± | 1 16 | 0 40 | 0 35 | 1 08 |
| CD (0 05) | - | | | |
| $N_0$ | 5 72 | 3 09 | 2 37 | 7 64 |
| $N_1$ | 6 11 | 2 57 | 1 91 | 8 60 |
| $N_2$ | 7 52 | 3 09 | 2 13 | 8 11 |
| F(2 6) | NS | NS | NS | NS |
| SEm± | 0 80 | 0 19 | 0 30 | 0 74 |
| CD (0 05) | - | | - | - |

NS - Not significant

4 6 Soil analysis

4 6 1 Available nitrogen

The mean values for available nitrogen in soils after the experiment is given in Table 13

It was observed that inoculation did not significantly influence the available nitrogen status in the soil

The different levels of nutrients significantly increased the available nitrogen status of soil The highest value of 248 27 kg ha $^{1}$ was recorded by the treatment $N_2$ which was significantly superior to $N_1$ and $N_0$

There was however no significant response in available nitrogen status of the soil due to the interaction between inoculation and nutrient levels

4 6 2 Available phosphorus content in soil

The mean values for available phosphorus content in the soil after the experiment are given in Table 13

Inoculation significantly decreased the available phosphorus content in the soil The control treatment recorded the highest available phosphorus in the soil followed by $I_3$ The lowest value was recorded by the treatment $I_1$

There was significant increase in available phosphorus content of the soil due to different nutrient levels The treatment $N_1$ recorded the highest available phosphorus content of 42 19 kg ha $^{1}$ which was significantly superior to $N_2$ and $N_0$

4 6 3 **Available potassium content in soil**

The mean values of available potassium content in the soil are given in Table 13

There was no significant increase in available potassium content of the soil due to inoculation However treatment $I_3$ recorded the highest available potassium content of 36 53kg ha $^{-1}$

The different nutrient levels did not significantly influence the potassium content of the soils The treatment $N_1$ recorded the highest available potassium content in the soil followed by $N_0$ and $N_2$

4 6 4 **Available calcium content in soil**

The mean values of available calcium content in the soil after the experiment are given in Table 13

There was no significant difference in the available calcium content in the soil due to inoculation However the highest content was recorded by the treatment $I_0$ and least by the treatment $I_1$

The different nutrient levels tried had also no significant influence on available calcium content in the soil

4 6 5 **Available magnesium content in soil**

The mean values for available magnesium content in the soil after the experiment are given in Table 13

Inoculation did not significantly increase the available magnesium content in the soil However the highest value was recorded for the treatment $I_2$ and least for the treatment $I_1$

Table 13 Available soil nutrient status after the experiment as influenced by inoculation and nutrient levels (kg ha$^{-1}$)

| Treatment | Available nitrogen | Available phosphorus | Available potassium | Available calcium | Available magnesium |
|---|---|---|---|---|---|
| $I_0$ | 143 73 | 32 87 | 33 95 | 292 14 | 35 73 |
| $I_1$ | 143 73 | 28 24 | 33 88 | 261 34 | 33 75 |
| $I_2$ | 143 73 | 29 25 | 31 84 | 269 26 | 41 38 |
| $I_3$ | 143 73 | 29 27 | 36 53 | 281 39 | 37 91 |
| F(3 9) | NS | 6 515* | NS | NS | NS |
| SEm± | 16 997 | 1 210 | 5 19 | 30 48 | 3 35 |
| CD(0 05) | | 3 148 | | | |
| $N_0$ | 156 80 | 39 40 | 48 17 | 348 75 | 46 08 |
| $N_1$ | 169 87 | 42 19 | 50 67 | 357 72 | 46 65 |
| $N_2$ | 248 27 | 38 63 | 37 36 | 397 66 | 56 02 |
| F(2 6) | 34 399** | 7 279* | NS | NS | NS |
| SEm± | 10 62 | 0 87 | 6 59 | 18 64 | 3 57 |
| CD(0 05) | 29 188 | 2 404 | | | |

* Significant at 5 percent level

** Significant at 1 percent level

NS Not significant

The nutrient levels also did not have any significant influence on the available magnesium content in the soil

## 4 7 Crude Protein

The effect of different treatments on the crude protein content of the crop is presented in Table 14

There was no significant increase in crude protein content due to the effect of the different inoculants The different nutrient doses also did not show any significant influence on the crude protein content

## 4 8 Economics of cultivation

The results on the economics of cultivation of <u>Stylosanthes</u> under different inoculation and fertilizer treatments is presented in Table 15

The treatment combination $i_3n_1$ (VAM + <u>Rhizobium</u> + 50 percent recommended dose) recorded the highest net returns of Rs 3743 09 and Benefit cost ratio of 2 01 This was followed by the treatment $i_2n_0$ (VAM+ no nutrients) with a Benefit cost ratio of 1 99 and $i_2n_1$ (VAM + 50 percent of the recommended dose ) with a net profit of Rs 2467 20 The control treatment $i_0n_0$ registered the lowest net income of Rs 711 70 and Benefit cost ratio of 1 33

Table 14 Effect of inoculation and nutrient levels on the crude protein (%) in the crop

| Treatment | Crude protein (%) |
|---|---|
| $I_0$ | 14 05 |
| $I_1$ | 15 84 |
| $I_2$ | 14 42 |
| $I_3$ | 15 11 |
| F (3 9) | NS |
| SEm± | 0 543 |
| CD(0 05) | — |
| NO | 13 82 |
| $N_1$ | 14 16 |
| $N_2$ | 16 59 |
| F(2 6) | NS |
| SEM± | 0 938 |
| CD(0 05) | — |

NS Not significant

Table 15 Economics of cultivation

| Treatments | Cost of Cultivation (Y) (Rs ) | Yield (Kg ha⁻¹) | Gross income (X) (Rs ) | Net income (Rs ) (X Y) | B/C ratio (X/Y) |
|---|---|---|---|---|---|
| $i_0n_0$ | 2159 00 | 9569 | 2870 70 | 711 70 | 1 33 |
| $i_0n_1$ | 2597 00 | 11852 | 3555 60 | 958 60 | 1 37 |
| $i_0n_2$ | 3035 00 | 13808 | 4142 40 | 1107 40 | 1 36 |
| $i_1n_0$ | 2226 70 | 11666 | 3499 80 | 1273 10 | 1 57 |
| $i_1n_1$ | 2664 70 | 15494 | 4648 20 | 1983 50 | 1 74 |
| $i_1n_2$ | 3092 70 | 14795 | 4438 50 | 1345 80 | 1 44 |
| $i_2n_0$ | 2239 20 | 11509 | 4452 70 | 2213 50 | 1 99 |
| $i_2n_1$ | 2677 20 | 17148 | 5144 40 | 2467 20 | 1 92 |
| $i_2n_2$ | 3105 20 | 18503 | 5550 90 | 2445 70 | 1 79 |
| $i_3n_0$ | 2251 70 | 11011 | 3303 30 | 1051 60 | 1 46 |
| $i_3n_1$ | 2689 70 | 18109 | 5432 79 | 2743 09 | 2 01 |
| $i_3n_2$ | 3117 70 | 19054 | 5716 50 | 2598 50 | 1 83 |

