

FIELD TESTING OF THE EFFICIENCY OF SOME *cb*

SELECTED RHIZOBIUM CULTURES

BY

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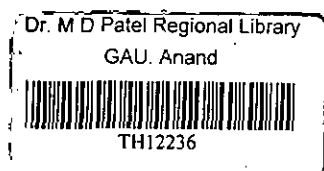
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卐 ॐ श्री सद्गुरवे नमः 卐

॥ ॐ भूर्भुवः स्वः तत्सवितुर्वरेण्यं भर्गो देवस्य
धीमहि धियो योनः प्रचोदयात् ॐ ॥

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Chapter - I

Introduction.

Following the fundamental discoveries, made principally in the last two decades of the nineteenth century, which revealed the essential part which soil bacteria play in the maintenance of soil fertility, considerable attention was devoted to the question of adding culture of bacteria to the soil or applying them to the seed with the object of exerting a stimulating effect on plant growth. Leguminous crops were the chief subject of study on account of the peculiar relationship which these plants bear to special groups of soil bacteria.

Many of the earlier attempts at soil or seed inoculation were unsuccessful due to lack of intimate knowledge of the bacteria concerned and of suitable methods for applying them. About the present century, however, it was first demonstrated in the field as well as in the laboratory, that practical use could be made of adding cultures of certain bacteria to soil or seed to make up for a natural deficiency of these bacteria in the soil. In later years, further investigations have helped to explain why many of the earlier attempts, conducted both in Europe and America, were unsuccessful, and have developed improved methods of preparing and distributing cultures with the result that

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to-day the inoculation of legumes is a recognized aid to farming.

For many centuries it has been recognized that plants belonging to the leguminous family differ in a striking fashion from other cultivated plants in the effect which the crop growth produces upon the fertility of the soil. The custom of including a legume crop in the rotation was based upon the observations that the productivity of the soil is noticeably better following a good growth of legumes - beans, peas, clovers, vetches, alfalfa etc. - than after any non-leguminous crop. Even in the time of the Romans, it was noticed that, when certain legumes were grown in a field, a subsequent crop of non-legumes showed improved growth. The explanation of the difference between legumes and non-legumes, however, was not found out until towards the end of last century, when it was shown that these two groups of plants differ with respect to the manner in which they obtain their supply of nitrogen. It was discovered that whereas all non-legumes are dependent upon the supply of nitrogen in the soil, legumes under certain conditions are able to make use of the nitrogen of the atmosphere. Four-fifths of the air is composed of nitrogen and legumes, by drawing on this supply which is quite unavailable to other plants, are able to

conserve in a large measure the supply of that element in the soil. Thus, biological nitrogen fixation is one of nature's important efforts for the maintenance of soil fertility.

This biological nitrogen fixation is mainly carried out by microorganisms. Two major groups of bacteria, usually designated as symbiotic and asymbiotic forms are primarily concerned in the process. Besides these, some fungi and blue-green algae are also known to fix atmospheric nitrogen in varying quantities.

Rhizobia fix up nitrogen symbiotically in association in the roots of legumes. The root nodule, which is the site of their activity, can be called miniature nitrogen factory of nature. Asymbiotic nitrogen-fixing microorganisms do the same job independently by their own enzymic activity.

The importance of biological nitrogen fixation in relation to soil fertility has been recognized since the discovery of nitrogen-fixing microorganisms. As reported by Waksman (1952), Hiltner obtained an increase of 17 - 30 times the yield for lupines and 15 - 80 times for serradella by inoculating with rhizobia. He has also reported the results of nitrogen-fixation varying from 15 to 350 pounds per acre. From times immemorial, the practice of mixed cropping

is followed in India generally with legume as one of the mixed crops. This suggests that even ancient Indian farmers were aware of some benefits of growing legumes.

The soils of the tropics and subtropics have very poor nitrogen and organic matter contents as compared to those of temperate climate. Soils of Gujarat, in general, are highly deficient in nitrogen and organic matter. The level of nitrogen in Gujarat soil varies from 0.03 to 0.05 per cent (Yadav, 1962) as against 0.14 per cent in European and American soils (Pillai, 1964). The organic carbon content of our soil is also low and is of the order of 0.6 per cent (Yadav, 1962) as against 3.0 per cent in Europe (Pillai, 1964). The temperature in many parts of India, including that of Gujarat is high during April, May and part of June. Under such hot climate, organic matter burns like fuel and, unless this is replenished every year, the land will cease to be fertile. It is a fact that farm-yard manure and compost are not available in sufficient quantities to meet the farmers' needs to keep up the fertility of the soil.

The combined goal of maintaining higher status of soil nitrogen and organic matter can be achieved by the practice of green-manuring, in which incorporation of leguminous crops in the soil provides the necessary chemical, physical and biological conditions for optimum growth of crops, including specific supply of adequate nitrogen in available forms.

The principal leguminous crops most widely used for this purpose are :

1. Sannhemp (Crotalaria juncea),
2. Dhaincha (Sesbania aculeata),
3. Guwar (Cyamopsis tetragonoloba).

Other crops like Sesbania aegyptiaca and Sesbania speciosa, which are more resistant to water-logged and drought conditions respectively, are also used (Mirchandani and Khan, 1952).

Vachhani (1961) has reported that, for wet areas, crops like dhaincha (Sesbania aculeata) are grown. Besides this, new introductions like Aeschynomene americana and Phaseolus semi-erectus are available. For dry conditions, sannhemp (Crotalaria juncea) is recommended. Other quick-growing leguminous crops like mung (Phaseolus aureus), udid (Phaseolus mungo) and newly introduced crops like pilipesara, Crotalaria usaramoensis, and Cassia leachensultiana are also used.

For rabi season, cowpeas (Vigna sinensis) and senji (Melilotus sp.) can be grown and for kharif season sannhemp and guwar are suitable.

Green-manuring has been adopted throughout India for rice cultivation. Rice is cultivated under water-logged

condition in many states of India. In the coastal tracts, the land is under water-logged condition and both salinity and alkalinity develop in these soils. Green-manuring is practised under such conditions also as a remedial measure. In Madras, Tephrosia purpurea, Sesbania aculeata, Sesbania speciosa, Phaseolus trilobus, Indigofera tinctoria and Crotalaria juncea are used as green-manure crops for rice soils (Chalam, 1961).

The main advantages of green-manuring are :

1. It protects the surface soil :

A green-manure crop protects the soil from the beating action of rains, thereby preventing erosion. It also obstructs the surface flow of water and thus permits more water entry into the soil.

2. It checks weed growth :

A green-manure crop grows very rapidly and thus suppresses the weed growth.

3. It improves soil structure :

After turning in, an average green-manure crop gives 8-10 tons of succulent organic matter, which quickly decomposes in the soil and improves soil structure.

4. It increases availability of plant nutrients :

Green-manure crops absorb nutrients from the soil and the leguminous ones, which are always preferred, fix nitrogen from the air. These nutrients are retained in the plant tissue and are made available to the succeeding crop as they get concentrated in the surface soil when the green manure is ploughed under. When fresh organic matter decomposes, carbon dioxide is evolved which makes the plant nutrients more soluble and therefore readily available to plants.

5. It increases the biological activity :

The decomposing organic matter enhances the microbial activity thereby liberating more plant food for consumption by crops.

6. It reduces loss of nitrogen applied as fertilizer salt :

It has been observed that if nitrogenous fertilizers like sodium nitrate and ammonium sulphate are applied to alkali soils, there is an appreciable loss of nitrogen. This loss is, however, checked appreciably when organic matter in the form of a green manure such as Crotalaria juncea is added to the soil (Mitra and Harishanker, 1955).

For obtaining beneficial results, however, it is necessary that the green-manure crop should be ploughed into the soil at least six weeks before sowing so as to provide time for satisfactory decomposition and nitrification. Secondly, there should be sufficient moisture supply to the soil. When the plants undergo decomposition in the soil, the soil is benefited by the humus compounds, which increase the adsorptive capacity of the soil, promote aeration, drainage and granulation conditions that are extremely important in successful plant growth (Lyon and Buckman, 1943).

Experiments on the study of the effect of green-manuring on the succeeding crops have shown that increased yields may be obtained for as long as five years after application. Joachim (1931), however, states that under tropical conditions the effect of green-manuring may last for only about six months so far as soil nitrogen is concerned. Similarly, it has been reported by Fuggles and Comhman (1939) that in East Africa the effect of green-manuring with gannhemp (Crotalaria juncea) lasts only for one year.

In the practice of green-manuring, the stage of growth at which the green manure is ploughed into the soil is

very important. If the green-manure is ploughed in before it becomes fibrous, i.e. when it is about two months old, it has a narrow C:N ratio and nitrogen in it becomes easily available in the soil. When the green matter is allowed to become fibrous, its C:N ratio is wide and it decomposes with great difficulty in the soil.

During the process of decomposition of green matter, considerable quantities of carbon dioxide and some acids are produced. The carbon dioxide, when dissolved in soil water along with acids, helps to bring into solution minerals containing plant nutrients from their own tissues, mobilises the dormant material in the soil and makes it available to the succeeding crop. Other products of decomposition are mainly ammonia, nitrites and nitrates (Lyon and Buckman, 1943).

The composition of plant material plays an important part in the decomposition process. It has been found that for the liberation of ammonia and nitrate from green material buried in the soil, its nitrogen content should not be less than 2 per cent. Most green-manure materials have nitrogen content considerably higher than this minimum; hence their incorporation in the soil should result in the liberation of large amounts of available nitrogen (Waksman, 1929).

The leguminous crops fix nitrogen from the atmosphere through the root-nodules, which harbour nitrogen-fixing bacteria. For enhanced microbial activity to fix atmospheric nitrogen, bacteria need an adequate supply of phosphorus and molybdenum.

Since phosphorus is found in every living cell, it is essential for photosynthesis, break-down and synthesis of carbohydrates and transfer of energy through the formation of energy-rich phosphate bonds within the plants. Phosphorus influences the vigour of the plants and improves the quality of crops. When supplied to leguminous plants, it hastens and encourages the development of nitrogen-fixing bacteria. It promotes root-growth and encourages formation of new cells, which result in higher amount of nutrient uptake and increased yield.

Molybdenum is considered particularly beneficial to leguminous crops. It is essential for the fixation of atmospheric nitrogen in the root nodules of legumes.

The present study is based on the investigation of "Biological Nitrogen Fixation in Goradu Soils" carried out by Patel (1965) at the Institute of Agriculture, Anand. He studied the functional and physiological characters of rhizobia and isolated various efficient cultures of bacteria

from the root nodules of various legume crops such as Sannhemp (Crotalaria juncea), Guwar (Cyamopsis tetragonoloba), Chauli (Vigna sp.), and Val (Dolichos lablab L.).

The selected bacterial cultures isolated by him were : Sannhemp C₁, Guwar G_{279/1}, Chauli C₃₆ and Val V₄₄.

These cultures were used for inoculation of respective legume seeds prior to sowing. He reported that soil nitrogen did not increase by the use of green-manuring practice along with bacterial inoculation. However, it must be stated that the small amount of increase in the nitrogen content of the soil may not be reflected in the determination of soil nitrogen made by the well-known Kjeldahl method. But the increase in nitrogen content of the soil is likely to be reflected in improved growth of the succeeding cereal crop. Therefore, the present investigation, involving the growth of wheat crop after decomposing the green-manure crop grown after inoculation with the selected cultures of sannhemp and guwar, was undertaken.

Chapter - II

Review of literature.

Green-manuring in the scheme of soil fertility for better crop production has been appreciated for a long time, especially by progressive farmers whose experiences have contributed a great deal in the solution of the problems involved. The scientific agriculturists have lagged behind in interpreting gains made and in explaining failures, thereby causing confusion and misunderstanding on the value of green manures. Doubts are often expressed on the usefulness of green-manuring. Since legumes are preferred as green-manure crops, the work done on the effectiveness of legume green-manuring is reviewed first.

A. Effectiveness of green-manuring with leguminous crops:

Sen and Bains (1952) reported from their field experiments at I.A.R.I. Farm, New Delhi, that the yields of cereals obtained from green-manured plots were significantly higher than those obtained from non-green-manured plots.

Response of wheat yields to different green-manure crops was studied by Agarwal (1956) at Agra. He studied the five kharif legumes (moong T.1; sanai, guwar, cowpea and china moong) and two methods of burial (whole plant and root only). Sanai proved to be most suitable green-manure crop for wheat for the tract.

Khan and Mathur (1957) studied the effect of burying annabemp as green manure on the yield of wheat at I.A.R.I., New Delhi. He concluded that green-manuring is a practice of 'timings'. In case of failures correct timings should be looked into. The whole technique is based on the correct intervals between sowing of green-manure, its burying - under and sowing of wheat crop succeeding it (8 weeks in both cases). Application of phosphate in conjunction with green manure was found profitable.

Chandnani (1958), working at I.A.R.I., New Delhi, found increase in yield of wheat crop succeeding green-manuring.

