RESPONSE OF GREENGRAM (*Vigna radiata* L.) TO COMBINED EFFECT OF VA-MYCORRHIZA AND RHIZOBIUM INOCULATION IN SOIL.

A THESIS

SUBMITTED TO

THE ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, BHUBANESWAR

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

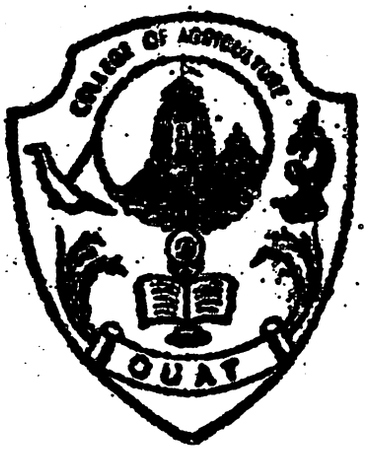
FOR THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

(AGRICULTURAL CHEMISTRY AND SOIL SCIENCE)

By

Padmanava Sahoo



Department of Agricultural Chemistry, Soil Science and Biochemistry

COLLEGE OF AGRICULTURE

Orissa University of Agriculture and Technology

BHUBANESWAR

1993

THESIS ADVISOR

Dr. P. K. DAS

Dedicated to
My
Beloved Parents

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CERTIFICATE

This is to certify that the thesis entitled, **"RESPONSE OF GREENGRAM (*Vigna radiata* L.) TO COMBINED EFFECT OF VA-MYCORRHIZA AND RHIZOBIUM INOCULATION IN SOIL"** submitted in partial fulfillment of the requirements for the Degree of **MASTER OF SCIENCE IN AGRICULTURE** (Agricultural Chemistry and Soil Science) of the Orissa University of Agriculture and Technology is a faithful record of bonafide research work carried out by **Sri Padmanava Sahoo** under my guidance and supervision during the academic session 1991-93. The results of the investigation reported in this thesis have not so far been presented for any other Degree or Diploma. The assistance and help received as well as sources of information availed during the course of investigation have been duly acknowledged.

Dated, the 7th Aug 1994

(DR. P.K.DAS)

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Padmanava Sahoo

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RESPONSE OF GREENGRAM (*Vigna radiata* L.) TO COMBINED EFFECT OF VA-MYCORRHIZA AND RHIZOBIUM INOCULATION IN SOIL.

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ABSTRACT

Response of greengram (*Vigna radiata* L.) to inoculation of VA-mycorrhiza (*Glomus fasciculatum*) and Rhizobium bacteria (M-14 strain) with P fertilization in light textured acid lateritic soil (Ustalf ochrepts) and their effects on the plant growth, dry matter yield, nutrient uptake, root volume, nodule formation, content studies of haemoglobin in nodules, chlorophyll in leaves and infection of roots of greengram plants were evaluated in both pot culture studies during the Rabi season, 1993-94 in two locations viz: Baghamari and Pubusahi in Khurda district. Treatments consisted of control (C), Mycorrhiza (M) Rhizobium (R) and R+M. Phosphate was applied in pots @ 20kg P ha^{-1} and no fertilizer was applied to field soils. Under field experiments, the growth attributes of greengram (cv. Nayagarh local) like shoot and root length, nodule number, number of pods per plant, dry weight of pods and N-P-uptake increased over uninoculated control as a result of dual inoculation of micro organisms. The grain yield increased by 50% at Baghamari Soil (6.31 Q ha^{-1}) of the same was about 3 times in R+M (10.40 Q ha^{-1}) of that due to uninoculated control (3.19 Q ha^{-1}) at Pubusahi location. In the pot culture experiments, the dual inoculation of VA-mycorrhiza and Rhizobium bacteria to greengram cv Sujata grown in acid lateritic soil ammended with lime and FYM increased the dry matter over uninoculated control (4.4 to 12.9 g pot^{-1}), root volume (2.6 to 22.8 cc per pot), the number nodules (6.65 to 45 per plant), N-uptake (0.0718 to 0.27 g N per pot), content of haemoglobin in nodule (1.227 to 3.008 mg g^{-1} fresh weight), Chlorophyll in leaves (0.826 to 1.254 mg g^{-1} fresh weight) during the period of seven weeks growth under study. The mycorrhizal inoculated green gram roots were found heavily infected as evidenced from the vesicles formed in the cortical cells.