Price (Rs ) 1Kg N 8 70 1Kg $P_2O_5$ 9 38 1Kg $K_2O$ 3 33 1Kg green fodder 0.30

Labour Cost Rs 54 60/ Labourer

# DISCUSSION

# DISCUSSION

A field experiment was conducted in the Instructional farm Vellayani to study the effect of different microbial inoculants in Stylosanthes guianensis cv Schofield for increasing the herbage production The results obtained from the study are discussed below

## 5 1 Growth characters

### 5 1 1 Height of plants

The results presented in Table 1 revealed that there was no significant effect due to dual inoculation with VAM + Rhizobium on the height of the plant at first and second months after transplanting This is in agreement with the observations of Carling et al (1980) in soybean where the response to mycorrhizal inoculation was evident only about 6 weeks after planting At the first cut (90 days after planting) there was significant difference in height between the treatments The maximum height (45 56 cm) was recorded in the dual inoculation treatment Similar increase in shoot growth due to dual inoculation was reported by Azcon et al (1979) in lucerne This is attributed to a synergestic effect due to interaction between the micro symbionts A similar trend was noticed in the second cut also But the increase in height due to inoculation was not statistically significant Similar reduction in response after the initial shoot harvest has been observed in white clover by Powell and Daniel (1979)

It was observed that full fertilizer dose ($N_2$) significantly increased the plant height over control at the first and second months after transplanting This might be due to the fact that increased supply of nitrogen phosphorus and potassium increased the height of plants The plants in unfertilized plot recorded the lowest height at all stages As the level of NPK increased from 0 kg $ha^{-1}$ to 20 80 30 kg $ha^{-1}$ the plant height increased progressively The influence of nitrogen in promoting the vegetative growth of plants is well established and as such the increase in plant height with incremental doses of nitrogen is quite natural (Tisdale et al 1985)

Phosphorus promotes root development (Tisdale et al 1985) and promotes meristematic activity (Black 1968) The increased root growth in turn would enhance the uptake of nutrients resulting in rapid growth and development including shoot growth Similar increase in plant growth due to phosphorus application was reported in Stylosanthes gracilis by Mariyappan (1978) Lekha Sreekantan (1981) and Balachandran Nair (1989)

The increasing levels of potassium from 0 kg $K_2O$ $ha^{-1}$ to 30 kg $K_2O$ $ha^{-1}$ was also found to contribute to the increasing plant height Potassium is important in the photosynthetic process thus leading to greater carbon assimilation and growth (Russell 1973)

The fertilizer dose 50 per cent of the recommended dose and 100 percent recommended dose were found to be on par This might be due to the comparatively better fertility status of the experimental soil

**5 1 2 Number of branches**

The results presented in Table 2 showed that there was no significant increase in the number of branches due to inoculation The treatment VAM alone recorded the highest number of branches in both cuts This was on par with the treatment VAM + Rhizobium and Rhizobium treatment alone Increase in the number of branches due to VAM inoculation may be related to enhanced nutrient uptake and translocation by fungal activity which enhances the utilization of photosynthate in the aerial part of the plant (Smith et al 1981)

The increasing fertilizer dose from control to full recommended dose significantly increased the number of branches in the first cut Application of nitrogen phosphorus and potassium might have increased the vegetative growth of plants Similar results have been reported with increasing nitrogen levels in lupin by Moursi et al (1976) increasing phosphorus levels in Stylosanthes gracilis by Balachandran Nair (1989) and increasing potassium levels by Annamma George (1980) in blackgram

5 1 3 Length of branches

The results presented in Table 2 showed that there was no significant increase in the length of branches due to different inoculants The treatment VAM + Rhizobium recorded the maximum length of branches in the first cut In the second cut also a similar trend was noticed The synergistic effect due to the dual inoculation treatment might have influenced the meristematic activity due to the better acquisition of nutrients resulting in increased length of branches (Barea 1991)

The increasing fertilizer levels did not have any significant influence on the length of branches However there was an increasing trend with increase in nutrient levels Similar increase in length of branches with increasing nitrogen levels was reported by Moursi et al (1976) in lupin and with increasing phosphorus levels in Stylosanthes guianensis by Balachandran Nair (1989) The increase in length of branches might be attributed to the fact that at higher nutrient levels, the uptake of nutrients was higher (Table 11 and 12) This would have stimulated meristematic activity, resulting in increased length of branches

5 1 4 Leaf stem ratio

Results presented in Table 4 showed that there was no significant difference in leaf stem ratio between the different inoculation treatments at the first cut However at the second cut, it was found that inoculation significantly increased the leaf stem ratio over control Leaf stem ratio is a measure of the quality of fodder and hence determines its effect on animals There was gradation in the leaf stem ratio, the highest being recorded by VAM + Rhizobium treatment, followed by treatment VAM alone Thus due to the combined treatment, the quality of forage has been improved Similar improvement in quality of fodder due to dual inoculation was reported by Bagyaraj et al (1979) in cowpea and Rinj et al (1987) in Leucaena leeucocephala The fungal hyphae increases the uptake increases the uptake of phosphorus (Table 11) and phosphate is known to increase the leaf area

The different nutrient levels were found to have significant influence on the leaf stem ratio of the plant at the first cut Though this effect was not so pronounced, the same trend continued in the second cut also The role of nitrogen in increasing the leafiness of crops is well known (Russell, 1973) Chandini (1980) and Lekha Sreekantan (1981) also reported an increased leaf stem ratio with increase in levels of phosphorus application upto 120 kg $P_2O_5$ ha $^{-1}$ Phosphorus application might have stimulated better root and nodule development which resulted in increased nitrogen availability and hence better leaf stem ratio (Russell 1973)

5 1 5 Spread of plants

The results presented in Table 3 showed that there was no significant difference between the different inoculation treatments However the treatment VAM + Rhizobium recorded the highest spread followed by VAM treatment alone in both the cuts This increase might be due to enhanced mineral nutrition of the plant resulting from greater exploring ability of the extramatrical mycelium of vesicular arbuscular mycorrhiza which overcomes the limitations on acquisition of ions that diffuse slowly in soil solution to the rhizosphere (Barea 1991) The increase in the length of branches and general height of the plants might also have contributed to the wider spread