Mann (1958) made an extensive study on green-manuring. According to him, green manure, and especially a highly nitrogenous green manure, has an appreciable manurial value when it is utilized at once before its value is partially lost either through drainage or from other causes, and it will therefore be most effective if the crop, which is intended to benefit, is grown very soon after the green manure is buried in a soil in which conditions are favourable for its rotting. He further concluded that the effect of the burial of lupins as a green-manure crop was very temporary and disappears completely after the second crop. There is a slight beneficial effect to a

succeeding crop from the mere growing of a green-manure crop of lupins or vetches. He also stated that the addition of farm-yard manure to green manures at the time of their burial did not materially affect the immediate benefits obtained. That the effect of green-manuring is temporary was shown earlier also by Crowther and Mann (1933), who concluded that the manurial value of green-manure crops must be utilized at once, for otherwise little beneficial effect will be left due to leaching of nutrients.

Relwani and Ganguly (1959) reported significantly higher yields of paddy after green-manuring with dhaincha (Sesbania aculeata).

(1962)
Davidescu et al. / have concluded that highest yields on marshy alluvial soil were produced by ploughing down peas as green manure, with vetch and sweet clover as second best in efficiency. On a leached chernozem, plowing-down of PK-fertilized lupin on the stubble was more effective than complementing the green mass of lupin with 10 tons/hectare manure.

Schaaffhausen (1963) worked on economical methods for using the legume Delichos lablab for soil improvement, food and feed and concluded that the legume crop doubled the yield of maize.

Sen (1963) has shown, in his study of the effect of Hubam clover as a green-manure crop in wheat-maize rotation, that best yields resulted when the wheat was grown with Hubam clover fertilized with phosphorus and the clover was then buried for green-manuring before the maize crop.

Ambika Singh (1963) has observed that 50 per cent of the benefits of green-manuring could be obtained in the form of increased sugar-cane yields even when the above-ground portion was removed. Green-manuring with sannhemp left little or no residual effect on the soil as measured by the amount of organic carbon and the nitrogen in the soil after harvest of the following crop. A similar result was also reported earlier by Mehta et al. (1956), who stated that organic matter content of goradu soil of Kaira district in Gujarat, which was about 0.5 per cent before monsoon, was found to be 0.9 per cent four weeks after sannhemp was buried, but the increase in the organic matter content was temporary and practically whole of the added organic carbon disappeared in nine months.

Apltauer and Skopalikova (1963) studied the effect of green-manuring on the microflora of the soil. They concluded that the microflora of the soil depend as much on a narrow C:N ratio (as existing with white clover and field pea) as on the amount of green manure ploughed down.

Pillai (1964) has shown, in his study on the effect of organic manure as pre-requisite for increased application of chemical fertilizers, that a basal application of 4000 to 6000 lb. of green manure per acre is essential for a maximum yield of paddy, even with readily available nutrients in the form of inorganic fertilizers like ammonium sulphate and superphosphate. He has also reported that organic manures also add the necessary minor elements which are usually lacking in various chemical fertilizers. Their residual effect is also very great. In most of the experiments, combination of organic manures and chemical fertilizers have brought economical yield for a long period. He has also recommended, for the soils containing organic matter below optimum, heavier doses of organic manures before applying any amount of chemical fertilizers.

Singh and Sinha (1964) reported Results of green-manuring of wheat in Bihar and optimum dose of green matter to be ploughed under. They conducted the experiments for a period of four years on green-manuring of wheat with lopings of the perennial plants. They obtained non-significant differences in wheat yields.

Buczak (1964) studied the effect of green manures in the rotation on yields of some vegetables. He showed that, on fairly compact clayey soils, ploughing down in summer of

properly fertilized spring mixtures of vetch-pea-bean or blue lupine-oats or winter vetch in pure stand, supplied large amounts of nitrogen and was a good substitute for manure to vegetable crops.

The fertilizing value of some organic manures was studied by Shavarbi and Hamissa (1964). They found that dung and green manure were the least effective sources of nitrogen on the clay loam, while green manure was satisfactory on the sandy soil. The effects were assessed in pot experiments with rice, maize, wheat and cotton.

Todorova (1966) studied the effect of dung and green manure on crop yield and development of some groups of microorganisms. He found that on alluvial meadow soil, dung and green manure in the first year of application were equally effective for rye. They increased the numbers of ammonifying and nitrifying bacteria, azotobacter and fungi several times. Dung and green manure increased the content of hydrolysable nitrogen and the nitrifying capacity of the soil.

Shubin (1966) reported studies on green-manuring in the Altai region. He concluded that sweet clover as green manure increased yield of spring wheat when the latter was grown in a sufficiently wet year but not when it was grown in a dry year. Weediness was controlled by ploughing in the sweet clover at the end of June and beginning of July.

B. Phosphate fertilization in green-manuring :

Successful growth of legumes depends upon favourable soil conditions and adequate supply of phosphorus either naturally present in the soil or supplied through phosphatic fertilizers.

Sen and Sundara Rao (1953), in a general review of the results of experiments conducted at different places in India on the phosphorus fertilization of legumes, reported that the response to phosphate has been very conspicuous in soils poor in available phosphorus. In phosphate-rich soils the results were not significant.

Vyas and Desai (1953) studied the effect of different doses of superphosphate on the fixation of nitrogen through pea in Delhi soil. They reported that application of phosphate gave significant increase in the number, weight and nitrogen content of nodules, yield of total dry matter produced and nitrogen content of the crop. They also reported that nitrogen content of the soil was not increased if the aerial part of the crop was completely removed.

Study on the building of soil fertility by phosphatic fertilization of legumes and the influence of a legume rotation on the microbiological activity of the soil was reported by Acharya and Jha (1954). They found that field plots, where berseem had been grown in rotation with wheat and

cowpea, showed higher microbial activity in respect of bacterial numbers, rate of carbon dioxide evolution, ammonifying and nitrifying powers than plots where no berseem had been grown. The microbial activity was higher in plots where berseem had been treated with superphosphate. The same research workers (1953) had earlier studied the building of soil fertility by the phosphatic fertilization of legumes and influence of a legume rotation on the organic matter level of the soil. They stated that the soil fertility was improved as shown by the increase of soil organic matter from 0.696 per cent to 0.989 per cent over an experimental period of ten years. The organic matter level was found to be significantly higher in plots in which the berseem crop had been treated with superphosphate (32 to 64 lb. P_2O_5 per acre) as compared to the plots in which the berseem had not been treated. The treatment did not alter C:N ratio of the soil.

Raychaudhuri and Subbiah (1954), while studying on phosphate manuring of legumes and its role in building up of soil fertility, have established the beneficial effect of phosphate-manuring of legumes.

Chandnani and Oberoi (1956) were of the opinion that application of phosphate to green-manure crops increased only quantity of green matter in summer legumes (sannhemp and cluster bean) and green matter and P_2O_5 content in

winter legumes. They have also reported that the application of P_2O_5 to green-manure crops did not increase the yield of succeeding crops of maize or wheat.

Phosphate-manuring of sannhemp green manure was also studied by Anant Rao et al. (1957). They applied varying doses of P_2O_5 (0, 80 and 160 lb. per acre) to sannhemp crop grown on sandy loam soil. They found that application of 160 lb. of P_2O_5 per acre at sowing of sannhemp crop for green manure and turning in after 49 to 56 days growth stage proved to be the efficient practice for wheat crop for the tract.

Shinde and Sen (1958), while conducting the experiments on phosphate-manuring of legumes on sandy ^{loam} soil, have reported that phosphate application to guwar crop showed an increase in the nitrogen content of plants grown in the control plots and also increased the green-matter yield by about 31 per cent. They showed higher per cent pore space as compared to other treatments. The aggregation of soil was significantly influenced by growing guwar in conjunction with phosphate.

Jadeja and Patel (1958) conducted the experiments for four years to study the economic dose of superphosphate

required by lucerne. They concluded that with increase in dose of phosphorus application, lucerne yields were increased, but the increases were not in direct proportion to the increase in the dose of P_2O_5 .

Venkata Rao and Shanker (1960) reported the results of a study in small pots containing red sandy loam soils poor in P_2O_5 on the influence of increased soil phosphate status brought about by continuous application of increasing levels of P_2O_5 up to 160 kg./ha., on the nitrogen of sannhemp. The nitrogen content of the crop at flowering stage was significantly superior at all the phosphate doses. Similarly, the yield of sannhemp crop and nitrogen contents of grain and straw at maturity were also increased; and the increase of 30 - 70 per cent in the nitrogen recovery by the crop was obtained due to the residual effect of P_2O_5 applications. Building up of soil phosphorus reserves through repeated heavy applications of phosphatic fertilizers as a means of nitrogen mobilization through legumes either as green-manures or in rotation was indicated.

Influence of green manures on phosphate utilization by crops was studied by Venkata Rao and Govind Rajan (1960) by conducting two pot experiments. They reported that application of green manure and phosphate together to ragi

crop increased yields and increased utilization of phosphorus by the crop not only from the added fertilizer but also from the reserve supplies of soil phosphorus.

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Venkata Rao and Shanker (1960) have reported beneficial effect of phosphate application on the yield of sannhemp crop, and the nitrogen composition of the crop at the following stage. This was also supported by Sens, Bains and Mathur (1962).

While studying the effect of different fertilizers added to a green-manure crop, Srivastava and Ram (1962) in Bihar concluded that the green-matter yield was highest from sannhemp treated with nitrogen and phosphate. Phosphate significantly increased the yield of sannhemp but not of dhaincha when P_2O_5 was applied at the rate of 40 lb. per acre and nitrogen at the rate of 10 lb. per acre.

Sankaran et al. (1963) studied the influence of phosphate on nitrogen fixation by rhizobia species. They conducted pot experiments in unsterilized black soil and sterile sand. Sannhemp was grown with P_2O_5 application at the rate of 15, 30, and 45 lb. per acre. They concluded that 15 lb. P_2O_5 per acre increased the total protein content of plants in sterile sand. In the black soil it was without effect.

Patel et al. (1963) reported that significant increase in organic carbon and nitrogen status of the soil by phosphate manuring of berseem at 72 kg. P_2O_5 per hectare was obtained. The C:N ratio of the soil remained constant. Soil fertility was also improved. The after-effects of the treatments on the yield of an unmanured crop of wheat were found to be highly significant. Phosphate-manuring of berseem markedly improved the nitrogen and phosphorus contents of the wheat crop.

Soil aggregation is improved if phosphorus is applied to the legume crop. Biswas et al. (1963) reported that phosphorus, supplied to berseem grown in brownish soil of pH 7.6 from I.A.R.I., Delhi, increased soil aggregation and the effect became more significant at higher phosphorus levels. It had greater effect on aggregation than farm-yard manure at the equivalent levels of phosphorus. Yield of berseem and of a subsequent unmanured wheat crop followed the same general trend as soil aggregation.

Shawarbi and Ibrahim (1964) in Egypt studied nitrogen fixation by Vicia faba as affected by superphosphate application. In their pot experiments with alkaline loam (pH 8.1) application of superphosphate up to 200 kg./fed. significantly increased total nitrogen in the tissues of plants, but 300 kg. had no further beneficial effect.

Rewari et al. (1965) reported that the amount of nitrogen fixed by cluster bean (*Cyamopsis tetragonoloba*) was positively and significantly related to its phosphate uptake from the soil. They further stated that the rate of increase of fixation of nitrogen with the uptake of phosphate was higher in a poor soil than in a rich one.

From their investigation at I.A.R.I., New Delhi, Gupta and Sen (1966) concluded that phosphate utilization is different in different rhizobium species in relation to their efficiencies. They have shown a positive and highly significant correlation between the amounts of phosphorus utilized and efficiencies of the isolates. Therefore, it could be inferred that strains of high efficiency utilized large amounts of phosphorus.

Marked increase in the yield of legumes and the yield of following wheat crop as well as increased content of protein was also observed by Panos (1959), Parr and Rose (1945), Sen (1964), Jha and Ram (1966), when the green-manure crop was fertilized with phosphate.

C. Other factors influencing symbiotic nitrogen fixation :

The chief soil factors which influence nitrogen fixation by legumes are - 1. fertility level, 2. soil salinity, 3. moisture, 4. soil air, 5. temperature, 6. organic matter, 7. H⁺ ion concentration, and 8. cultural factors.

The effect of phosphate has been considered in detail in the preceding paragraphs. The other soil mineral nutrients which have received much attention in relation to the growth of plants through nitrogen fixation are lime, molybdenum and boron.

Batta and Gurubasava Raj (1953) studied the effect of boron and molybdenum fertilizer on Berseem (Trifolium alexandrinum) on I.A.R.I. farm at Delhi. They noted from two years' results that boron and molybdenum exercised a significant influence on the fodder, seed and 'bhusa' yields of berseem. The possibility of using boron and molybdenum as fertilizers in conjunction with phosphates to obtain increased yields of leguminous fodder and seed on soil types similar to those of Delhi was therefore indicated.

Kliwar and Kennedy (1960) worked on the studies on response of legumes to molybdenum and lime fertilization on Mardin silt loam soil. They concluded that lime increased the availability of molybdenum in this soil, which, in turn, increased symbiotic nitrogen fixation. Lime actually corrected nitrogen deficiency in the plant. They also claimed that lime when applied at a rate of 2 tons per acre always gave maximum yields for the four legumes studied. Application of molybdenum increased the size of the nodules, reduced the total number to one-fourth or one-half and changed the

nodules to a pinkish colour. Legumes studied in the pot experiment were alfalfa, birds-foot trefoil and ladino red clover.

In a trial with Kenya white clover, Bumpus and Poulitney (1961) reported highly significant effects when magnesium and boron were applied in presence of phosphate and gypsum. This legume responded to zinc applications when accompanied by 3 cwt./acre triple super, but not when given with 1 cwt./acre super.