CHAPTER-I

INTRODUCTION

INTRODUCTION

In the eighth plan document of India among other objectives, the priority has been laid down on growth and diversification of agriculture to achieve self sufficiency in food grain and generate surplus for exports. Now the level of food grain production has been reached 183 million tons due to introduction of advanced technology based on high yielding variety crop cultivation and emphasis on optimum and balanced use of inputs. Still 230 million people remain below the hunger line getting less than 1500 cal./day.

Although food production in Orissa is satisfactory to feed a population of about 34 million which may rise to 38 million by the turn of this century, it is hoped that 99.43 lakh tons of food grain can be produced by 2000 A.D. in the state. Yields per hectare and total production of rice in Orissa has been some what static since large area of upland is of low productivity. Therefore, the suggested approaches to achieve the goal of such a production level are:(I) increasing the net area of crops through intensification of cropping (II) increasing the productivity of dryland through moisture

conservation, (III) Optimum use of irrigation potential, (IV) increasing efficiency of inputs, (V) increasing productivity and stability of yields of pulses and oil seeds. Stress on the integrated nutrient management (INM) using chemical fertilizers organic manures, biofertilizers, etc. should be given to generate viable techniques for the sustainable agricultural system.

Sustaining agricultural productivity at reasonably high level to meet the requirements of changing time is difficult in any soil but it is particularly so in the acid lateritic soils (ustalfs-Ochrepts) which cover about 70% of the total geographical area of Orissa. These soils are characterised by undulating and folded topography which suffer from low fertility, acidic nature with low base saturation, especially in uplands, meagre irrigation facilities and high degree of erosion. Agricultural productivity in an area is the result of soil productivity, climatic and weather conditions and ecological environments. Soil productivity is not merely a result of soil fertility as determined by its nutrient status but is also governed by in no small measures by its organic matter content, physical conditions, biological ecosystem and management over the years.

The pattern of rainfall and its distribution in most parts of our state is quite uneven (Patra, 1988). It greatly

varies not only from year to year but also within the rainy season of the year. Even if the total precipitation may be adequate the eratic distribution of annual rainfall leads to frequent crop failure (Nanda & Patro, 1973). In most areas of the state monocrop of rice cultivation is practised under such rainfed situations except the areas having few irrigation facilities to go for multiple cropping. Crop production has been largely limited to the areas having irrigation facilities and thus there has been no break through in the productivity of rainfed crops though it occupies nearly 70% of the gross cropped area. Basing upon the importance & limitations of different soil properties for increasing productivity of crops under irrigated areas, attempt has also been made for suitable classification of soil (Mishra & Nanda, 1984). Taking advantage of the residual soil moisture the farmers of Orissa grow major pulse crops like green gram and black gram mostly during rabi reason. Out the total cultivated area of 6304 thousand hectares, the pulse growing area in the state was 1463.33 thousand hectares and 50% of it was used for greengram cultivation in an area of 735.28 thousand hectares during the year 1991-92. The achieved green gram production during the year was 376.23 thousand tons which seems insufficient to meet the requirement of the people of Orissa. Moreover, the pulse grain is the major source of protein for the poor people of the state & for which it is known as the "Poor man's meat".

The native source of soil organic matter becomes inadequate to meet the nitrogen demand of the high yielding variety of crops. Nitrogen as a major nutrient plays an important role in crop growth. It not only acts as a structural ingredient of chlorophyll and protein but also takes part functionally in most biochemical enzymatic reactions during plant growth.

It is a blessing of the nature that most of the legume crops derive atmospheric elemental form of N_2 through symbiotic association of host plant roots and the Rhizobium bacteria. The nodules which are formed on the roots of legume plants act as the site of nitrogen fixation by the help of the bacteriods and contribute nitrogen to the growing parts of the legume plants.

Legumes inoculated with Rhizobium have been found to increase in yield (Basu *et al* 1989, Subba Rao, 1982). Response of legume crops has been improved when such inoculated legume crops were supplemented with application of essential utrients like phosphorus (Samal, 1992) and micronutrient like molybdenum (Banasri et al : 1989, Samal, 1992). Dual inoculation of Rhizobium and VA-mycorrhiza especially in soils of low fertility status increased growth attributes and grain yield of legumes like greengram due to their synergistic effect in making symbiosis. (Adholeya *et al* :1988).