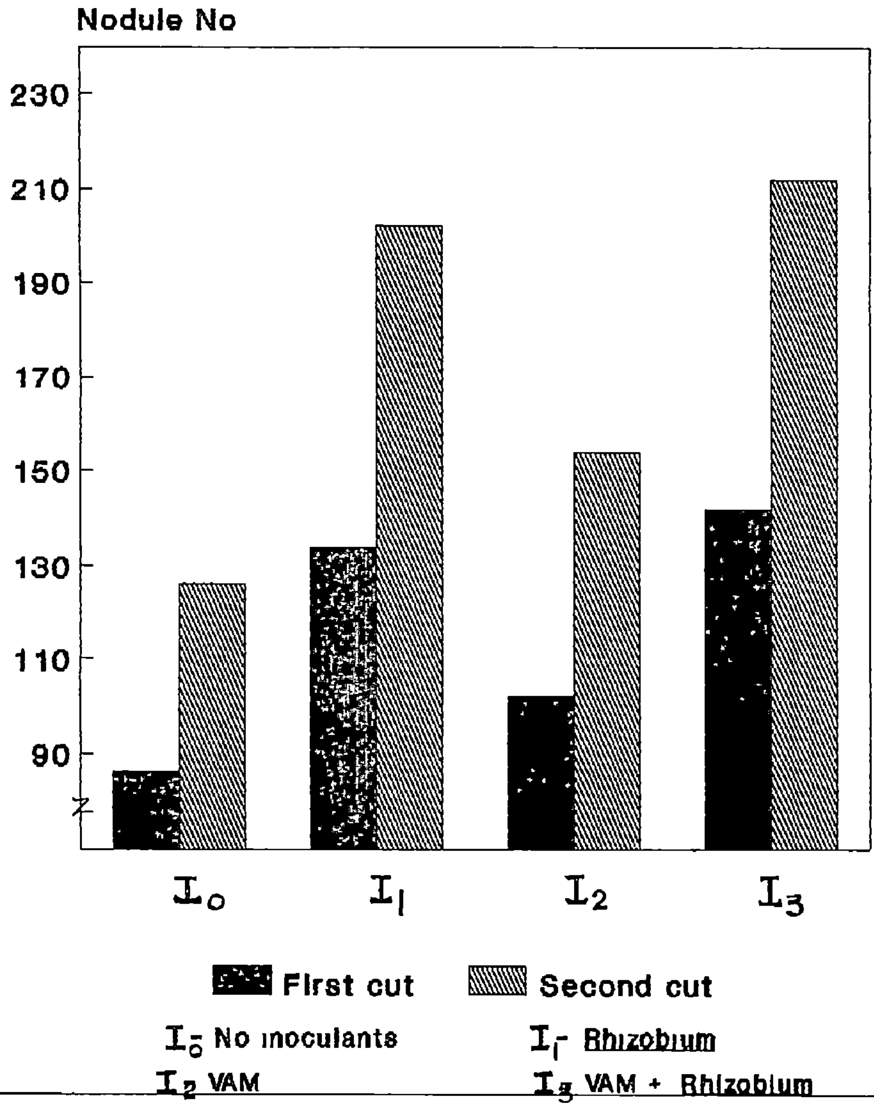
The fertilizer levels had significant influence on the spread of plants The treatments 50 per cent recommended dose and 100 per cent recommended dose were on par and significantly superior to control The effect of graded doses of N P and K in increasing the vegetative growth of plants has already been discussed The difference in spread of plants might be due to the difference in the length of branches Moursi et al (1976) also reported an increase in the spread of lupin with increase in nitrogen application Increased plant spread due to phosphorus application was reported by Sharma and Lavania (1980) in Vicia hirsuta and Vica sativa and in Stylosanthes by Lekha Sreekantan (1981) and Balachandran Nair (1989) The increase in spread noticed in the study might be due to increased meristematic activity as a result of enhanced uptake of nutrients (Table 11 and 12)

### 5 1 6 Number of nodules

Inoculation significantly increased the number of nodules as evidenced from the result presented in Table 6 and Fig 4 the Rhizobium treatment alone and dual inoculation treatment (VAM + Rhizobium) recorded the highest number of nodules Similar results were reported by Rawat et al (1991) and Rinj et al (1987) in Leucaena leucocephala A good supply of phosphorus is essential for effective nodulation (Munns and Mosse 1980) The increased nodulation could be attributed to the fact that specific root exudates on mycorrhizal legumes may act as chemotactic attractants of Rhizobium (Gitte et al 1978) Sivaprasad and Rai (1987) effectively proved that increased nodulation of Cajanus cajan in tripartite symbiosis was due to increased cytokinin activity in stem exudates and roots of mycorrhizal plants VAM fungi thus first stimulate the nodule bacteria and their activity by increasing tissue phosphorus concentration (Smith and Daft 1977 Mosse et al 1976) resulting in increased nitrogen fixation and consequently enhanced plant growth

There was no significant difference in nodule number due to different nutrient levels However the treatment full recommended dose showed a slight increase in nodule number when compared with the control Graded dose of N P and K are known to increase

FIG 4 NUMBER OF NODULES AS INFLUENCED BY INOCULATION



nodulation in legumes (Tisdale et al 1985) The limited increase in nodulation may be due to the medium nitrogen status (313 8 kg ha $^{-1}$) of the soil Nitrogen fixing bacteria when supplied with readily available forms of nitrogen become inactive and less efficient in fixing atmospheric nitrogen thereby retarding the formation of nodules (Russell 1973)

**5 1 7 Weight of nodules**

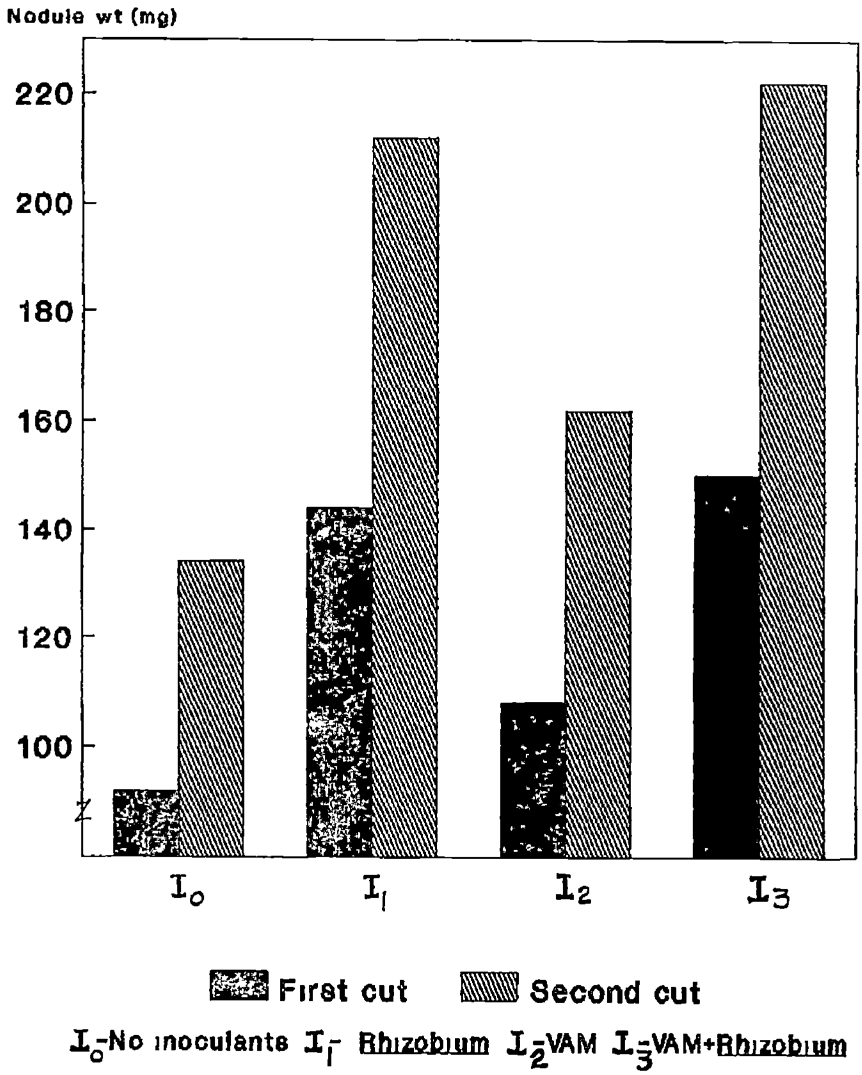
The results regarding the weight of nodules is presented in Table 7 and Fig 5 The same trend shown by the number of nodules is repeated in the character also Inoculation with Rhizobium and VAM + Rhizobium significantly increased the weight of nodules over control Rai (1988) observed similar increase in nodule dry weight by dual inoculation in *Cicer arietinum* Similarly Pacovsky et al (1986) reported increased nodule weight and hence high nitrogen fixation due to dual inoculation in soybeans The increase in nodule number recorded in this study might also have had a positive role in increasing nodule weight