Yadav (1962) has concluded that the yield of the crop sannhemp (Crotalaria juncea) recorded both as green matter and on oven-dry basis showed a reduction with increase in salinity.

Patel (1962) studied the effect of the application of phosphorus, molybdenum, boron and green-manuring on yield and quality of alfalfa grown on goradu soil. He concluded that application of soluble phosphates has a beneficial effect on the yield of alfalfa and total uptake of phosphate and nitrogen and that boron, molybdenum and green-manuring in goradu soil did not give any additional effect.

Krylova (1963) made a study on the role of micro elements in nitrogen fixation. He concluded that molybdenum increases nitrogen fixation by nitrogen-fixing organisms when the iron

concentration in the medium is optimal. Molybdenum can be partly replaced by vanadium. Tungsten (W) inhibits while molybdenum and boron increase nitrogen fixation.

Ratner (1964) studied the effect of molybdenum application on biological nitrogen fixation. He found that, in acid derno-podzolic soils, molybdenum activated the nodule bacteria and thus increased legume yields. He has emphasized the importance of molybdenum to the inoculated seeds of legumes.

Salazar (1965) studied the factors affecting legume nodulation in the tropics. He concluded that there exists strong relationship between size and weight of nodules in tropical legumes and deficiency or excess of minor elements, especially boron and molybdenum.

Ecological factors also play a role in the stability of symbiotic nitrogen fixers. Antagonistic organisms may inhibit nitrogen fixation. Soil moisture is another important factor affecting the activity of symbiotic microorganism. Extreme soil moisture, pH and temperature conditions may adversely affect the activity of symbiotic nitrogen fixers. Too low temperature or too high temperature may also affect adversely the activity of nitrogen-fixing bacteria. The symbiotic nitrogen fixers are more active in aerated soils.

Rajgopalan and Sadasivan (1964) reviewed the general aspects of root-nodules in tropical legumes. They reported

that inoculation with effective rhizobia was observed to lead to different responses in nodulation of groundnut in red and black soils - two of the major soil types in South India. An effective strain (R_4) induced heavy nodulation in Arachis hypogaea in red soils, which are acid to neutral (pH 5.5 - 7.0), more than in black soils, which are distinctly alkaline (pH 8.2 - 10.5), in spite of the fact that red soils contain less exchangeable cations than black soils. In sterile cultures, pH levels of 4, 5 and 6 were found suitable for plant growth, optimum nitrogen fixation and haemoglobin formation with a most favourable effect at pH 5, while fixation deteriorated towards pH 3, 7 and 8.

Cultural practises such as tillage operation, irrigation etc. have several physical and chemical effects on soil which are reflected on nitrogen fixation. Some crop rotations bring about stimulatory effects on microorganism. The types of chemicals excreted by the plants affect the selective stimulation of rhizobium bacteria by the growth-promoting substances produced. Extremes of soil reaction could adversely affect both the nodule formation and nitrogen fixation.

D. Inoculation with nitrogen-fixing bacteria :

Nitrogen-fixing bacteria are of two types - symbiotic and asymbiotic in nature. Rhizobia bacteria fix atmospheric

nitrogen symbiotically in the nodules of leguminous plants. Lockhead (1932) has reviewed beneficial effects of inoculation, effect on soil fertility, conditions affecting success of inoculation etc. In his review, he emphasizes that the purpose of inoculation is not to supply an easy way to grow legumes, but to provide for a better growth through the addition of bacteria which help the plants to make the most of ^a good environment. The legume bacteria can not supply the crop with lime or with such plant food as potash or phosphates. Inoculation, therefore, is most effective on soils sufficiently well-limed and containing adequate supplies of plant mineral food. In Canada, reports are distinctly favourable to inoculation. This is seen not only in case of alfalfa, the most outstanding case of success, but also in other legume crops. It is stated that inoculation is more successful where the legume is grown for the first time. In many cases, where no benefit is noted, the apparent non-success may be due to the soil being already sufficiently inoculated. In such cases the treated and untreated plants grow equally well. Adverse climatic or soil conditions may also be responsible for the lack of success. Drought, poor drainage, soil sourness and inferior seed quality are outstanding causes for legume failures even with treated seed, the adverse condition affecting the bacteria as well as the plants themselves.

Whiting (1925) worked on the relation of inoculation of soil or seed to yield and quality of peas. He concluded that the green peas from the inoculated plants were of better quality and had a higher protein content than from the uninoculated plants. Inoculation increased the yield and protein contents of vines. Inoculation produced increased yield on land already containing some pea bacteria. The increases due to inoculation occurred under a variety of conditions such as soil type, soil fertility, acidity and climate and with different varieties of peas.

Wilson and Leland (1929) conducted the experiments on the value of supplementary bacteria for legumes, and concluded that alfalfa grown on limed soil produced more dry weight when supplementary bacteria were applied at seed-time, although 46 days-old roots failed to indicate any value of the extra bacteria as measured by the number of nodules. Red clover similarly showed 40 per cent more dry weight on limed soil when supplementary bacteria were applied at seed time. Red kidney beans also showed increased dry weight on the limed soils. In case of peas, the same beneficial effect of supplementary bacteria was observed.

Hoffer (1943) carried out inoculation tests on 50 pea fields in New York State. He found that the results of pea inoculation in the State, though reasonably good, were

variable. He concluded that it would be wise to recommend inoculation for all fields on which peas or beans had not been grown for several years.

Greaves and Jones (1950), from the results of pot experiment, showed that the growing of alfalfa for 16 years on the soil and removal of the crop did not measurably increase the total nitrogen content of the soil. Inoculation of alfalfa, even where the plants were turned under, was without effect on soil nitrogen.

Hyka (1962) studied the effect of seed inoculation with lucerne-field soil on the growth of lucerne (Medicago sativa). He claimed that seed inoculation with an equal volume of lucerne-field soil greatly increased seedling height, fresh-root volume and dry weight of roots.

Obaton and Blachere (1963) reported their observations on inoculation of legumes in France. They stated that acid soils did not contain any Rhizobium meliloti, and many non-calcareous soils contained an insufficient number for lucerne. Inoculation was found worthwhile because even if the rhizobia did not survive in the soil they did survive in the rhizosphere.

Giddens (1964) reported the effect of adding molybdenum compounds to soybean inoculant. He stated that inoculant and

molybdenum compound should be mixed only at the time of seed treatment. Molybdenum compounds reduced nodulation when incorporated into the inoculant and stored.

Inoculation with isolates of cowpea rhizobium increased the nitrogen content of cowpea plants. Ezedinana (1964) stated that symbiotic nitrogen fixation alone did not provide the plants with their optimal nitrogen requirements. Application of 100 p.p.m. of nitrate increased the number and weight of nodules and the amount of nitrogen fixed. Higher levels tended to give lower results. There was no evidence of excretion of symbiotically fixed nitrogen.

Abel and Erdman (1964) studied the response of Lee soybeans to different strains of Rhizobium japonicum. They found that, in soils free from soybean rhizobia, some strains were more effective than others in increasing seed yield, protein per cent of seed, root-nodulation, green colour of leaf and fresh plant weight and in decreasing oil content of seed. In contaminated rhizobia, inoculation treatments produced no measurable differences in any of these characteristics.

Rhizobia may occur in the soil provided legumes are grown on the soil every few years. This was observed by Nowak and Lehner (1965), studying on re-isolation tests with nodule bacteria (Rhizobium sp.) from soil.

Hansen (1965) observed that inoculation with Rhizobium leguminosarum did not prove beneficial on seed yields, number and weight of root nodules when inorganic nitrogen at the rate of 100 - 200 kg. calcium nitrate was applied per acre.

Brakel and Manil (1965), working on symbiotic fixation of nitrogen by the bean plant (Phaseolus vulgaris L.), have stated that in one of the three experimental sites, the increase in yield due to inoculation was similar to that due to 50 kg./ha. nitrogen fertilizer. Response to inoculation was nil on the other two sites, which had a high available nitrogen content. On all sites, inoculation caused rapid and abundant nodulation. Variety had no significant effect.

Bonnier and Lebrun (1965), working on the effect of the number of rhizobia used for inoculation on yield of lucerne, have found that the number of rhizobium used for inoculating lucerne seed was important for maximum yield in the first year under Ardennes conditions. The concentrated inoculum used provided 30,000 live bacteria per seed at sowing. Diluting the inoculum gave lower yields in the first year.

Kick (1966), in his pot and field experiments for testing the effectiveness of legume inoculants, reported that there was no significant response to inoculation in field trials with Trifolium resupinatum, serradella, bush bean and lucerne.

Sen (1966) reported results of field experiments at various sites in India and showed that strains isolated from plants grown at different places showed great variations in some biochemical characteristics and in ability to fix atmospheric nitrogen. An effective rhizobium strain should only be used at places that have soil and climatic conditions similar to those of the original habitat.

E. Comparison of relative efficiency of rhizobium strains :

Rajagopalan (1938) compared six isolates of groundnut rhizobium strains, which were found morphologically alike but physiologically different. The characteristics of one strain were high gum-production, high sensitivity of hydrogen ions, high 'ferment', high nitrogen fixation and production of large number of small and medium-sized nodules, which were concentrated more on the tap-root than on the laterals. The other strains showed the reverse characteristics. The efficiency of the strains as nitrogen-fixers appeared to depend more on the location of the nodules than on their number and size.

The nitrogen-fixing ability of various strains of nodule bacteria was studied by Yakovleva (1960). It was demonstrated that the nitrogen-fixing ability was innate not only in bacterioids but, to an even large degree, in the small cells from the apical region. In evaluating the activity of these and other forms of bacteria, attention

should obviously be given not only to the energy of fixation of molecular nitrogen, but also to the bacteroid capable to easily give up the nitrogen fixed by them to the plants.

Fedorov and Nitse (1961) studied physiological differences between strains of root-nodule bacteria of pea and vetch differing in N-fixing activity. They stated that strains of pea and vetch root-nodule bacteria, having different nitrogen-fixing activity in nodules of these plants, differed from one another in number of physiological characteristics, which were easily determined in laboratory cultures of these bacteria. The strains that fixed molecular nitrogen actively in nodules synthesized their cellular material more efficiently on various carbon and nitrogen sources. They have reported a higher protein content also.

Krasil'nikov and Melkumova (1963) reported that some strains of lucerne-nodule bacteria produced nodules actively on roots, accelerated the growth of lucerne and increased the crop growth by 100 - 250 per cent or more. With other less active strains the increase in crop yield did not exceed 20 - 50 per cent, and with still other inactive strains there was no benefit. They stressed the importance of selecting effective strain for artificial infestation.

Linta (1963) observed variations in different strains of pea and vetch nodule bacteria. He reported that active strains of rhizobia not only produced greater number of nodules of larger size and fixed higher amounts of nitrogen than less active strains, but also caused an increase in the proportions of protein nitrogen and amounts of amino acids.

Abel and Erdman (1964) observed that in soils free from soybean rhizobia, some strains were more effective than others in increasing seed yield, protein percentage of seed, root-nodulation, green colour of leaf, and fresh plant weight. In rhizobia-contaminated soils, infestation treatments produced no measurable differences in any of these characteristics.

F. Work Done under Different I.C.A.R. Schemes on Biological Nitrogen Fixation

Investigations have been done at Ludhiana (Punjab), Poona (Maharashtra), Bapatla (Andhra Pradesh) and Anand (Gujarat) on symbiotic nitrogen fixation under the ICAR - Coordinated Scheme on Biological Nitrogen Fixation. The results achieved earlier at Anand have been referred in the introduction. The present investigation is a part of the scheme.

Investigations done at Bapatla

The work done at Bapatla (Andhra Pradesh, 1959-62) involved isolation and testing of different strains of

bacteria isolated from pillipesara and groundnut. The strains BGP-215, BMP-219 and BSP-225 isolated from pillipesara from Gannavaran, Maruten and Samalkot areas were found to be superior to the others. Application of molybdenum spray at 8 p.p.m. gave outstandingly high green-matter yields of pillipesara. Application of lime was not beneficial in increasing the yield of pillipesara. Further, out of twelve cultures isolated from groundnut, the four cultures BNAh 261 (Nellore), BEAL 252 (Dapatla), BEAL 258 (Elur) and BHIAL 260 (Vijaywada) were found to be efficient.

Investigations done at Ludhiana (1960-65) :

At Ludhiana, effects of different treatments were studied on the growth, nodulation, nitrogen fixation by dhaincha and on the yield of wheat crop that followed green-manuring. Indications were that nitrogen alone at 50 lb. N/acre in the form of ammonium sulphate depressed nodulation. The total nitrogen content and total dry weight of different plant parts of dhaincha were the lowest in the case of control without fertilizers.

Nitrogen, phosphorus and potash treatments alone or with molybdenum or with all micro nutrients increased the yield of dhaincha.

The beneficial effect of green-manuring on the yield of wheat, in absence of fertilization to the green-manure crop,

was of small order. The highest yield of wheat was obtained with NPK + Mo or all micro nutrients applied to the green-manure crop.

The soil left after harvesting of wheat was comparatively richer with respect to total nitrogen than the original soil used for growing dhaincha.

No direct relationship was observed between leghaemoglobin content of the nodules and the nitrogen fixed by them. However, a positive correlation coefficient was observed between these two factors.