In Orissa, the growth of legume erops, especially in acid laleritic light textured soil becomes poor and productivity is low varying from as low as 2-4.9 Q/ha during rabi season. Very few advanced farmers now use Rhizobium as seed inoculant in legume cultivation. But dual inoculation of Rhizobium and VA-mycorrhiza remains in dream. Mycorrhizal inoculation may be highly beneficial for the legume corps grown in acid laleritic soil which fixes the soluble form of applied P to the extent of about 91% (Das *et al* 1993). Under this situation an attempt has been made through the present investigation to find out the effect of single and dual inoculation of Rhizobium & VA-mycorrhiza (*Glomus fasciculatum*) on the growth & yield of green gram (*Vigna radiata L.*) grown under both field situation & poteulture experiments.

Thus the experiments conducted under present investigation was based on the following objectives :

I) To compare the symbiotic efficiency of the inoculants viz. Rhizobium & VA-mycorrhiza towards the growth attributes & yield of green gram.

II) To observe the effect of dual inoculation of Rhizobium and VA-mycorrhiza on growth & yield of greengram grown in lateritic soil)

CHAPTER-II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The scientific investigation regarding value of legume plant in contributing to the nitrogen nutrition of plants and the role of legumes in enriching the fertility of soil opened the eyes of microbiologists only after the isolation for the first time of the symbiotic bacteria from the root nodule of legume by Beijerinck (1888) which he named as *Bacillus radicicola* Subsequently it was changed to *Rhizobium leguminosarum* (Frank). A number of scientific reports based on findings of the research work about biological nitrogen fixation (BNF) done by several scientists working in India and abroad through last one century have ben compiled (Dadarwal *et al.* 1987). The successful symbioses between legume and Rhizobium can be improved when VA-mycorrhiza is associated with crop root system. Mycorrhizal foundation research was done a century ago by Frank (1885). A number of scientific reports based on mycorrhizal research in the field of agriculture and allied fields have been enumerated. (Mahadevan *et al* 1988).

ECOLOGICAL DISTRIBUTION

The beneficial effect of VAM on plant growth has been documented (Mosse, 1973) and it has been attributed to increased P-uptake. The population of VA-endophytes varies greatly in size and species composition (Mosse, 1973) and are affected by a number of factors such as fertility level, soil type, light and temperature (Hayman, 1975, Kruckelmann, 1975).

The dependence of leguminous plants on mycorrhizal fungi for optimum growth in a given environment is influenced by three major factors: Plant species, the endogonaceous fungal species and also availability of soil P. Since it has been demonstrated that species & strains of fungi differ in their ability to stimulate plant growth (Abbot & Robson, 1978), The proper selection of efficient VAM fungi for a crop and soil type is therefore of prime importance towards establishing a successful application of mycorrhizal research to crop productivity (Rhodes, 1984).

Gerdemann (1968), reported the occurrence of VA-mycorrhizal associations widely throughout the plant kingdom. They have been reported to be present in Bryophytes, Pteridophytes, Gymnosperms and Angiosperms in nearly all the geographical regions of the world.

Khan (1974) reported that a limited number of plant species especially belonging to hydrophtes, halophytes & xerophytes were regarded as non-mycorrhizal. In few families, viz, crucifeae, chenopodiaceae, caryophyllaceae, cyperaceae, VAM infection is rare or absent.

Isabelle Louis & G.Lim (1988) reported the effect of storage of the inoculum (mycorrhizal & roots in soil) as the prerequisite treatment for increasing axenic spore germination taking a tropical isolate of *Glomus clarum*. Prolonged dry storage of the inoculum, for upto 6 months at 25-30°C increased the percentage of sporegermination significantly on water agar. If following dry storage, the extracted spores were then stored at 40°C for atleast 2 weeks, germination was further enhanced. Incubation temperature of 25-35°C and medium p^H of 5-8 were most favourable for germination.

Mackee (1961) reported that the growth of survival of seedlings & nodulation were satisfactory on soil p^H of above 6.2 But in soils of lower p^H adequacy of nodulation declined. Jones & Thomas (1966) found that *Trifolium repens* seeds inoculated with effective strains of *Rhizobium trifolii* with lime pellets & sown in mountain conditions gave significantly better establishment than uninoculated control.