The different fertilizer levels had no significant influence on weight of nodules But there was a gradation the weight increasing from the control treament to full recommended dose High doses of phosphorus and potassium are known to increase nodulation (Russell 1973) The increase in nodule number (Table 6) might have increased the nodule weight also

**5 2 Green matter yield**

The results on green matter yield presented in Table 8 and Fig 6 showed that the treatment VAM + Rhizobium was significantly superior to uninoculated control The dual

FIG 5 WEIGHT OF NODULES AS INFLUENCED BY INOCULATION

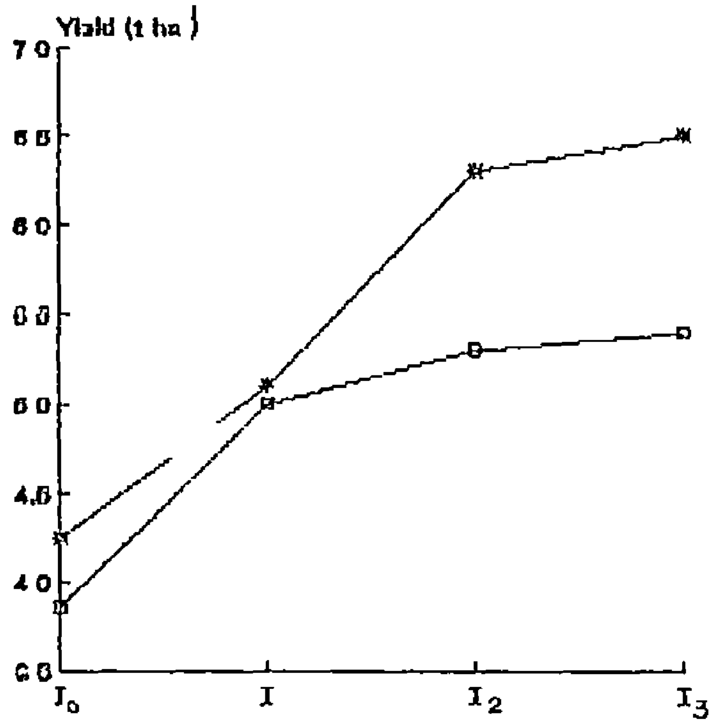


inoculation treatment showed 52 percent higher yield than the uninoculated control in the first cut In the first cut the height spread leaf stem ratio number and length of branches were higher for the dual inoculation treatment This might be the reason for enhanced green matter yield The synergestic effect might have induced more uptake of nutrients (Table 11 and 12) by way of enhanced nitrogen fixation and extramatrical net work of VAM hyphae which functions synonymous to the root hair (Barea 1991) which in turn increased the vegetative growth reflecting in increased green matter yield

The effect due to inoculation was not so pronounced in the second cut though the treatment VAM + Rhizobium recorded 34 percent higher yield than control This might be due to an overall reduction in growth parameters also in the second cut Similar depression in yield after the first cut was reported by Lim and Cole (1984) in white clover where the magnitude of mycorrhizal response decreased with successive harvests

The fertilizer level 100 percent of the recommended dose recorded the highest green matter yield which was on par with 50 percent of the recommended dose As the level of nitrogen increases the carbohydrates synthesised in the leaves are converted to aminoacids mainly in the leaf The extra protein allows the leaves to grow larger and have more photosynthetic area leading to higher yield (Russell 1973) Increase in top growth of lupin and hence green matter yield with increasing nitrogen rates was reported by Mourai et al (1976) Balachandran Nair (1989) also reported an increase in green matter yield with increasing phosphorus levels in Stylosanthes