In case of berseem, application of phosphate showed maximum beneficial effect in regard to the production of fresh green fodder. NPK with Mo improved the performance of phosphate alone.

Some efficient strains of root-nodule bacteria of dhaincha and berseem were isolated. Gum-production and glucose-consumption of these isolated strains were also studied in order to find out the correlation, if any, between these characteristics and their effectiveness. The results showed that the isolates, which produced more gum, fixed less nitrogen, while the isolates, which consumed more glucose, fixed more nitrogen indicating

thereby that these laboratory tests can be used for testing the effectiveness of root-nodule organisms.

Investigations done at Poona

Large number of rhizobia cultures were isolated from groundnut, sannhemp, maiz, garden peas, gram, tur and soyabean. The efficient cultures were then tested simultaneously in pot culture and in the field experiments. The methods for quick selection of effective Rhizobium cultures of tur have been worked out.

Chapter - III

Methods and Materials.

A field experiment was undertaken to study the effects of inoculation and application of phosphate to two leguminous crops viz. sannhemp and guar on their green-matter yield and on the organic matter and nitrogen content of the soil at the Institute of Agriculture, Anand. This experiment was carried out for four seasons from 1964-67. The wheat crop was taken as a following crop on the same experimental plots in which sannhemp and guar crops were grown and ploughed under. Wheat crop was taken to study the after-effects of green-manuring in increasing wheat yield for the years 1966 and 1967.

Sannhemp (Crotalaria - juncea) and guar (Cyamopsis tetragonoloba) were sown at the seed-rate of 56 kg. per hectare. The plan of the experiment was as shown below :

- Treatments :** All combinations of
- A. Culture -**
1. Uninoculated (C_0)
 2. Inoculated (C_1)
- B. Phosphate -**
1. Without phosphate (P_0)
 2. With 56 kg. P_2O_5 per hectare in the form of single superphosphate (P_1).
- Lay-out -** Randomized blocks;
- Replications -** 6.
- Plot size -** Gross 2.5 m. x 6 m.
Net 2 m. x 5 m.

Technique :

After good showers of rain, the land in the experimental field was prepared by ploughing, harrowing and planking. Markings were then made to indicate the area required for each crop and for each plot. Soil samples were collected from ten random places from each area for analysis of nitrogen and organic matter prior to sowing of the crops. In each plot furrows were opened at a distance of 30 cm. with a country plough for sowing the seeds.

Seeds required for each plot were treated with 70 per cent alcohol for five minutes for disinfection. As per requirement of the plan, seeds of each crop were infested with the respective selected cultures. For control plots disinfested seeds were kept separate. Infested and disinfested seeds for each plot were placed in separate polythene bags. Superphosphate at the rate of 56 kg. per hectare in the form of single superphosphate required for each plot (560 gms. per plot) was weighed and packed separately in paper bags.

At the time of sowing, seeds and phosphate fertilizer bags were first placed in front of respective plots according to the plan of the experiment. Superphosphate was sprinkled evenly with hand in the furrows opened in the plots before sowing the seeds.

seeds were then sown uniformly in the furrows with hand taking all precautions to avoid mixing of infested and disinfested seeds. Hands were washed every time before sowing each plot. After sowing the seeds, furrows were covered with soil from the same plot by the help of wooden rake kept separately for each treatment.

There was good germination of seeds in both the legume crops. The plants attained very good vegetative growth during forty days after sowing and started flowering during this period.

At the end of eighth week after sowing, when most of the plants attained flowering stage, the plants were uprooted and the average weight of fresh green plants was taken from two patches 0.6 m. x 0.6 m. at random from each plot in each crop. Furrows were opened in all the plots and then the plants were placed in the furrows of respective plots, and were covered with soil for decomposition. The yields of the green matter (average weight of the plants from two patches of 0.6 m. x 0.6 m. from each plot) for the four successive years (1964-67) were taken.

Description of the soil

The experiment was conducted on a loamy sand of Anand, locally called geradu, which is very deep and well-drained. It is deficient in organic matter and nitrogen but rich in

potash and therefore does not respond to potash fertilization. The chemical and mechanical composition of the goradu soil profile is given in table 1.

Climatological data :

The climate at Anand is sub-tropical with dry and hot summers from March to June. Temperature sometimes goes up to 118°F. The winter lasts from November to February with lowest temperature up to 40°F. which leads to frost. The average annual precipitation is 75 cm; but it is erratic.

Green-matter yield :

The green-matter yields of sannhemp and guar were taken from a patch of 0.6 m. x 0.6 m. from each plot at the time of flowering i.e. after about 8 weeks of growth. At the flowering stage, the plant height of sannhemp was about 1.8 to 2 meters. The growth of guar was found luxuriant when the green weight was recorded. Two such patches at random were selected and their average green-matter weights were recorded. The plant samples were then dried in sun and then in oven at 70°C. till free from the traces of moisture.

Chemical studies :

The plants were then cut into small pieces and ground in mortar and pestle. The plant samples so prepared were

1. Particle-size distribution and chemical characteristics of goradu soil profile.

(air-dry basis)

Constituents	Depth in cm.					
	0-15	15-30	30-60	60-90	90-120	120-150
Coarse sand p.c.	0.42	0.48	0.47	0.35	0.54	0.47
Fine sand p.c.	80.07	86.00	81.61	71.01	70.87	71.56
Silt p.c.	10.75	9.75	9.50	22.50	20.50	21.50
Clay p.c.	5.50	1.00	3.75	2.50	2.25	2.75
Loss on ignition p.c. (after removing moisture).	1.24	0.97	0.97	1.46	1.50	1.48
Organic matter p.c.	1.32	1.17	1.60	2.07	2.67	2.91
Sulphates p.c.	Nil	Nil	Nil	Nil	Nil	Nil
Water soluble salts p.c.	0.016	0.011	0.096	0.098	0.032	0.026
Total nitrogen p.c.	0.036	0.036	0.030	0.034	0.033	0.029
Total phosphorus (100 g.).	434.00	434.00	322.00	294.00	356.00	403.00
Plant available phosphorus (mg./100 g.) (Muller's method).	9.7	9.7	2.8	7.1	7.9	10.5
Water-soluble phosphorus (mg./100 g.)	0.28	0.28	0.18	0.28	0.09	0.09
Total potassium (100 g.).	275.6	254.7	286.5	327.4	312.4	333.5
Plant available potassium (100 g.)	26.2	17.8	17.8	19.0	21.2	19.0
Total manganese (100 g.).	49.1	48.3	46.8	45.4	44.8	46.8
Plant available manganese (100 g.).	0.78	0.59	0.70	0.58	0.54	0.50
Plant available boron p.p.m.	0.3	0.3	0.3	0.3	0.5	0.5
Plant available zinc p.p.m.	2.05	2.05	1.45	1.80	1.20	1.20
Plant available cobalt p.p.m.	12.40	12.40	20.50	18.75	19.50	20.00
Plant available cobalt p.p.m.	0.60	0.60	0.37	0.20	0.10	0.10
Plant available copper p.p.m.	0.29	0.29	0.15	0.10	0.25	0.10
Total copper p.p.m.	35.00	35.00	30.00	55.00	30.00	25.00
Cation exchange capacity (100 g.).	9.8	9.2	10.5	16.4	17.00	17.9

used for nitrogen estimation by Winkler's boric acid modification of the Kjeldahl method. Total nitrogen uptake of sauhenp and that of guwar was then calculated.

Analysis of soil samples for nitrogen and organic matter in the field trial experiment before sowing and after decomposition of buried organic matter :

Soil samples were collected from 10 random places from each crop area before sowing the seeds. One composite soil sample was made from these 10 sub-samples and analysed for nitrogen and organic matter.

Soil samples were also collected after 8 weeks of decomposition of the buried plant residue from the depth of 0-23 cm. from 10 random places from each plot. Out of these sub-samples one composite soil sample was made for analysis of nitrogen and organic matter.

Analysis of soil for nitrogen :

Soil samples collected before sowing of the crop and after decomposition of plant material were analysed for nitrogen content by Winkler's boric acid modification of the Kjeldahl method (1913), adding salicylic acid during digestion and using the mixed indicator for the final titration. The boric acid used in the Winkler's method has five marked advantages : (1) It eliminates error in

measuring standard acid in the receiver. (ii) The boric acid solution need not be accurately measured. (iii) By using a suitable aliquot of the sample, the percentages of nitrogen can be read directly from the burette. (iv) Only one solution need be standardised. (v) Even if there is suck-back of boric acid, the experiment is not spoiled and the distillation can be continued.

The mixed indicator gives a sharp end-point and hence it was utilized in nitrogen estimation. The mixed indicator is prepared by dissolving 0.5 g. of bromo-cresol green and 0.1 g. methyl red in 100 ml. of 95 per cent ethanol and adjusting the solution to the bluish-purple mid colour at pH 4.5, with dilute NaOH or HCl. This indicator is pink at pH 4.2 and below, and bluish-green as the pH rises to pH 4.9 and above.

Analysis of plants for nitrogen :

The method for nitrogen estimation of the plant material was similar to that of soil analysis. Quantity of the plant material taken for analysis was 1.0 g.

Soil organic matter :

A quick and simple method of determining soil organic matter has been worked out by Shah and Mehta (1955). A slight modification of this was adopted.

Reagents

- (1) N potassium dichromate solution :
49.04 g. $K_2Cr_2O_7$ was dissolved in distilled water and diluted to 1 litre.
- (2) Concentrated sulphuric acid.
- (3) Glucose (analar).

Procedure for standardisation

1.0 g. of "analar" glucose was weighed and dissolved in water and made up to 1000 ml. From this solution, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 ml. were taken in 250 ml. pyrex beakers and evaporated to dryness on water bath. It was then allowed to cool and 10 ml. of 1 N $K_2Cr_2O_7$ solution was added. Then 20 ml. of concentrated sulphuric acid was added rapidly and the mixture was stirred for 10 seconds. It was then allowed to stand for 10 minutes and 100 ml. of distilled water was added. A blank was similarly prepared without glucose. After it cooled well, the green colour of the solution was measured on Klett-Summerson photoelectric colorimeter using a 655 m μ (red) filter after adjusting the blank reading. Colorimetric readings were then plotted against the concentration.

Determination of organic matter from soil

1.0 g. of the air-dried soil was weighed and transferred to 250 ml. pyrex beaker. 10 ml. of 1 N $K_2Cr_2O_7$

solution was then added to it. Then 20 ml. of concentrated sulphuric acid was added rapidly and the mixture stirred for 10 seconds. It was then allowed to stand for 10 minutes and 100 ml. of distilled water was added and stirred well. It was then allowed to stand overnight. The supernatant liquid was decanted carefully and the green colour was measured by Klett-Summerson photoelectric colorimeter using 655 m μ (red) filter after adjusting blank (prepared by the same procedure taking 1.0 g. of the ignited soil).

Yield of wheat

Wheat was sown to study the effect of sannhemp and guar green-manuring. 100 kg./ha. of extra nitrogen in the form of urea was applied in the first trial and 45 kg.N/ha. as urea was applied in the second trial. In the first year, 100 kg. of N per hectare was applied as it is the recommended dose. But as no significantly higher yield was obtained with inoculated plots, in the next season a lower dose of 45 kg. N per hectare was applied, because it was thought that at the optimum dose of fertilizer nitrogen, inoculation may not prove effective. After the sannhemp and guar plants were buried in the plots and allowed to decompose, the same plots were used for wheat crop. Wheat was sown in November-December, and seven irrigations were given as per the requirement of the crop. The dry matter yield was taken at the time of maturity of seeds.

The seeds and straw were separated from the air-dried plant. From the nitrogen contents and yields of each, the total uptake of nitrogen was calculated for a plot of 0.6 m. x 0.6 m. This was done for two patches from each plot in a replication and an average was taken.

Chapter IV

Results and Discussion.

Inoculation of the seed with efficient strain of legume culture may increase the yield of the leguminous plant or the nitrogen content of the plant or both. Therefore, yield, nitrogen content and the total nitrogen uptake of the plants were determined. As the symbiotic nitrogen fixers require liberal quantities of phosphorus, it was included in the treatment. Although nitrogen fixation by these bacteria is improved by the application of molybdenum, it was not included in the treatments as the available molybdenum in the Institute soil is fairly high (Reddy and Mehta, 1958) and it has been shown by Patel (1962) that there was no response to apply molybdenum to a leguminous crop in a field trial.

Green-matter yield

The results for the green-matter yield of sorghum under the different treatments are given in table 2. These data reveal that, on an average, the culture in combination with phosphate (P_1C_1) gave the highest yield. Phosphate without culture (P_1C_0) also gave higher yield than the control (P_0C_0) receiving neither phosphate nor the culture, but was not much different from the yield obtained under the treatment containing the culture but without phosphate (P_0C_1). The yield under P_0C_1 was also higher than P_0C_0 .

Table 2. Green-matter yield (g.) of sannhemp (C. luncea) from a patch of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment:	Y	E	A	R	S	: Average
:	1964-65:	1965-66:	1966-67:	1967-68 :	:	:
P ₁ C ₁	1404	1105	1176	1103		1197
P ₁ C ₀	1279	1016	1089	1089		1118
P ₀ C ₁	1200	990	1106	1068		1113
P ₀ C ₀	1178	1006	1012	1039		1059

C₁ = inoculated; C₀ = non-inoculated.
P₁ = with phosphate; P₀ = without phosphate.