Paulin (1989) found that the absence of lime growth & nodulation of pigeonpea were drastically reduced, mainly due to aluminium toxicity in acid soils. There was a remarkable response to lime application in form of increased plant growth, nodulation & uptake of P & Ca. Response to P application was more efficient in the presence of lime.

Peter *et al* (1990) observed that cowpea rhizobia isolated from hot, dry areas were more tolerant to temperature and desication than strains isolated from cooler regions having high humidity.

Parrek *et al* (1990) conducted pot experiment taking sandyloam soil at hot humid Tarai regions, at four temperatures, (ambient temperature, 21±2°C, 27±2°C and 32 ± 2°C maintained in the thermostatically controlled water bath) observed that the temperature at 21 ±2°C was the most congenial for nodulation, while the highest temperature (33±2°C) didn't allow nodulation and significantly reduced the plant dry matter weight.

Narendra Kumar *et al* (1986) reported that the survival & the subsequent growth of the inoculated Rhizobium is greatly affected by soil moisture. Maximum cells died at 10% moisture and survivors also couldn't multiply As the soil moisture was increased (up to 50%) both the strains (G4 and

TAL 620) of chickpea favourably responded and mortality on second day reduced considerably. Maximum survival of G4 and TAL 620 was obtained at 30 and 50 % water holding capacity of the soil, respectively. However, their original cell population (107 cell/ seed) couldn't be attained. Pareek *et al* (1990) reported that increasing soil moisture from 20 to 50% of W.H.C. increased nodule number and dry weight significantly.

Sen & Bains (1955) observed by taking a field trial conducted with 16, 32 & 64 lbs per acre of $P_2 O_5$ as FYM alone, superphosphate alone or both together that all the treatments increased the yield of berseem fodder and seed significantly over control. However, the phosphatic fertilizer alone or in combination with-FYM was superior to FYM alone. The similar trend was observed for nodulation & available P was more with added phosphate fertilizer.

Iswaran *et al.* (1970) reported that lime pelleting of soybean seeds followed by inoculation found to increase nodulation and grain yield of soybean under both acidic as well as saline soil conditions (PH 4.9 and 8.1 respectively). soil conditions (P^H 4.9 and 8.1 respectively).

Thompson (1988) reported the symbiotic, ecological & cultural characteristics for strain selection of Rhizobium for legume inoculation.

CHARACTERISTICS AND TAXONOMY OF RHIZOBIUM AND VAM FUNGI.

Morton & Benny (1990) have classified the VAM-fungi as follows.

Order	Sub-Order	Family	Genus
1.Endogonales		Endogonaceae	Endogone.
2.Glomales	(i)Glominae	(i)Glomaceae	Glomus Sclerocystis
		(ii) Acaulosporaceae	Entrophospora Acaulospora
	(ii)Gigasporinae	(I)Gigasporaceae	Gigaspora. Scutellispora

Thaxter Sensu Gerd. has identified the characters of *Glomus fasciculatum* as mentioned below:

SPORE:

Sporeshape - Globose, often mixed with subglobose, ovate, ellipsoid or irregular.

Longest dimension at maturity-50-75/µm or 75-100 /µm.

Hyphal envelope enclosing individual spores-"Absent".

Surface ornamentation at maturity- (all smooth in young). Smooth to dull rounded.

Distinct wall layers - Single or double (outer one is thicker than inner).

Wall colour in cross section at maturity with transmitted tungsten illumination (all hyaline in young). All layers yellow, or yellowish brown to brown.

Composite wall thickness at maturity 5-8µm or 9-12µm or 13-20 µm

Contents of intact, healthy, mature spores white to hyaline globules or granules.

SUBTENDING HYPHAE:

Number-single.

Shape-cylindrical or flared towards the point of attachment.

Diameter at point of attachment-
5-8µm or 13-20 µm.

Wall colour at maturity-(all hyaline in young)
yellow or brown.

Wall thickness at maturity-(all thin in young)
2-4 µm or 5-8 µm.

Alignment with spore axis-Straight

Closure at spore wall-Occlusion by spore wall thickening or plug.

SPOROCARPS

Existence- Present/absent.

Shape - Round or lobed or convoluted or irregular aggregation.

Largest dimension-Less than 1 mm or 1-10 mm

Surface texture & configuration-Exposed spores.