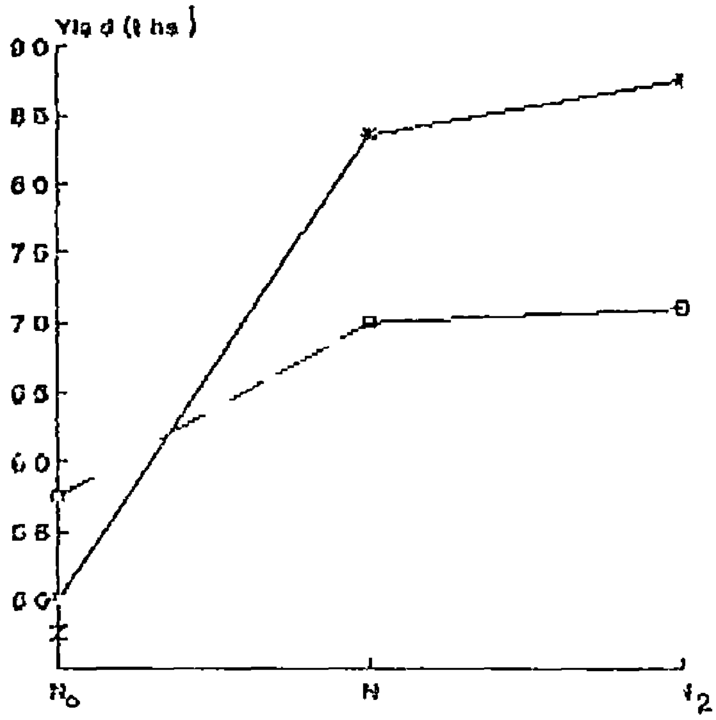
FIG 6 EFFECT OF INOCULATION AND FERTILIZERS ON GREEN MATTER YIELD




First cut   Second cut

$I_0$ No inoculants   I Rhizobium

$I_2$ VAM   I VAM Rhizobium




First cut   Second cut

$N_0$ No nutrients   N 50 % recommended dose

$N_2$ 100 % recommended dose

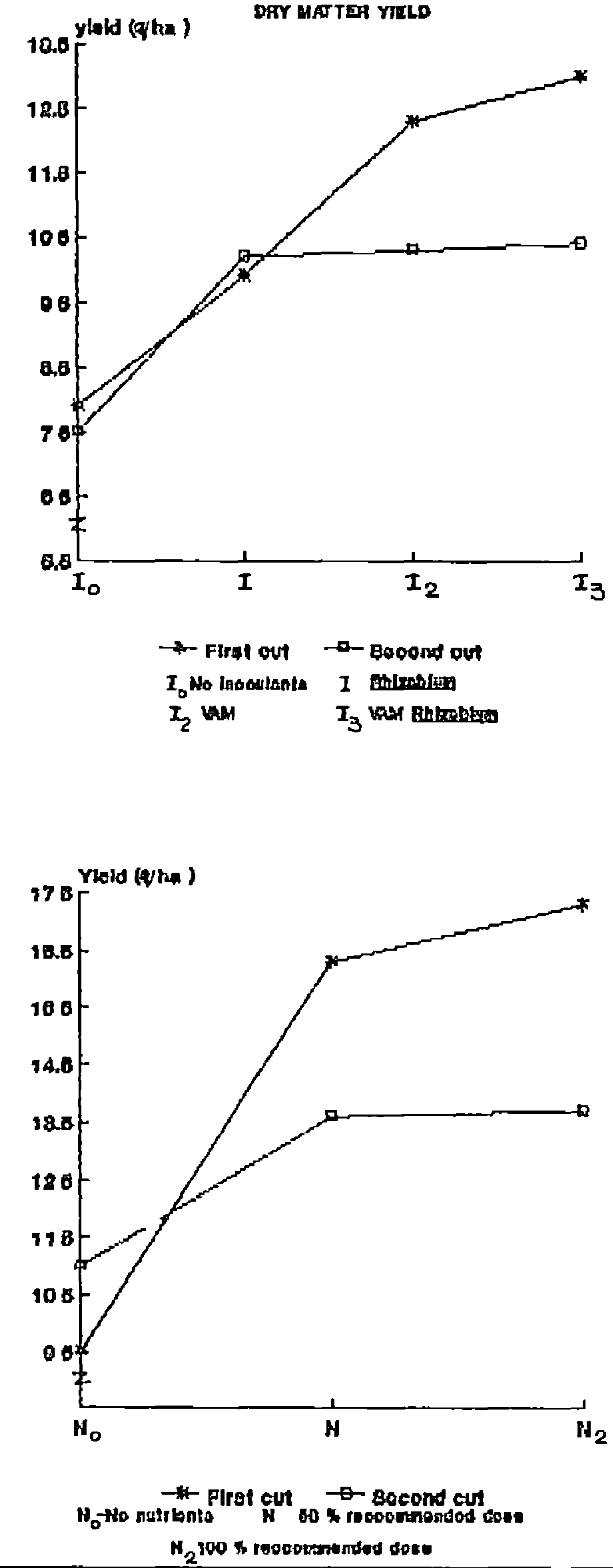
The role of phosphorus in increasing the cell division and activity of meristematic tissues thus increasing growth and yield is well established (Russell 1973) Potassium has been shown to increase yields in <u>Trifolium alexandrinum</u> (Robinson and Savoy 1989) In general the higher fertilizer dose given in this trial might have increased the overall vegetative growth of the plant and this could be the reason for higher green matter yield Green matter yields obtained showed that 50 percent recommended dose is as efficient as 100 percent recommended dose in producing high green matter yield

5 3 Dry matter yield

The results on dry matter yield presented in Table 9 and Fig 7 showed that there was significant response to inoculation with VAM and <u>Rhizobium</u> over control at both cuts Similar results were reported in lucerne by Barea et al (1980) in <u>Glycine max</u> by Pacovsky et al (1986) and in pigeonpea by Sivaprasad and Rai (1987) Increase in height spread number and length of branches might have contributed to high dry matter yield The enhanced vegetative growth and high green matter yield might also contributed to greater dry matter yield

The different fertilizer levels gave significantly higher yields compared to control The treatments 50 percent of the recommended dose and 100 percent of the recommended dose were on par and significantly higher than control Similar trend was observed in the case of height spread and length of branches The increasing dose of fertilizers might have increased the green matter yield, reflecting in higher dry matter yield also The treatments 50 percent recommended dose and full fertilizer dose

FIG 7 EFFECT OF INOCULATION AND FERTILIZERS ON DRY MATTER YIELD



were on par suggesting that 50 percent recommended dose is sufficient beyond which no significant yield increase occurs

### 5 4 Mycorrhizal colonization in the root

The results presented in Table 10 showed that at transplanting the mycorrhizal colonization in VAM inoculated plants was 29 33 percent The mean value for mycorrhizal colonization at the close of the experiment showed that the treatment $i_3n_1$ (VAM + Rhizobium + 50 percent recommended dose) registered the highest value followed by $i_3\,n_0$ (VAM + Rhizobium + no nutrients) Saif (1986) also reported stimulated mycorrhizal infection with low levels of applied phosphorus Addition of combined nitrogen decreased mycorrhizal development in young clover roots (Chambers et al 1980) Elias and Safir (1987) found that the preference of VAM fungus to low phosphorus concentration is because at low phosphorus concentrations the exudates from plants stimulate hyphal elongation of VAM fungus

The lowest value was reported for the treatment No inoculants + 100 percent recommended dose ($i_0n_2$) This indicates that native VAM is suppressed at higher doses of fertilizer application But the inoculation responses were not related to infection level Stylosanthes responded most from inoculation in soil with most available phosphorus Similar results were obtained in lucerne by Owusu Bennoah and Mosse (1979) VAM fungi are especially affected by soil fertility factors Limonnard and Ruissen (1989) found that effect of nitrogen on VAM development was even more significant than that of phosphorus Even at high phosphorus levels much VAM could be formed provided the soil nitrogen level was low

## 5 5 Nutrient uptake

### 5 5 1 Nitrogen uptake

It is evident from the results presented in Table 11 and Fig 8 that inoculation did not significantly increase the nitrogen uptake by the plant However the treatment Rhizobium alone recorded the highest nitrogen uptake which was closely followed by VAM + Rhizobium Rhizobium treatement recorded higher nodule number and nodule weight hence the uptake was also higher Smith et al (1981) showed that VAM increased nitrogen inflow into the plant The VAM fungus can absorb nitrate ions from beyond the more deficient cells around the roots Smith and Gianinazzi Pearson (1988) observed that indirectly VAM increased the nitrate reductase in the plant which is necessary for nitrate assimilation

The different nutrient levels also had no significant effect on the nitrogen uptake The increasing nutrient levels however showed an increasing trend in the nitrogen uptake The insignificant result might be due to the medium nitrogen status (313 8 Kg ha $^{1}$) of the experimental site

### 5 5 2 Phosphorus uptake

The results on the phosphorus uptake by plants is presented in Table 11 and Fig 8

The treatments VAM alone and dual inoculation recorded on par values for phosphorus uptake which was highly significant over control The reason for enhanced phosphorus uptake by VAM plants is still controversial In any case VAM hyphae can take advantage of their geometry and better distribution than roots to acquire phosphate from transitory localised and diluted sources of the element (Harley and Smith 1983) There are indications that VAM hyphae are able to take up phosphate from soil solutions with low phosphate concentrations more efficiently than simple roots (Barea 1991) The qualitative and quantitative changes in the root exudation patterns (Harley and Smith 1983) and the differences between VAM and non mycorrhizal plants in the absorption of anions and cations which can change with the pH of the rhizosphere (Buwalda et al 1983) are the indirect mechanisms which increases phosphate availability to the plant

There was no significant increase in phosphorus uptake due to increasing fertilizer levels But progressive increase was noticed in the uptake pattern from the lowest dose to the highest dose of fertilizers Increase in phosphorus uptake with increasing doses of phosphorus was reported by Lekha Sreekantan (1981) Increasing the rate of phosphorus applied in the soil might have increased its availability and consequent assimilation by plants which results in higher phosphorus uptake values for the plant Similar results have been reported by Dhar (1978) and Mariyappan (1976) in the case of various legumes tried The increase in dry matter yields with increasing nutrient levels might have enhanced the total phosphorus recovery in the present study

5 5 3 Potassium uptake

The results presented in Table 11 and Fig 8 showed that there was significant increase in potassium uptake due to inoculation The highest potassium uptake value was recorded by