Table 3. Green-matter yield (g.) of guar (C. tetragonoloba) from a patch of 0.6 m x 0.6 m. (average of 6 replications).

Treatment:	Y	E	A	R	S	: Average
:	1964-65:	1965-66:	1966-67:	1967-68 :	:	:
P ₁ C ₁	585.7	595.0	996.0	560.0		684.2
P ₁ C ₀	524.0	593.0	933.0	557.0		652.0
P ₀ C ₁	524.2	545.0	963.0	555.0		646.8
P ₀ C ₀	504.0	483.0	881.0	542.5		602.6

The results of green-matter yield of GUAR (table 3) also showed the identical trend as that observed under sannhemp.

Dry-matter yield of sannhemp

Yields of green matter do not exactly reflect the total biomass synthesized in plants on account of varying moisture contents. Therefore, dry-matter yields were determined. The dry-matter yields of sannhemp and the combined analysis of the data for four years are recorded in tables 4 and 5 respectively.

Dry-matter yield of sannhemp in the year 1964-65 under treatment P_1C_1 was significantly more than that obtained under P_1C_0 or P_0C_0 , but no such significant difference was observed between the treatments P_1C_1 and P_0C_1 . The latter one also yielded significantly more than P_0C_0 . The yield differences between P_0C_1 and P_1C_0 as well as between P_1C_0 and P_0C_0 were not significant. In the year 1965-66, dry-matter yield of sannhemp under P_1C_1 was highest but the treatment differences were not significant. Among rest of the treatments very small differences were observed. In the year 1966-67, dry-matter yield of sannhemp was lowest under P_0C_0 and highest under P_1C_0 . P_1C_1 yielded little less than P_1C_0 but it was more than that under P_0C_1 but here again these treatment differences were not significant. In the year 1967-68, no material differences were observed in the dry-matter yields of sannhemp under the various treatments.

Table 4. Dry-matter yield (g.) of sannhemp (C. juncea) from a patch of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment:	Y	E	A	R	S	: Average
:	1964-65:	1965-66:	1966-67:	1967-68 :	:	:
P ₁ C ₁	360.6	324.1	333.6	205.0	307.8	
P ₁ C ₀	320.6	275.4	347.6	207.3	287.7	
P ₀ C ₁	338.1	267.9	307.2	199.5	278.2	
P ₀ C ₀	299.1	272.4	281.0	209.7	265.6	
S.E.M. for treatment	10.10	N.S.	N.S.	N.S.		
L.S.D. at 5 % for treatment	30.35	N.S.	N.S.	N.S.		
C.V. %	7.5	19.2	26.5	10.8		

Table 5. Pooled analysis of dry-matter yield of sannhemp (C. juncea).

Source	d.f.	S.S.	M.S.S.	Obs. F.	Table F.	5%	1%
Years	3	225165.49	75055.16	26.7**	2.76	4.13	
Treatments	3	20700.30	6900.10	3.1 N.S.	3.86	6.99	
a. Culture	1	5659.55	5659.55	<3 N.S.	5.12	10.56	
b. Phosphate	1	14862.82	14862.82	6.7*	5.12	10.56	
c. Interaction	1	177.93	177.93	<1 N.S.	5.12	10.56	
Y x T	9	19955.10	2217.23	<1 N.S.	2.04	2.72	
Pooled error	60	168022.03	2800.37	-	-	-	
Total	95						

** Significant at 1 % level

* Significant at 5 % level

On an average, the dry-matter yield of sannhemp under P_1C_1 was highest, while it was lowest under P_0C_0 . Not much difference was observed under P_1C_0 and P_0C_1 . Pooled analysis of dry-matter yields of sannhemp of all the four years did not show any significant difference under any of the treatments.

Dry-matter yield of guwar :

The results of dry-matter yields of guwar and the combined analysis of the data for four years are represented in tables 6 and 7 respectively. In the year 1964-65, though the differences observed were not significant, the yield under P_1C_1 was highest, while least was observed under P_0C_0 . P_1C_0 and P_0C_1 did not differ much. In the year 1965-66, P_0C_0 yielded the least and it was highest under P_1C_0 . However, the differences were non significant. Between P_1C_1 and P_0C_1 treatments, the yield differences were not much. Similarly, in the years 1966-67 and 1967-68, the yield differences were not significant although highest yield was recorded under P_1C_1 . Very little variations were observed in the yield of guwar under rest of the treatments.

On an average of the four years results, the yield of guwar under treatment P_1C_1 was maximum and that of P_0C_0 was

Table 6. Dry-matter yield (g.) of guar (C. tetragonoloba) from a patch of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment:	Y	E	A	R	S	Average
	1964-65:	1965-66:	1966-67:	1967-68:		
P ₁ C ₁	118.0	130.5	149.7		66.3	116.1
P ₁ C ₀	108.3	134.6	128.2		62.0	108.3
P ₀ C ₁	107.7	126.1	137.6		63.3	108.7
P ₀ C ₀	103.1	118.6	134.3		63.7	104.9
S.Em. for treatment	N.S.	N.S.	N.S.		N.S.	
L.S.D. at 5 % for treatment	N.S.	N.S.	N.S.		N.S.	
C.V. %	9.6	13.5	14.9		12.2	

Table 7. Pooled analysis of dry-matter yield of guar (C. tetragonoloba).

Source	id.f.	S.S.	M.S.S.	Obs. F.	Table F.
					5% 11%
Years	3	76700.99	25566.79	**	2.76 4.13
Treatments	3	1603.80	534.60	3.38	N.S. 3.86 6.99
a.Culture	1	790.62	790.62	5.0	N.S. 5.12 10.56
b.Phosphate	1	717.78	717.78	< 5	N.S. 5.12 10.56
c.Interaction	1	95.40	95.40	< 1	N.S. 5.12 10.56
Y x T	9	1422.69	158.08	< 1	N.S. 2.04 2.72
Pooled error	60	13308.34	221.81	-	- -
Total	95				

** Significant at 1 % level

minimum. The yields under P_1C_0 and P_0C_1 treatments can be considered practically equal. A similar trend as that observed under sannhemp due to different treatments was maintained in the production of dry matter of guar.

Total nitrogen content

By inoculating seeds of leguminous plants, there can be an increase in the dry-matter production, nodule-formation, nitrogen content or total nitrogen uptake of a plant. Hence total nitrogen content was determined and the total nitrogen uptake was calculated. The results for nitrogen content of sannhemp (table 8) under different treatments did not show any regular trend. The nitrogen percentage under P_1C_1 was not necessarily the highest and that under P_0C_0 was also not necessarily the lowest.

The results of analysis for total nitrogen content of guar plants are presented in table 9. Here, too, there is no clear trend of treatment differences yearwise. On an average, there was practically very little difference in the nitrogen contents under different treatments.

Total uptake of nitrogen

The results for total uptake of nitrogen by sannhemp plants and the combined analysis for the four years data are

Table 8. Total nitrogen per cent of sannhemp plant material from a patch of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment:	Y	E	A	R	S	Average
:	1964-65:	1965-66:	1966-67:	1967-68 :	:	:
P_1C_1	2.93	3.29	2.13	1.57		2.63
P_1C_0	2.82	3.34	2.08	1.77		2.50
P_0C_1	2.64	3.25	2.08	1.79		2.44
P_0C_0	2.85	3.31	2.11	1.73		2.50

Table 9. Total nitrogen per cent of guar plant material from a patch of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment:	Y	E	A	R	S	Average
:	1964-65:	1965-66:	1966-67:	1967-68 :	:	:
P_1C_1	4.32	4.13	3.67	3.05		3.58
P_1C_0	4.35	4.31	3.01	3.11		3.69
P_0C_1	4.08	4.17	2.85	2.92		3.51
P_0C_0	4.32	4.25	2.70	2.83		3.52

presented in tables 10 and 11 respectively. In the first two years of the experiment, the total uptake of nitrogen by sannhemp plants under P_1C_1 treatment was highest followed by P_1C_0 . The lowest uptake was observed under P_0C_0 during the first year of the experiment and under P_0C_1 during the second year. However, these differences in the uptake of nitrogen were significant only for the first year. In the year 1966-67, very little difference was observed in the uptake of nitrogen by sannhemp plants under the treatments P_1C_1 and P_1C_0 . Least uptake was found in P_0C_0 but here also treatment differences were not significant. In the last year 1967-68, it was strange that highest nitrogen uptake was found under P_1C_0 and lowest under P_1C_1 . The uptake difference between P_0C_1 and P_0C_0 was not much.

On an average, total nitrogen uptake was maximum under treatment P_1C_1 and minimum under P_0C_0 . The nitrogen uptake by sannhemp plants under P_0C_1 was slightly more than that of P_0C_0 . Thus, inoculation of culture with phosphate application seemed to be beneficial in increasing the uptake of nitrogen of sannhemp plants but the combined analysis (table 11) of total uptake of nitrogen by sannhemp plants did not show any significant difference among the different treatments.

Table 10. Total uptake of nitrogen (g.) of sannhemp plant material from a patch of 0.6 m x 0.6 m. (average of 6 replications).

Treatment:	Y	E	A	R	S	Average
	1964-65:	1965-66:	1966-67:	1967-68:		
P ₁ C ₁	10.53	10.65	7.11	3.24		7.89
P ₁ C ₀	9.03	9.21	7.18	3.75		7.29
P ₀ C ₁	8.90	8.72	6.40	3.54		6.89
P ₀ C ₀	8.51	8.99	5.92	3.61		6.76
S.Em. for treatment	0.29	N.S.	N.S.	N.S.		
L.S.D. at 5 % for treatment	0.87	N.S.	N.S.	N.S.		
C.V. %	7.6	19.5	27.9	31.7		

Table 11. Pooled analysis for total nitrogen uptake of sannhemp plants from a patch of 0.6 m. x 0.6 m. (average of 6 replications).

Source	d.f.	s.s.	m.s.s.	obs. F.	Table F.
					5% 1%
Years	3	5395.72	1798.57	**	2.76 4.13
Treatments	3	0.52	0.14	< 1 N.S.	3.86 6.99
a.Culture	1	0.08	0.08	< 1 N.S.	5.12 10.56
b.Phosphate	1	0.47	0.47	< 1 N.S.	5.12 10.56
c.Interaction	1	0.05	0.05	< 1 N.S.	5.12 10.56
Y x T	9	34.73	3.86	< 2 N.S.	2.04 2.72
Pooled error	60	128.33	2.14	-	- -
Total	95				

** Significant at 1 % level

In case of guar, total nitrogen uptake (table 12) was maximum under P_1C_1 for all the years except 1965-66. The minimum uptake was obtained under P_0C_0 for all the four years. Between the treatments P_1C_0 and P_0C_1 , the greater uptake was always found under the former treatment except in the year 1966-67 when it was practically equal in both the treatments. But these results were statistically significant only for the first year.

On an average, maximum uptake was recorded under the treatment P_1C_1 , and minimum under P_0C_0 . The uptake of nitrogen under P_1C_0 was slightly more than that obtained under the treatment P_0C_1 . Here also inoculation of legume seeds in presence of phosphate seems to be advantageous in increasing the uptake of nitrogen. But the combined analysis (table 13) for total nitrogen uptake by guar plants under different treatments did not show any significant differences.

Soil organic matter

Organic matter content of soils under different treatments before sowing sannhemp did not show any wide differences from year to year as seen from the table 14.

Table 12. Total uptake of nitrogen (g.) of guwar plant material from a patch of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment:	Y	E	A	R	S	: Average
	: 1964-65:	1965-66:	1966-67:	1967-68:		:
P ₁ C ₁	3.44	5.42	4.29	2.01		3.79
P ₁ C ₀	3.04	5.81	3.88	1.94		3.67
P ₀ C ₁	2.85	5.21	3.91	1.86		3.46
P ₀ C ₀	2.94	5.05	3.71	1.79		3.37
S.E.M. for treatment	0.13	N.S.	N.S.	N.S.		
L.S.D. at 5 % for treatment	0.39	N.S.	N.S.	N.S.		
C.V. %	10.5	17.8	26.5	16.5		

Table 13. Pooled analysis for total nitrogen uptake of guwar plants from a patch of 0.6 m. x 0.6 m. (average of 6 replications).

Source	d.f.	s.s.	m.s.s.	obs. F.	Table F.	
	:	:	:	:	: 5%	:1%
Years	3	1345.86	448.62	**	2.76	4.13
Treatments	3	0.70	0.23	< 1 N.S.	3.86	6.99
a.Culture	1	0.01	0.01	< 1 N.S.	5.12	10.56
b.Phosphate	1	0.06	0.06	< 1 N.S.	5.12	10.56
c.Interaction	1	0.0	0.0	< 1 N.S.	5.12	10.56
Y x T	9	4.34	0.48	< 1 N.S.	2.04	2.72
Pooled error	60	33.11	0.55	-	-	-
Total	95					

** Significant at 1 % level

After green-manuring, the organic matter content increased in all the years except 1965-66. On an average, the organic matter content of soil after green-manuring with sunhemp plants under P_1C_1 showed a maximum increase, while minimum increase was found under P_0C_0 . Thus, there seemed to be a slight improvement in the organic matter content of soil which was green-manured every year with sunhemp under all the treatments, but the differences were not of high magnitude.

Results of organic matter content of soils after green-manuring with guar (table 15) showed that on an average there was a slight increase under the treatments P_1C_1 and P_0C_1 , but no such improvement was observed under treatments P_1C_0 and P_0C_0 after four years of green-manuring.

Nitrogen content of green-manured soil

The total nitrogen content of the soil was of the order of 0.032 per cent before sowing. Even though green-manuring was done for four years, there was no substantial improvement in the nitrogen content of soil (tables 16 and 17).

Table 14. Per cent soil organic matter before and after decomposition of buried plants of sannhemp.

Year	O.M. %	O.M. per cent after decomposition			
	before	P_1C_1	P_1C_0	P_0C_1	P_0C_0
	sowing				
1964-65	0.60	0.80	0.65	0.70	0.60
1965-66	0.52	0.51	0.53	0.55	0.52
1966-67	0.58	0.67	0.60	0.64	0.64
1967-68	0.51	0.73	0.76	0.76	0.72
Average	0.55	0.68	0.64	0.66	0.62

Table 15. Per cent soil organic matter before and after decomposition of buried plants of guwar.

Year	O.M. %	O.M. per cent after decomposition			
	before	P_1C_1	P_1C_0	P_0C_1	P_0C_0
	sowing				
1964-65	0.70	0.90	0.70	1.0	0.70
1965-66	0.52	0.57	0.56	0.55	0.52
1966-67	0.61	0.67	0.58	0.61	0.61
1967-68	0.65	0.58	0.51	0.52	0.50
Average	0.62	0.68	0.59	0.67	0.58

Table 16. The effect of seed infestation with rhizobium culture with and without P_2O_5 on nitrogen content of soil (%) after decomposition of sannhemp plants (average of 6 replications).

Treatment:	Before:	Y	E	A	R	S
:	sowing:	1964-65:	1965-66:	1966-67		: 1967-68
P_1C_1	0.032	0.032	0.031	0.029	0.039	
P_1C_0	0.032	0.029	0.030	0.029	0.036	
P_0C_1	0.032	0.031	0.031	0.029	0.034	
P_0C_0	0.032	0.030	0.030	0.029	0.032	

Table 17. The effect of seed infestation with rhizobium culture with and without P_2O_5 on nitrogen content of soil (%) after decomposition of guar plants (average of 6 replications).

Treatment:	Before:	Y	E	A	R	S
:	sowing:	1964-65:	1965-66:	1966-67		: 1967-68
P_1C_1	0.032	0.043	0.031	0.034	0.032	
P_1C_0	0.032	0.038	0.030	0.032	0.032	
P_0C_1	0.032	0.035	0.030	0.032	0.034	
P_0C_0	0.032	0.034	0.030	0.030	0.031	

Dry-matter yield of wheat

The improvement in symbiotic nitrogen fixation brought about by inoculation may not be reflected in the nitrogen content of soil as the amount of nitrogen is small and is not likely to be clearly indicated by the Kjeldahl method. However, this additional nitrogen is likely to benefit the succeeding cereal crop. Therefore, wheat was grown on the same green-manured plots by applying the recommended dose of nitrogen viz. 100 kg./ha. in the form of urea in the first year. The total dry matter produced is reported in table 18.

In case of wheat following sannhemp green-manuring in the year 1966-67, the least yield was recorded under P_0C_0 . It was highest under P_1C_0 . Treatments P_0C_1 and P_1C_1 also yielded more than P_0C_0 but all these differences were not significant.

It was thought the differences due to inoculation treatment may not be reflected at the high level (100 kg. N/ha.) of applied fertilizer which is the optimum dose. Therefore, next year, a smaller dose of nitrogen viz. 45 kg. N/ha. was applied to wheat crop and the effects of treatments studied. It was observed that P_1C_1 treatment yielded highest, while lowest yield was recorded under P_0C_1 but the results were not significant.

On an average, P_1C_1 gave the highest yield, while lowest yield was recorded under P_0C_0 . P_1C_0 and P_0C_1 also yielded slightly higher than P_0C_0 . The combined analysis (table 19) of the yield data for two years did not show any significant treatment differences.

When guar was used as a green-manuring crop, the dry-matter yield (table 20) of wheat in both the years was highest under P_1C_1 , while it was lowest under P_0C_0 . P_1C_0 yielded slightly higher than that observed under P_0C_0 , but the results were statistically significant only for the year 1967-68. Amongst P_0C_1 and P_0C_0 treatments, no material differences were obtained in the dry-matter yield of wheat during both the years. Pooled analysis (table 21) for the two years yield data did not bring out any significant differences due to any of the treatments.

Yield of wheat seed

Yield data of wheat seed from the field green-manured with sambhar are given in table 22. The results revealed that lowest production of grain was recorded in the treatment P_0C_0 , and P_0C_1 was highest in the first year and P_1C_1 in the second year. But all these differences were not significant either for individual years or for the combined results of two years (table 23).

(9.)

Table 18. Dry-matter yield of wheat plants grown on plots green-manured with sannhemp from a patch of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment	Y E A R S	Average	
	: 1966-67	: 1967-68	
P ₁ C ₁	411.0	357.5	384.2
P ₁ C ₀	434.0	327.6	380.8
P ₀ C ₁	423.0	319.5	371.2
P ₀ C ₀	386.0	337.6	361.8
S.E.m. for treatment	N.S.	N.S.	
L.S.D. at 5 % for treatment	N.S.	N.S.	
C.V. %	9.8	19.2	

Table 19. Pooled analysis of wheat yield preceded by sannhemp green-manuring.

Source	d.f.	s.s.	m.s.s.	Obs. F.	Table F.
					5% 1%
Years	1	80031.33	80031.31	26.5**	4.17 7.56
Treatments	3	3561.71	1187.24	< 3 N.S.	9.28 29.46
a.Culture	1	450.00	450.00	< 2 N.S.	10.13 34.12
b.Phosphate	1	2992.34	2992.34	7.0 N.S.	10.13 34.12
c.Interaction	1	119.37	119.37	< 1 N.S.	10.13 34.12
Y x T	3	1273.46	424.49	< 1 N.S.	2.92 4.51
Pooled error	30	90278.83	3009.29	-	-
Total	47				

** Significant at 1 % level

Table 20. Dry-matter yield^(9.) of wheat plants grown on plots green-manured with guar from a patch of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment	Y E A R S	Average	
	1966-67	1967-68	
P ₁ C ₁	446.0	449.0	447.5
P ₁ C ₀	431.0	368.0	399.5
P ₀ C ₁	425.0	350.6	387.8
P ₀ C ₀	425.0	349.0	387.0
S.E.m. for treatment	N.S.	20.5	
L.S.D. at 5 % for treatment	N.S.	61.6	
C.V. %	14.8	13.3	

Table 21. Pooled analysis of wheat yield preceded by guar green-manuring.

Source	d.f.	s.s.	M.S.S.	obs. F.	Table F.
					5% 1%
Years	1	33814.08	33814.08	10.2 **	4.17 7.56
Treatments	3	28166.50	9388.83	< 3 N.S.	9.28 29.46
a. Culture	1	6674.08	6674.08	< 2 N.S.	10.13 34.12
b. Phosphate	1	15051.08	15051.08	< 4 N.S.	10.13 34.12
c. Interaction	1	6441.34	6441.34	< 2 N.S.	10.13 34.12
Y x T	3	11587.62	3862.54	< 2 N.S.	2.92 4.51
Pooled error	30	99070.13	3302.34	-	- -
Total	47				

** Significant at 1 % level

Table 22. Wheat-grain yield (g) taken from sannhemp green-manured plots of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment	Y E A R S	Average	
	1966-67	1967-68	
P ₁ C ₁	109.0	180.0	144.5
P ₁ C ₀	116.0	176.0	147.0
P ₀ C ₁	121.0	172.0	146.5
P ₀ C ₀	106.0	156.0	132.5
S.Em. for treatment	N.S.	N.S.	
L.S.D. at 5 % for treatment	N.S.	N.S.	
C.V. %	10.8	21.8	

Table 23. Pooled analysis of wheat-grain yield taken from sannhemp green-manured plots.

Source	id.f.	s.s.	m.s.s.	Obs. F.	Table F.
					5% 11%
Years	1	42186.02	42186.02	**	4.17 7.56
Treatments	3	1667.23	555.74	< 3 N.S.	9.28 29.46
a.Culture	1	379.69	379.69	< 2 N.S.	10.13 34.12
b.Phosphate	1	462.60	462.60	< 2 N.S.	10.13 34.12
c.Interaction	1	824.94	824.94	< 4 N.S.	10.13 34.12
Y x T	3	732.73	244.24	< 1 N.S.	2.92 4.51
Pooled error	30	23882.29	796.08	-	-
Total	47				

** Significant at 1 % level

Table 24. Wheat-grain yield (g) taken from guwar green-manured plots of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment	Y E A R S	Average	
	1966-67	1967-68	
P ₁ C ₁	126.0	223.0	174.5
P ₁ C ₀	109.0	195.0	152.0
P ₀ C ₁	126.0	187.0	156.5
P ₀ C ₀	128.0	168.0	148.0
S.E.m. for treatment	N.S.	N.S.	
L.S.D. at 5 % for treatment	N.S.	N.S.	
C.V. %	10.9	18.9	

Table 25. Pooled analysis of wheat-grain yield taken from guwar green-manured plots.

Source	d.f.	s.s.	m.s.s.	obs. F.	Table F.
					5% : 1%
Years	1	90208.33	90208.33	**	4.17 7.56
Treatments	3	4754.83	1584.94	<1 N.S.	9.28 29.46
a.Culture	1	2821.33	2821.33	<2 N.S.	10.13 34.12
b.Phosphate	1	1386.75	1386.75	<1 N.S.	10.13 34.12
c.Interaction	1	546.75	546.75	<1 N.S.	10.13 34.12
Y x T	3	6057.84	2019.28	2.4 N.S.	2.92 4.51
Pooled error	30	25223.83	840.79	-	- -
Total	47				

** Significant at 1 % level

When the green-manuring was done with guwar, the grain yield of wheat (table 24) in the year 1966-67 showed very little differences amongst the treatments P_1C_1 , P_0C_1 and P_0C_0 . Lowest yield was obtained under P_1C_0 but the results were non-significant. In the ~~next year~~, the highest grain yield was recorded under P_1C_1 and lowest under P_0C_0 . P_1C_0 and P_0C_1 also yielded higher than that under the control but all these differences were non-significant. Combined analysis of yield data of two years (table 25) also did not indicate any significant treatment differences.

Total nitrogen content of wheat

Nitrogen content of wheat fodder from plots green-manured with sannhemp, presented in table 26, showed that it was highest under treatment P_0C_0 in the year 1966-67, while it varied narrowly amongst rest of the treatments. In the subsequent year, practically the same trend was maintained. In the case of wheat grain, the nitrogen content was highest under P_1C_1 and practically equal under rest of the treatments.

In the case of wheat following guwar green-manuring, the nitrogen content (table 27) was found to be highest both in grain and fodder under the treatment P_1C_0 and practically lowest in P_1C_1 and P_0C_1 .

Table 26. Total nitrogen per cent of wheat fodder and grain from sannhemp green-manured plots (average of 6 replications).

Treatment:	N % of wheat fodder		Average:	N % of wheat grains		Average:
	:1966-67:	1967-68:		:1966-67:	1967-68:	
P ₁ C ₁	0.53	0.42	0.48	2.60	2.51	2.56
P ₁ C ₀	0.58	0.38	0.48	2.52	2.27	2.39
P ₀ C ₁	0.48	0.39	0.44	2.54	2.26	2.40
P ₀ C ₀	0.65	0.47	0.56	2.58	2.19	2.39

Table 27. Total nitrogen per cent of wheat fodder and grains from guar green-manured plots (average of 6 replications).

Treatment:	N % of wheat fodder		Average:	N % of wheat grains		Average:
	:1966-67:	1967-68:		:1966-67:	1967-68:	
P ₁ C ₁	0.55	0.41	0.48	2.43	2.18	2.31
P ₁ C ₀	0.69	0.43	0.56	2.55	2.32	2.44
P ₀ C ₁	0.57	0.38	0.48	2.39	2.24	2.32
P ₀ C ₀	0.60	0.44	0.52	2.45	2.28	2.37

Total nitrogen uptake of wheat

The nitrogen percentage of the plant does not necessarily indicate the available nitrogen in the soil because of variations in the yield. A better measure of the availability would be the total nitrogen uptake which is determined by multiplying the nitrogen percentage by the total dry-matter produced, ^{and dividing by 100.} In the present case, the fodder was separated from grain and the nitrogen percentages of both were determined. They were multiplied by the respective dry weights and added together. In 1966-67, the total nitrogen uptakes by wheat under treatments P_1C_0 and P_0C_1 following sannhemp green-manuring crop (table 28) were practically equal and it was lowest under P_1C_1 , but these differences were non-significant. In the following year, highest uptake was observed under P_1C_1 , while lowest was recorded under P_0C_0 . Here also the differences were non-significant. On an average, highest uptake of nitrogen was found under P_1C_1 followed by P_1C_0 , P_0C_1 and P_0C_0 in that order. Thus, treating legume seeds with culture in presence of phosphate seemed to increase nitrogen uptake, but the combined analysis (table 29) did not show any significant effect on nitrogen uptake due to any of the treatments.