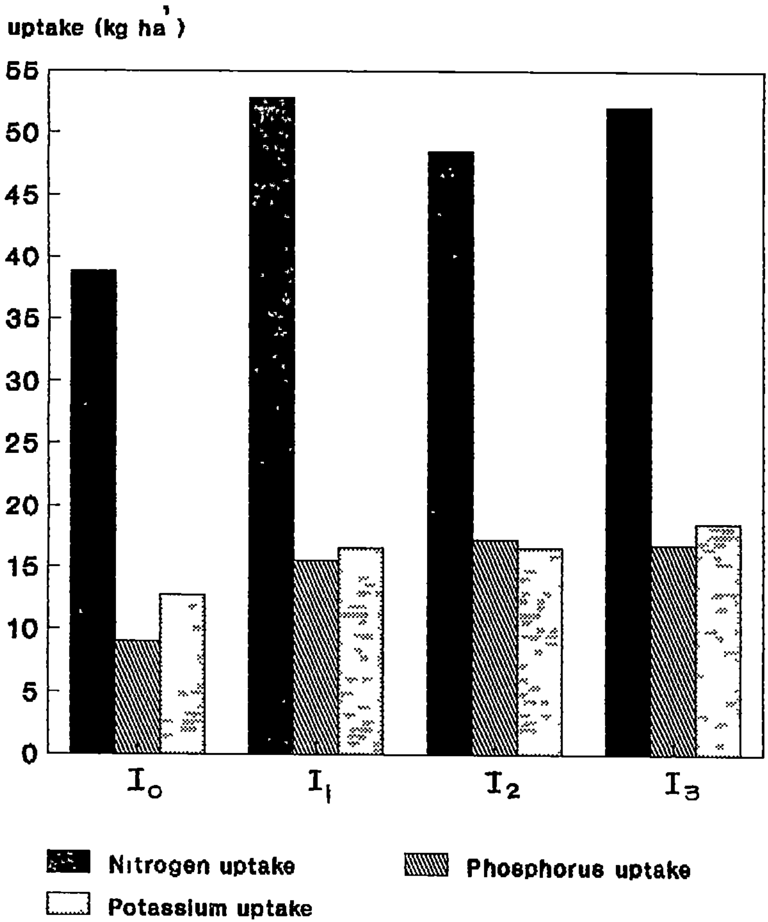
VAM + *Rhizobium* treatment VAM increases potassium uptake resulting in an increment in shoot growth (Nielson and Jenson 1983) Mycorrhizal infection has been found to improve the potassium nutrition of *Trifolium subterraneum* when internal potassium concentrations were generally low The increased potassium uptake might also be the result of improved phosphorus nutrition (Smith et al 1981) The *Rhizobium* treatment might have contributed to better nodulation and hence better availability of nutrients to the crop

There was significant increase in uptake of potassium with increasing levels of nutrients A stimulated growth under higher levels of fertilizer application might have resulted in better proliferation of root system and increased intake efficiency of plants Similar results with increasing potassium concentration was reported by Robinson and Savoy (1989) in *Trifolium repens* and Lekha Sreekantan (1981) in *Stylosanthes* with increasing phosphorus doses The higher dry matter production with 100 percent recommended dose might have contributed to the enhanced uptake of nutrients

### 5 5 4 Calcium and magnesium uptake

It was observed (Table 12) that there was no significant difference in the uptake of calcium and magnesium by the plant due to different inoculation treatments However the VAM + *Rhizobium* treatment recorded the highest calcium uptake followed by *Rhizobium* treatment Similar increase in uptake of calcium was reported by Huang et al (1983) in *Leucaena leucocephala* Smith and Gianinazzi Pearson (1988) suggest an association of $Ca^{2+}$ distribution in plants with the synthesis and breakdown of

FIG 8 EFFECT OF INOCULATION ON UPTAKE OF MAJOR NUTRIENTS




$I_0$-No inoculants $I_1$- *Rhizobium* $I_2$-VAM $I_3$-VAM+*Rhizobium* polyphosphate granules since the cation is a secondary constituent of these granules

Magnesium uptake was also higher for the treatments with mycorrhiza Mycorrhizal inoculation was shown to significantly increase magnesium uptake of lucerne by Nielson (1990) The enhanced magnesium uptake may be an effect of the extensive mycelial network and increased dry matter production of plants

The nutrient levels also did not affect the uptake of calcium and magnesium However with increasing nutrient doses an increasing trend was noticed in the uptake value Similar increase in uptake of cations with increase in the dose of phosphorus applied was reported by Balachandran Nair (1989) The indirect effect of enhanced uptake may be due to the higher dry matter production at higher nutrient levels

## 5 5 5 Micronutrient uptake

Inoculation did not significantly increase the micronutrient uptake as evidenced by results presented in Table 12 However the treatments with mycrorrhizal inoculation registered the highest micronutrient uptake The VAM are known to be involved in the uptake of zinc and copper trace elements having low mobility in the soil (Rhodes and Gerdemann 1980 Tinker and Gildon 1983 Harley and Smith 1983) An increase in iron uptake due to VAM inoculation has also been shown by Rai (1988) in <u>Cicer arietinum</u> Plants colonized by VAM fungi exploit a much larger soil volume and so are capable of mining more immobile trace elements than nonmycorrhizal ones (Pacovsky 1986)

## 5 6 Soil Analysis

### 5 6 1 Nitrogen content in the soil

The results presented in Table 14 showed that there was no significant difference in the soil nitrogen content due to different inoculants The high nitrogen uptake by the crop when inoculated with VAM+Rhizobium and the consequent high dry matter production might have led to a reduction in the nitrogen status of the soil But in the no inoculation treatment the low uptake of nitrogen and the comparatively less dry matter production might have resulted in limited utilization of nitrogen reserves in the soil

However with increasing fertilizer levels from control to 100 percent recommended dose there was significant increase in the soil nitrogen status The moderately high (313 $kgha^{-1}$) nitrogen status of the soil might have enhanced the dry matter production of the plant resulting in less utilization of applied nitrogen The increased soil nitrogen status might also be attributed to the utilization of fixed nitrogen by the crop leading to the increase in residual nitrogen status Increase in soil nitrogen status with increased application of nitrogenous fertilizers had been reported by Lee et al (1990) in *Medicago sativa*

### 5 6 2 Phosphorus content in the soil

The results presented in Table 14 show that the control treatment (no inoculation) recorded the highest available phosphorus content in the soil This could be attributed to less uptake of phosphorus by the plant and hence less utilization of native and applied phosphorus THe lowest value was recorded by the treatment *Rhizobium* VAM + *Rhizobium* inoculation treatment recorded available phosphorus content of 29 27 kg ha $^{-1}$ in the soil Inoculation with mycorrhiza increases the phosphorus uptake by way of extensive mycelial net work and also adds phosphorus to the available pool (Barea 1991) However the higher green matter yield due to acquisition of nutrients like phosphorus would have resulted in low available phosphorus in the soil

Significant increase was observed in available phosphorus content due to different fertilizer levels The treatment 50 percent of the recommended dose recorded the highest available phosphorus content followed by full fertilizer dose Increase in available phosphorus content in soil with increase in the dose of phosphorus applied was reported by Mariyappan (1978) Lekha Sreekantan (1981) and Balachandran Nair (1989)

**5 6 3 Potassium content in soil**

The results presented in Table 14 showed that there was no significant difference in potassium content of the soil due to inoculation The treatment VAM + *Rhizobium* recorded the highest available potassium content in the soil followed by the control treatment This might be due to the fact that could VAM could enhance potassium content of the soil by hyphal exploration for further utilization and better plant growth