Table 28. Total uptake of nitrogen (g.) by wheat from sannhemp green-manured plots of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment	Y E A R S	Average	
	1966-67	1967-68	
P ₁ C ₁	4.43	5.26	4.85
P ₁ C ₀	4.79	4.58	4.69
P ₀ C ₁	4.74	4.41	4.58
P ₀ C ₀	4.56	4.32	4.44
S.Em. for treatment	N.S.	N.S.	
L.S.D. at 5 % for treatment	N.S.	N.S.	
C.V. %	35.0	21.3	

Table 29. Pooled analysis for total uptake of nitrogen by wheat from sannhemp green-manured plots.

Source	d.f.	s.s.	m.s.s.	obs. F.	Table F.
					5% 1%
Years	1	0.05	0.05	< 1 N.S.	4.17 7.56
Treatments	3	1.35	0.45	< 1 N.S.	9.28 29.46
a.Culture	1	0.09	0.09	< 1 N.S.	10.13 34.12
b.Phosphate	1	1.19	1.19	< 2 N.S.	10.13 34.12
c.Interaction	1	0.07	0.07	< 1 N.S.	10.13 34.12
Y x T	3	2.34	0.78	< 1 N.S.	2.92 4.51
Pooled error	30	53.41	1.78	-	- -
Total	47				

Table 30. Total uptake of nitrogen^(g.) by wheat from guar green-manured plots of 0.6 m. x 0.6 m. (average of 6 replications).

Treatment	Y E A R S	Average	
:	1966-67	1967-68	
P ₁ C ₁	4.81	5.45	5.13
P ₁ C ₀	4.92	5.24	5.08
P ₀ C ₁	4.71	4.78	4.75
P ₀ C ₀	4.92	4.59	4.76
S.E.m. for treatment	N.S.	N.S.	
L.S.D. at 5 % for treatment	N.S.	N.S.	
C.V. %	12.6	13.5	

Table 31. Pooled analysis for total uptake of nitrogen by wheat from guar green-manured plots.

Source	d.f.	s.s.	m.s.s.	obs. F.	Table F.
:	:	:	:	:	5% : 1%
Years	1	0.35	0.35	< 1	N.S. 4.17 7.56
Treatments	3	1.55	0.52	< 2	N.S. 9.28 29.46
a.Culture	1	0.01	0.01	< 1	N.S. 10.13 34.12
b.Phosphate	1	1.53	1.53	3.06	N.S. 10.13 34.12
c.Interaction	1	0.01	0.01	< 1	N.S. 10.13 34.12
Y x T	3	1.50	0.50	1.1	N.S. 2.92 4.51
Pooled error	30	12.53	0.42	-	- -
Total	47				

In the year 1966-67, total uptake of nitrogen by wheat following guar green-manuring was highest under treatments P_1C_0 and P_0C_0 (table 30), while it was least under P_0C_1 and intermediate in case of P_1C_1 . However, these differences were non-significant. In the subsequent year also, treatment differences were non-significant, but the uptake was highest under P_1C_1 and lowest under P_0C_0 . The uptake of nitrogen under different treatments can be arranged in the decreasing order as $P_1C_1 > P_1C_0 > P_0C_1 > P_0C_0$.

On an average, almost similar order was maintained with very little difference between the last two treatments viz. P_0C_1 and P_0C_0 . Combined analysis of the data for two years (table 31) did not bring out any significant difference due to any of the treatments.

Chapter V

General Discussion.

The present investigation was started in order to test the effectiveness of selected cultures of azotobacter and gynura in fixing nitrogen and improving growth of these leguminous green-manure crops. Various investigators have reported that inoculation with legume culture improves nitrogen fixation and also the yield of the crop. Thus Whiting (1935), Wilson and Leland (1939), Hofer (1943), Greaves and Jones (1950), Hayeka (1962), Obaton and Blacher (1963), Giddens (1964), Abel and Erdman (1964), Nowak and Netzsck - Lehner (1965), Hansen (1965), Bonnier and Lebrun (1965), Kick (1966) and Sen (1966), to mention only some of them, have shown that inoculation with legume culture is beneficial. The selected strains put under the test in the present investigation were S₁ of azotobacter and G_{279/1} of gynura. These were selected on the basis of nodule count, weight of nodules, location of nodules, colour of the nodules and green matter produced in pot experiments. (Patel, 1965).

When they were tested on the field, it was found that P₁C₁ treatment, involving the inoculation with the selected culture and application of phosphate, gave the highest yield

in the first year and the results were significant. The trials were carried out for three years more but it was found that in none of these three years the results were significant. Also the results were not significant in pooled analysis of the data of all the four years both in the case of sorghum and guar. Total nitrogen uptake by the plants was significant only for the first year, but they were not significant for the next three years. Also they were not significant when the pooled analysis of the data of all the four years was carried out. The plants were buried in the soil at flowering time and, after thorough decomposition had taken place, the nitrogen content of the well-mixed soil was determined. There were no substantial differences between the various treatments. Thinking that the small improvement in nitrogen fixation, which inoculation may bring about, may not be reflected in the total nitrogen content of the soil determined by the chemical method, it was decided to study the effect on the subsequent crop of wheat. In the first year, wheat was grown on the green-manured field by applying the recommended dose of 100 kg. N/ha. in the form of urea. It was found that there was practically no benefit due to inoculation. The nitrogen uptake was not higher in the P_1C_1 treatment and the results were not statistically significant.

The chief factors which influence nitrogen fixation by legumes are soil fertility, soil moisture, soil aeration,

temperature, soil pH, cultural factors, presence or absence in the soil of symbiotic nitrogen fixers of the specific group and inoculation with efficient strains of the specific legume culture. So far as fertility level is concerned, it is reported that if the nitrogen status of the soil is high, there will not be material symbiotic nitrogen fixation.

But the gorady soil, on which the present investigation was carried out, has poor nitrogen status and therefore, if the selected culture is efficient, a beneficial effect should be reflected in the total nitrogen uptake by the crop or total nitrogen content of the soil after decomposition of the buried green-manure crop. Even if it is not reflected in the total nitrogen content of the soil determined by the Kjeldahl method, the subsequent cereal crop must be benefited. In the first year, the wheat crop was not benefited presumably on account of the high dose (100 kg. N/ha.) of fertilizer nitrogen added. In the subsequent year, when a smaller dose of nitrogen (45 kg. N/ha.) was applied, P_1C_1 was highest so far as the yield and nitrogen uptake are concerned, but the results were statistically significant only in the case of yield. It appears that a further trial using a lower dose (45 kg. N/ha.) of nitrogen may bring out clear trend.

A legume culture requires liberal quantities of phosphate for adequate nitrogen fixation. Therefore, phosphate was included in the treatments. However, in none of the years, a significantly superior effect of fertilizer phosphorus was observed. This may be due to the fact that original available phosphate status of the soil was high. This fact becomes clear from the analytical figures given in table 1.

Molybdenum benefits nitrogen fixation by legumes. But in the present case it was not included in the treatments as molybdenum status of Goradu soil is fairly high (Reddy, 1958). Further field experiments carried out by Patel (1962) by applying molybdenum salt in a soil growing a legume crop have indicated no beneficial effect of molybdenum application.

Moisture content of the soil was kept optimum throughout. Goradu soil is well-aerated. The pH of the soil is near neutrality and improved recommended cultural practices were adopted. Hence these factors are not likely to affect adversely the nitrogen fixation in the present case. Sen (1966) stated that an effective rhizobium strain should only be used at places that have soil and climatic conditions similar to those of the original habitat. The

selected rhizobium strains of sorghum and guar used in the present investigation were isolated from plants growing in soil and climatic conditions similar to those prevailing at Anand.

The doubtful performance of the selected cultures in the field may be due to the fact that there are already almost equally effective nitrogen-fixing bacteria present in goradu soil or there are some other dominant antagonistic organisms which do not allow the selected cultures to manifest their optimum performance. But before such a generalization is made, it would be necessary to take further trial with wheat crop using a smaller dose of fertilizer nitrogen.

Chapter VI

Summary.

To test the efficiency of two selected cultures of sannhemp and guar, viz. S₁ and G_{279/1} respectively, the seeds of these legumes were inoculated and sown in goradu soil with and without phosphate for four seasons under field conditions. When plants reached the flowering stage, they were ploughed in and were allowed to decompose for eight weeks. Wheat crop was taken in the same plots for two years. The results achieved are summarized below :-

1. Green-matter yield of sannhemp and that of guar was maximum in the phosphate-culture (P₁C₁) treatment and minimum under the control (P₀C₀) without either culture or phosphate. The culture without phosphate (P₀C₁) and phosphate without culture (P₁C₀) were also better than P₀C₀.
2. The dry-matter yields of these crops were not significant except sannhemp grown in the first year in which P₁C₁ was superior. Pooled analysis of the data for four years did not show a significant trend.
3. There was no clear trend in the case of nitrogen contents of the two crops or soil nitrogen contents

after they were ploughed into the soil and allowed to decompose well in the soil.

4. The nitrogen uptake by the sannhemp crop under the different treatments showed a different trend in different years. Pooled analysis did not show significant results. The nitrogen uptake by the guwar crop under the P_1C_1 treatment was highest. Neither for individual years except the first nor for the pooled data of all the four years the results of both the crops were statistically significant.

5. The total dry matter of wheat plants grown in sannhemp green-manured plots to which 100 kg. N/ha. was applied as a fertilizer, was highest ^{in P_1C_0 .} In the next year, in which a smaller dose of fertilizer ^{nitrogen} (45 kg. N/ha.) was applied to wheat crop, P_1C_1 was highest. But in both the years the results were not significant. In the case of guwar, it was highest in P_1C_1 and lowest in P_0C_0 in both the years, but the results were ^{significant} only for the second year. The results for wheat grain yield showed that P_0C_1 , in the first year, and P_1C_1 , in the second year, were highest, but there were no significant differences.

6. In the first year the uptake of nitrogen by wheat under P_1C_1 in gannhemp was lowest and in the second year it was highest. On an average, it was highest under P_1C_1 followed by P_1C_0 , P_0C_1 and P_0C_0 in that order. In the case of guar, the trends for the two years were not similar but, the average of two years' data showed P_1C_1 to be highest and P_0C_0 lowest. These results were also not statistically significant.

7. In the second year, ^{in which} the applied fertilizer dose was low, the nitrogen uptake for P_1C_1 was highest and P_0C_0 lowest in both the green-manure crops but the results were not significant. A further trial with a lower dose of nitrogen is indicated to bring out the trends clearly.

Bibliography.

1. Annonymous. 1964-1968 "Biological nitrogen fixation under different conditions". Ann. Rep., Inst. of Agri., Anand (Gujarat).
2. _____ 1959-1962 "Biological nitrogen fixation under different conditions". Final Rep., Bapatla, (Andhra Pradesh).
3. _____ 1960-1965 "Biological nitrogen fixation under different conditions". Final Rep., Ludhiana (Punjab).
4. _____ 1960-1967 "Biological nitrogen fixation under different conditions". Ann. Rep., Poona (Maharashtra).
5. Abel, G.H., and Erdman, L.W. 1964 Response of Lee soybeans to to different strains of Rhizobium japonicum. Agron. J. 56 : 423-424.
6. Acharya, C.N. and Jha, J. 1954 Studies on the building up soil fertility by phosphatic fertilization of legumes. Influence of a legume rotation on the microbiological activity of the soil. Ind. J. Soil Sci. 2 (1) : 21-29.

7. Agarwal, J.P. 1956 Response of wheat yields to different green-manure crops. Allahabad Farmer. 30 (3) : 87-90.
8. Ambika Singh. 1963 A critical evaluation of green-manuring experiments on sugarcane in North India. Empire J. Expt. Agric. 31 : 205-212.
9. Anant Rao, N.K., Agarwal, J.P., Pawar, K.S., and Yadav, N.R. 1957 Phosphatic manuring of ~~sorghum~~ green-manure. Ind. J. Agron. 1 (4) : 215-221.
10. Apltauer, J. and Skopalikova, O. 1963 The effect of green-manuring on the microflora of soil. Vet. Ved. Inf. MZL VII; 36 : 764-767 (Read from Soils and Fert. 27 (1) : 68, 1964).
11. Biswas, T.D., Sharma, D.C., and Naskar, G.C. 1963 A preliminary study on the effect of phosphate fertilization of a legume on the water stable structure of a sandy loam soil. J. Ind. Soc. Soil Sci. 11 : 239-246.

12. Bonnier, C., and
Lebrun, F. 1965 Effect of the number of
rhizobia used for inoculation
of yield of lucerne. Suppl.
Annls. Inst. Pasteur, Paris
109 (3) : 95-102(F). (Read from
Soils and Fertl 29(2) : 177,
1966).
13. Brakel, J., and
Masil, P. 1965 Symbiotic fixation of nitrogen
by the bean plant (Phaseolus
vulgaris, L.). Inoculation
trials with Rhizobium
phaseoli. Bull. Inst. Agron.
Stn. Rech. Gembloux 33 : 3-25
(Read from Soils and Fert.
29 (2) : 176, 1966).
14. Boczek, E. 1964 The effect of green-manure
crops in the rotation on
yields of some vegetables.
Roczn. Nauk. 88A : 363-403.
(Read from Soils and Fert.
27 (6) : 516, 1964).
15. Pampus, E. D., and
Foultney, R. G. 1961 The problems of nitrogen
fixation by legumes in Kenya
in relation to soil nutrient

- status. Proc.eighth int. Grassl. Cong., Reading 1960, 244-247. (Read from Soils and Fert. 25 (1) : 42, 1962).
16. Chandnani, J.J. 1958 Studies on the value and economics of green-manuring. Ind.J.Agr. 2(4): 209-215.
17. _____, and Oberoi, S.R. 1956 Studies on the relative value of various legumes as a source of green-manure. Ind.J.Agron. 1 (2) : 95-105.
18. Chalam, G.V. 1961 "Green-manuring species suitable under flood and water-logged conditions and for different crop rotations". Paper read at the seminar on green-manuring, Bombay, Oct., 1961.
- * 19. Crowther, E.H., and Mann, H.H. 1933 Manurial value of green-manuring. J.Royal Agric.Soc. 94 : 128.
20. Datta, N.P., and Gurubasava Raj, H. 1953 Effect of boron and molybdenum fertilizer on berseem (Trifolium alexandrinum) Nature. 171 : 134-135.

24. Fuggles, and
Conchman, N.R. 1939 East African Agric. Jour.
5 : 208.
25. Giddens, J. 1964 Effect of adding molybdenum
compounds to soybean inoculant.
Agron. J. 56 : 362-363.
26. Greaves, J.E.,
and Jones, L.W. 1950 Influence of legumes on soil
nitrogen. Soil Sci. 69 :
71-76.
27. Gupta, K.G., and
Sen, S. 1966 Utilization of phosphates by
Rhizobium spp. in relation to
their efficiencies. Soil Sci.
Pl. Nutr. 12 (1) : 18-22.
I.A.R.I. (Read from Soils
and Fert. 29 (6) : 555, 1966).
28. Hansen, S.E. 1965 The effect of inoculation with
nodule bacteria and of nitrogen
fertilizer on the yield of
peas. Tidsskr. P. Av. 92 :
353-354 (Da.e) (Read from
Soils and Fert. 29 (3) :
274, 1966).
29. Hoffer, A.W. 1943 Effect of inoculation on
yield of canning peas in New
York State. Soil Sci. 56 :
117-126.

30. Hyeka, M. 1962 Effect of seed inoculation with lucerne field soil on the growth of lucerne (Medicago sativa) Exp. Anals. Dei. 7 : 99-103 (S.P.C. 1963). Read from Soils and Fert. 27 (1) : 46, 1964).
31. Jadoja, B.J., and Patel, J.R. 1958 Application of superphosphate to lucerne. Farmer. 9 (2) : 21.
32. Jha, H.N., and Ram, A. 1966 Effect of phosphate-fertilized green manures grown in association with maize on the yield of succeeding wheat. Proc. Bihar Acad. Agric. Sci. 14 : 17-23. (Read from Soils and Fert. 30 (2) : 180, 1967).
33. Joachim, A.W.H. 1931 A manual of green-manuring (Ceylon).
34. Kiek, H. 1966 Pot and field experiments for testing the effectiveness of legume inoculants. L and W Forsch 19 : 10-17. (Read from Soils and Fert. 29 (6) : 557, 1966).

35. Kliever, W.M., and Kennedy, W.K. 1960 Studies on response of legumes to molybdenum and lime fertilization on Mardin silt loam soil. Soil Sci. Am.Proc. 24 : 377-380.
36. Khan, A.R., and Mathur, B.P. 1957 Effect of burying sorghum as green manure on the yield of wheat, Part II. Ind. J. Agri. Sci. 27 : 171-176.
37. Krasil'nikov, N.A., and Melkumova, T.A. 1963 Variability of nodule bacteria inside nodules of legumes. Invest. Akad. Nauk. SSSR Ser. Bio. 28 : 693-706. (Read from Biol. Abst. 45 : 1967. 1964).
38. Krylova, N.B. 1963 Role of micro elements in nitrogen fixation. Izv. Akad. Nauk. Ser. biol. No. 5, 718-731. 1962. (Read from Soils and Fert. 26 , 1963).
39. Linta, L. 1963 The nitrogen-fixing capacity of bacteria in the nodules of pea and vetch and the dynamics of nitrogen accumulation in

plant physiological properties of rhizobium strains of high nitrogen-fixing capacity isolated from the nodules of pea and vetch. *Agrokem. Talajt.* 12 : 647-660. (Read from *Soils and Fert.* 27 : 402, 1964).

40. Lockhead, A.G.

1932

Legume inoculation with rhizobium strains. Dept. of Agri. Canada. Tech. Bull. 157 : 1-16.

41. Lyon, T.L., and
Buckman, H.O.

1943

The nature and properties of soils. Macmillan Co., New York.

42. Mann, H.H.

1958

"Field studies in green-manuring". *Empire J. Expt. Agri.* 26 : 274-282.

43. _____

1959

"Field studies in green-manuring". II. *Empire J. Expt. Agri.* 27 : 243-251.

44. Mehta, F.V.,
Parikh, N.M., and
Shah, C.C.

1956

Decomposition of organic matter in goradu soil of Gujarat. *B.A. Agri. Coll. Mag.*, 9 : 12-14.

45. Nirchandani, T.J., and Khan, A.R. 1952 "Green Manuring". I.C.A.R. Review No. 6.
46. Mitra, S.P., and Harishanker. 1955 "The relative effects of Trichinopoly rock phosphate and basic slag, when reinforced with organic matter like garrhemp and alfalfa, in retarding the nitrogen loss when nitrogenous fertilizers like ammoni sulphate and sodium nitrate are added to alkali soils". Proc. Natl. Acad. Sci., India, Sect. A, 24 : 665-669. (Read from Chem. Abst., 52 : 13167 (a) 1958).
47. Nowak, W., and Netzsch-Lehner, A. 1965 Re-isolation tests with nodule bacteria (Rhizobium spp.) from soil. Zentbl. Bakt. Parasitkde Abt. II. 119 : 570-578. (Read from Soils and Fert. 29 (3): 272, 1966).
48. Obaton, M., and Blachere, H. 1963 Observations on inoculation of legumes in France. Ann. Inst. Pasteur, 105 : 282-290 (F.E.). (Read from Soils and Fert. 27 (1) : 79, 1964).

49. Patel, I.R. 1965 Biological nitrogen fixation in goradu soils. M.Sc.(Agri.) thesis of Sardar Patel University.
50. Patel, N.A. 1962 Effect of P, Mo. B and green-manuring on yield and quality of alfalfa grown on goradu soil. M.Sc.(Agri.)thesis of Sardar Patel University.
51. Patel, S.P., Ghosh, A.B., and Sen, S. 1963 Effect of phosphate manuri of berseem on the fertility status of the Delhi Soil. Ind. J. Soil Sci. 2 : 225-38.
52. Panos, D.A. 1959 The growth of legumes in conjunction with phosphate fertilizer, as a means of increasing and stabilizing soil fertility and agricultural productivity in Greece. Empire J.Expt.Agri. 27 : 98-106.
53. Parr, C.H., and Bose, R.D. 1948 Phosphate manuring of legumes-II. Ind.Fng. 6 : 201-208.

54. Pillai, K.M. 1964 Organic manure as pre-requisite for increased application of chemical fertilizers. Fertilizer News. May : 11-20.
55. Rajgopalan, T. 1958 Studies on groundnut nodule organism. I. Isolation of strains and study of cultures. Ind.J.Agric.Sci. 8(3) : 331-348.
56. Rajgopalan, N., and Sadasivan, T.S. 1964 Some aspects of root nodulation in tropical legumes. Current Sci. 33 : 197-202.
57. Ratner, E.I. 1964 Molybdenum and problems of biological nitrogen in agriculture. Izv.Akad. Nauk Ser. Biol. 2 : 223-243 (R.E.). Read from Soils and Fert.27 (4) : 306, 1964).
58. Raychaudhuri, S.P., and Subbiah, R.V. 1954 Phosphate-manuring of legumes and its role in building of a soil fertility in India. Soils and Fert. 17 (9) : 180. No.440-907.
59. Reddy, G.R., and Mehta, R.V. 1958 Availability of molybdenum in Gujarat Soils. 'Vidya' J.Guj. Uni., 2 : 92-99.

60. Relwani, L.L., and
Ganguly, B.D. 1959 Effect of green-manuring in
conjunction with fertilizers
on paddy yields. Ind. J. Agri.
Sci. 29 (2) : 1-13.
61. Rewari, R.R., Sen,
A.N., and Sen, A. 1965 Nitrogen fixation by cluster
bean (Cyamopsis tetragonoloba, L.)
in relation to phosphate uptake
from soil. Ind. J. Agri. Sci.
35 (2) : 162.
62. Salazar, C.E. 1965 Factors affecting legume
nodulation in the tropics.
Turrialba, 15 : 252-253.
(Read from Soils and Fert.
29 (3) : 273, 1966).
63. Sankaran, A.,
Raju, P.V.L.N.,
and Kasiab, S. 1963 Influence of phosphate on
nitrogen fixation by rhizobium
spp. Ind. J. Micro. 3 (1) :
17-20.
64. Schaffhausen, R.V. 1963 Economical methods for using
the legume Dolichos lablab
for soil improvement food and
feed. Turrialba. 13 : 173-179.
(Read from Soils and Fert.
27 (3) : 243, 1964).

65. Sen, S., and
Bains, S.S. 1952 Roll of legumes and green-
manuring in mixed farming
holdings. Ind.J.Agric.Sci.
22 : 33-48.
66. _____, and
Sundara Rao, W.V.B. 1953 Phosphate fertilization of
legumes. I.C.A.R. Rev.Ser.3.
67. _____, Bains, S.S.,
and Mathur, B.P. 1962 Phosphate-manuring of legumes.
Ind.J.Soil Sci.10 (4) :
283-86.
68. _____ 1963 Roll of Hubam clover as a
green-manure crop in wheat-
maize rotation. J.Ind.Soc.
Soil Sci. 11 : 275-281.
69. _____ 1964 Phosphate fertilization of
legumes in building up soil
fertility. Proc.Symp.Fert.
Ind.Soils. 1962. Bull. Nat.
Inst.Sci.India. 26 : 330-335
(I.A.R.I, New Delhi). Read from
Soils and Fert. 29 (1) :
81, 1966).
70. Sen, A.N. 1966 "Inoculation of legumes of
influenced by soil and climatic
conditions". Ind.J.Agric.Sci.
36 (1) : 1-3.

71. Shah, C.C., and Mehta, B.V. 1955 "Colorimetric determination of soil organic matter". B.A. Agril.Coll. Mag. 8 (2) : 1954-55.
72. Shawarbi, M.Y., and R.Hamissa. 1964 The fertilizing value of some organic manures. J.Soil Sci. Uni. Arab. Republic. 4 : 141-150.
73. _____, and Ibrahim, M.E. 1964 Symbiotic nitrogen fixation by Vicia faba as affected by superphosphate application. J.Soil Sci. Un.Arab.Republic. 4 : 19-37.
74. Shinde, N.K., and Sen, S. 1958 Phosphate-manuring of legumes. Ind.J.Agron. 3 (2) : 89-99.
75. Shubin, M.M. 1966 Green manure in the Altai Region. Agrokhimiya. 5 : 64-66(R). (Read from Soils and Fert. 29 (5) : 475, 1966).
76. Singh, M.P., and Sinha, K.S. 1964 Studies in green-manuring of wheat in Bihar II. Optimum dose of green matter. Ind. J.Agron. 9 (2) : 138-44.

77. Srivastava, K.N., and Ram, A. 1962 Amount of green manure added by different green-manuring legumes under different fertilizer conditions. Proc. Bihar Acad. Agric. Sci. 99 : 101-117.
78. Todorova, B. 1966 Effect of dung and green manure on crop yield and development of some groups of micro-organisms. Pochv. Agrokhim. 1 (1) : 95-101. (read from Soils and Fert. 39 (5) : 450. 1966).
80. Venkata Rao, B.V., and K. Shanker. 1960 Influence of soil phosphorus status on the composition of sannhemp. J. Ind. Soc. Soil Sci. 8 : 129-132.
81. _____, and Govind Rajan, S.V. 1960 Influence of green manures on phosphate utilization by crops. J. Ind. Soc. Soil Sci. 8 : 145-149.
82. Vyas, N.D., and Desai, J.R. 1953 Effect of different doses of superphosphate on the fixation of atmospheric nitrogen through pea. Ind. J. Soil Sci. 1(1) : 32-40.

83. Wakeman, S.A. 1952 "Soil Microbiology". John Wiley and Sons., Inc. New York.
84. _____ 1929 Chemical and microbiological principles underlying the decomposition of green manures in the soil. *J. Am. Soc. Agron.* 21 : 1-18.
85. Whiting, A.L. 1925 The relation of inoculation on quality and yield of peas. *J. Am. Soc. Agron.* 17 : 474-87.
86. Wilson, J.K., and Leland, E.W. 1929 The value of supplementary bacteria for legumes. *J. Am. Soc. Agron.* 21 : 574-586.
87. Winkler, L.W. 1913 The volumetric determination of ammonia. *Budapest Z. angew. Chem.* 26 : 331-232. (Read from *Chem. Abst.* 7 : 2175, 1913).
88. Yadav, C.N. 1962 Effect of salinity on the growth and chemical composition of sannhemp grown on goradu soil. M.Sc. (Agri.) thesis of Sardar Patel University.
89. Yakovlevs, Z.M. 1960 The nitrogen-fixing ability of various strains of nodule bacteria. (Read from *Bio. Abst.* 37 (3): 1052, 1962).

* Original not referred.