The different nutrient levels did not show any significant influence on the available potassium content of the soil However the control treatment registered the highest potassium content probably due to limited utilization of the element for green matter production by the plants

### 5 6 4 Calcium and magnesium content in the soil

There was no significant difference in the available calcium and magnesium contents in the soil due to inoculation This might be due to increased uptake of these nutrients with inoculation

The different nutrient levels also did not influence the available calcium and magnesium status of the soil This showed that there was no additional benefit due to the application of NPK fertilizers on the calcium and magnesium contents in the soil However increase in cation exchange capacity with increase in dose of phosphorus was reported by Singh (1975) in Stylosanthes humilis and Bruce (1974) in Stylosanthes guianensis Hence the increasing trend shown by the increasing nutrient levels seems to suggest the influence of phosphorus in increasing the available calcium and magnesium contents in soil

### 5 7 Crude Protein

From the results presented in Table 13 it could be seen that there was no significant effect on the crude protein content due to inoculation The treatment Rhizobium recorded the highest crude protein content which may be due to the higher nitrogen content in the plant due to better assimilation of nitrogen This was followed by the treatment VAM + Rhizobium The Rhizobium symbiont in the dual inoculation treatment might have contributed to better nitrogen uptake and hence higher crude protein yield Increase in crude protein was noticed with Rhizobium inoculation in lucerne by (Jonsson 1982) and in soybean hay by Karyagin (1980)

The crude protein content was not found to vary significantly with increasing nutrient levels But an increasing trend was observed with the nutrient levels varying from control to 100 percent recommended dose Increase in nitrogen content in the plant has a positive effect on the crude protein content (Russell 1973) Phosphorus is known for the development of roots and rootlets Hence more area would be available for nodule formation leading to more nitrogen fixation Phosphorus was reported to increase the crude protein content in Stylosanthes by Lekha Sreekantan(1981) and Balachandran Nair (1989) Application of NPK fertilizers also increased the crude protein content in soybea (Girenko and Levenskii 1974)

# SUMMARY

## SUMMARY

An investigation was conducted to study the effect of different microbial inoculants (Rhizobium Vesicular arbuscular myrorrhiza (VAM) and VAM+Rhizobium) as well as different levels of nutrients (No no nutrients $N_1$ 50 percent of recommended dose $N_2$ 100 percent of recommended dose) and their interactions on increasing the forage production of *Stylosanthes guianensis* cv Schofield

The experiment was laid out in strip plot design with four replications The important results of the study are summarised below

1 Plant height significantly increased with the treatment, VAM + Rhizobium in the first cut All other treatments were on par In the second cut no significant increase was noticed in height due to inoc lation But the treatment VAM + Rhizobium was superior over the other treatments

The treatment 100 percent recommended dose was significantly superior to all other treatments in the first and second cuts

2 Inoculation and nutrient levels did not significantly increase the number of branches in the first and second cuts

3 The combination treatment VAM +Rhizobium registered the highest length of branches in both cuts but it was not statistically significant

The increasing levels of nutrients showed a consistent increase in the length of branches in the first cut and a significant response with higher nutrient levels was noticed in the second cut

4 The spread of plants was not increased significantly due to inoculation However the treatment VAM + Rhizobium registered higher spread in both the cuts

The spread of plants increased significantly with increasing nutrient levels The treatments 50 percent recommended dose and 100 percent recommended dose were on par and significantly superior to control

5 A significant increase in leaf stem ratio was noticed with inoculation But this was evident only in the second cut In both the cuts the treatment VAM+Rhizobium recorded the highest leaf stem ratio followed by the VAM treatment

The treatment 50 percent recommended dose recorded significant increase in leaf stem ratio in the first cut In the second cut however the effect was not significant

6 The treatment VAM + Rhizobium recorded significant increase in the number of nodules but it was on par with the Rhizobium treatment in both the cuts

The different nutrient levels did not show any significant influence on the number of nodules

7 There was significant increase in the weight of nodules due to inoculation with VAM + Rhizobium in both cuts but it was on par with the Rhizobium treatment

The nutrient levels did not show any significant increase i this character

8 The green matter yield was significantly increased due to inoculation with VAM + Rhizobium in the first cut But this effect was not significant in the second cut

A significant increase was noticed in the green matter yield with increase in nutrient levels There was a gradation in increase the highest value being for 100 percent recommended dose which was on par with 50 percent recommended dose

9 Inoculation with VAM + Rhizobium produced significant increase in dry matter yield in the first and second cuts over control

Similar significant response was noticed with increasing nutrient levels The highest dry matter yield was obtained with 100 percent recommended dose which was on par with 50 percent recommended dose

10 There was significant increase in phosphorus and potassium uptake by the crop due to inoculation with VAM + Rhizobium Nitrogen uptake was not significantly influenced by inoculation

11 A significant response was not noticed in the case of calcium magnesium and micronutrient uptake due to inoculation or different nutrient levels

12 No significant response was noticed in crude protein content due to different inoculants or fertilizer levels

13 The available nitrogen phosphorus potassium calcium and magnesium status of the soil was not significantly influenced by inoculation But with increase in nutrient doses there was corresponding increase in the available nitrogen and phosphorus status of the soil

14 The treatment combination VAM + Rhizobium + 50 percent of the recommended dose registered the highest net profit of Rs 2743 09 and B/C ratio of 2 01

Future line of work

The effect of dual inoculation with VAM + Rhizobium in increasing the fodder production under different cutting intervals and on seed production has to be investigated Different methods of application of mycorrhizal inoculum should be tried out and the best method should be evolved

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* Original not seen

# APPENDIX

## APPENDIX I

### WEATHER CONDITION DURING THE CROPPING PERIOD

(Weekly means)

Ist July 1990 to 7th January 1991

| Standard week | Period From | Period To | Temperature °C Maximum | Temperature °C Minimum | Rainfall (mm) | Relative humidity (%) |
|---|---|---|---|---|---|---|
| 26 | 5 06 90 | 1 7 90 | 9 11 | 23 9 | 11 1 | 85 50 |
| 27 | 02 07 90 | 8 7 90 | 9 69 | 23 89 | 01 64 | 8 93 |
| 28 | 9 07 90 | 15 07 90 | 28 39 | 2 19 | 02 16 | 86 5 |
| 29 | 16 07 90 | 22 07 90 | 28 01 | 23 43 | 0 7 | 78 29 |
| 30 | 23 07 90 | 9 07 90 | 9 38 | 23 46 | 08 6 | 86 |
| 31 | 30 07 90 | 05 8 90 | 9 34 | 23 57 | 19 9 | 81 9 |
| 32 | 06 08 90 | 12 08 90 | 29 77 | 23 71 | 00 83 | 7 9 |
| 33 | 13 08 90 | 19 08 90 | 8 55 | 23 03 | 10 4 | 8 79 |
| 34 | 20 08 90 | 26 8 90 | 9 79 | 2 23 | 02 9 | 77 57 |
| 35 | 27 08 90 | 02 9 90 | 9 22 | 23 6 | 14 | 8 79 |
| 6 | 0 09 90 | 09 9 90 | 0 42 | 2 74 | 1 54 | 74 |
| 37 | 10 09 90 | 16 09 90 | 31 0 | 24 36 | 07 54 | 6 14 |
| 38 | 17 09 9 | 3 09 90 | 50 | 23 9 | 00 | 79 |
| 9 | 24 9 90 | 0 10 90 | 31 0 | 24 50 | 0 0 | 78 17 |
| 4 | 01 10 90 | 7 10 90 | 3 89 | 24 61 | 00 | 79 71 |
| 41 | 08 10 90 | 14 10 90 | 30 29 | 23 61 | 01 56 | 79 |
| 4 | 15 10 90 | 21 10 90 | 30 29 | 2 21 | 08 63 | 8 57 |
| 4 | 22 10 90 | 28 10 90 | 28 66 | 23 19 | 09 9 | 80 14 |
| 44 | 29 10 90 | 4 11 90 | 29 60 | 23 73 | 07 50 | 83 |
| 45 | 05 11 90 | 11 11 90 | 0 09 | 23 34 | 01 56 | 83 57 |
| 46 | 12 11 90 | 18 11 90 | 29 82 | 23 24 | 28 77 | 87 74 |
| 47 | 19 11 90 | 25 11 90 | 0 38 | 22 63 | 00 | 77 21 |
| 48 | 6 1 90 | 2 12 90 | 30 51 | 23 56 | 0 0 | 78 0 |
| 49 | 03 12 90 | 9 12 90 | 0 58 | 20 3 | 0 | 0 43 |
| 5 | 10 12 90 | 16 12 90 | 0 55 | 21 31 | 00 00 | 6 7 |
| 51 | 17 1 90 | 2 12 90 | 0 40 | 23 70 | 01 11 | 4 4 |
| 52 | 24 12 90 | 12 90 | 1 60 | 24 64 | 02 9 | 8 86 |
| 01 | 31 12 90 | 06 1 91 | 0 12 | 21 56 | 5 0 | 7 |
| 02 | 07 01 91 | 13 01 91 | 29 26 | 19 54 | 0 0 | 72 6 |
| 03 | 19 01 91 | 0 01 91 | 29 26 | 20 90 | 0 | 7 36 |
| 4 | 21 1 91 | 01 91 | 31 13 | 19 53 | 00 | 7 9 |

Source Meteorological Observatory College of Agriculture Vellayani

## APPENDIX II

## AVERAGE WEATHER DATA FOR THE PAST FIVE YEARS (1985 89)

(Weekly means)

| Standard week | Period From | Period To | Temperature °C Maximum | Temperature °C Minimum | Rainfall (mm) | Relative humidity(%) |
|---|---|---|---|---|---|---|
| 26 | June 25 | July 1 | 30 02 | 22 52 | 06 26 | 81 93 |
| 27 | July 2 | July 8 | 30 44 | 23 56 | 05 29 | 76 78 |
| 28 | July 9 | July 15 | 30 24 | 23 48 | 05 68 | 81 73 |
| 29 | July 16 | July 22 | 30 12 | 23 44 | 07 07 | 81 46 |
| 30 | July 23 | July 29 | 29 84 | 23 48 | 01 92 | 77 98 |
| 31 | July 30 | Aug 5 | 30 08 | 23 58 | 04 52 | 79 34 |
| 32 | Aug 6 | Aug 12 | 29 40 | 23 20 | 10 16 | 79 12 |
| 33 | Aug 13 | Aug 19 | 29 58 | 22 92 | 05 60 | 80 24 |
| 34 | Aug 20 | Aug 26 | 30 17 | 23 56 | 03 72 | 77 96 |
| 35 | Aug 27 | Sept 2 | 30 46 | 23 66 | 02 68 | 78 12 |
| 36 | Sept 3 | Sept 9 | 30 56 | 23 30 | 05 24 | 76 62 |
| 37 | Sept 10 | Sept 16 | 30 90 | 23 66 | 03 58 | 78 12 |
| 38 | Sept 17 | Sept 23 | 30 12 | 23 54 | 08 56 | 77 62 |
| 39 | Sept 24 | Sept 30 | 29 98 | 23 44 | 05 78 | 80 06 |
| 40 | Oct 1 | Oct 7 | 30 54 | 23 80 | 05 20 | 77 46 |
| 41 | Oct 8 | Oct 14 | 30 62 | 23 80 | 02 36 | 80 52 |
| 42 | Oct 15 | Oct 21 | 30 84 | 23 96 | 05 04 | 89 86 |
| 43 | Oct 22 | Oct 28 | 30 46 | 23 72 | 05 48 | 82 10 |
| 44 | Oct 29 | Nov 4 | 30 40 | 23 24 | 14 98 | 79 86 |
| 45 | Nov 5 | Nov 11 | 30 46 | 23 36 | 08 94 | 80 22 |
| 46 | Nov 12 | Nov 18 | 30 74 | 22 24 | 03 64 | 76 52 |
| 47 | Nov 19 | Nov 25 | 30 86 | 22 66 | 02 14 | 73 32 |
| 48 | Nov 26 | Dec 2 | 30 98 | 22 28 | 04 64 | 76 92 |
| 49 | Dec 3 | Dec 9 | 30 96 | 22 72 | 02 24 | 74 18 |
| 50 | Dec 10 | Dec 16 | 31 18 | 22 42 | 04 60 | 71 14 |
| 51 | Dec 17 | Dec 23 | 31 10 | 22 34 | 01 26 | 71 24 |
| 52 | Dec 24 | Dec 30 | 31 54 | 22 94 | 02 68 | 71 88 |
| 01 | Dec 31 | Jan 6 | 31 38 | 21 98 | 00 54 | 73 34 |
| 02 | Jan 7 | Jan 13 | 31 02 | 21 28 | 00 80 | 76 20 |
| 03 | Jan 14 | Jan 20 | 31 44 | 21 13 | 00 02 | 75 50 |
| 04 | Jan 21 | Jan 27 | 31 58 | 21 28 | 00 38 | 74 92 |

Source Meteorological Observatory College of Agriculture Vellayani

# EFFECT OF MICROBIAL INOCULANTS ON STYLOSANTHES GUIANENSIS cv SCHOFIELD FOR HERBAGE PRODUCTION

BY

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# ABSTRACT

An experiment was conducted at the College of Agriculture Vellayani to study the effect of different inoculants (Rhizobium Vesicular arbuscular mycorrhiza (VAM) and VAM+Rhizobium) and different nutrient levels (no nutrients, 50 percent of the recommended dose and 100 percent of the recommended dose) in increasing the herbage productivity of *Stylosanthes guianensis* cv Schofield The recommended nutrient dose was N P K @ 20 80 30 kgha $^{1}$

The experiment was laid out in Strip Plot Design with four replications

The study revealed that co inoculation of Stylosanthes with VAM and Rhizobium significantly increased the plant height The nutrient dose 50 percent of the recommended dose and 100 percent of the recommended dose were found to be at par

A consistent increase in leaf stem ratio and spread of plants was noticed due to dual inoculation with VAM and Rhizobium

There was significant increase in nodulation and nodule weight on inoculation with VAM+Rhizobium The highest number of nodules ( 141 8 in first cut and 211 8 in second cut) was recorded by the dual inoculation treatment

Significant response to dual inoculation was observed with respect to green matter yield (52% increase over control in the first cut and 34% increase in the second cut) Similar results were obtained in the case of dry matter yield also

The treatments 50 percent recommended dose and 100 percent recommended dose were at par for green matter and dry matter yields

The phosphorus uptake values also showed significant increase with dual inoculation

The treatment VAM+ Rhizobium + 50 percent recommended dose recorded the highest B/C ratio of 2 01