Surface colour in sun or strong tungsten light-Brown.

Interior colour at maturity in Sun or strong tungsten light-Brown.

Gross morphology of interior-Undifferentiated

spore arrangement-Random.

Number of spores in sporocarps-6-10 or >10.

Peridium-Absent.

A.B. Frank (1885), the German Botanist was the first man to coin the term "Mycorrhiza" for designating a symbiotic relationship between fungi and plant roots

Vesicular arbuscular mycorrhiza (VAM) is characterised by the presence of two specialized structures: arbuscules and vesicles. Arbuscules are produced by the internal mycelium intracellularly in the form of highly ramified minute arborescences within a few days of infection (Rich & Bird, 1974) as hyphae penetrate mechanically and enzymatically into cortical cells (Kinden and Brown, 1975 C). As the arbuscule hyphae bifurcate repeatedly (Cox and Sanders, 1974), they are enveloped by a host-derived encasement layer (Scannerivi and Bonfante-Fasolo, 1979) and the continuously invaginating host plasmalemma (Cox and Sanders, 1974; Kinden and Brown, 1975 a,b, Dexheimer *et al.* 1979) and ensure an extremely wide contact between the two symbionts, but are short lived and digested by host, few days after there formation. Vesicles are also produced by the internal mycellium but mostly

intercellularly. They are regarded as structures of storage and are absent in certain forms.

Dangeard (1900) was the first to name a vesicular-arbuscular fungus.

Peyronel (1924) was the first to recognise the VAM fungi as endogone species.

Thaxter (1922) was the first to write a monograph of the family Endogonaceae where he described all the species known at that time.

Mosse (1956) was the first to demonstrate experimentally that Endogone species as inoculum, she was able to produce typical VA-mycorrhiza.

The importance of VA-mycorrhizae as a tool for improving the growth and productivity in diverse groups of plants was recognized only after the work of Gerdemann (1968), Baylis (1972) and Mosse (1972).

Gianinazzi *et al*, 1982; Harley and Smith, 1983, Gianinazzi-Pearson and Gianinazzi 1986, Graham, 1988) a lot of information has been gathered about the taxonomy, ecology, physciology and anatomy of VAM fungi and their relations with

their hosts especially with reference to uptake of water, phosphorus and other nutrients, hormone production and root diseases.

Abbott and Robson (1979) & Abbott (1982) studied the characters of endophytes in the roots, histochemistry chemotaxonomy, taxonomy and cytology of the VAM roots & reaction of the host to different strains of the same species of VAM fungi.

Beiierinck in Holland was the first to isolate and cultivate a microorganism from the nodules of legumes in 1888. He named it *Bacillus radicicola* which is now placed in Bergey's Manual of Determinative Bacteriology under the genus *Rhizobium*. The genus *Rhizobium* has been placed in Bergey's Manual of Determinative Bacteriology in such diverse families as Azotobacteriaceae, Mycobacterejaceae, Myxobacteriaceae and Pseudomonadaeae. Specification of Rhizobium based on the Linnaean concept has proved difficult and therefore, the cross-inoculation grouping is based on the classification studies of Fred, Baldwin and Mc Coy is being generally followed. The Principle of cross inoculation grouping is based on the ability of an isolate of Rhizobium to form nodules in a limited genera of species of legumes related to one another. All rhizobia that could form nodules on roots of certain legume types have been collectively taken as a species. This system of classification has provided a workable basis for the

agricultural at practice of legume inoculation. Under this scheme, seven species are generally recognized (Table-)

Rhizobium Spp	Cross-inoculation grouping	Legume types.
R.lequminosarum.	Pea group	Pisum, Vicia, Lens
R. phaseoli.	Bean group	Phaseolus.
R. trifolii	Clover group.	Trifolium.
R. meliloti.	Alfalfa group	Melilotus,Medicago, Trigonella.
R. lupini.	Lupini group.	Lupinus. Orinthopus.
R. japonicum.	Soybean group	Glycine.
R. Sp.	Cowpea group.	Vigna, Arachis.

As per the 8th edition of Bergey's manual the bacteria are divided into 20 parts (19+1). The taxonomical position of Rhizobium is enumerated as follows.

PART-7 GRAM-NEGATIVE AEROBIC RODS AND COCCI

Family-III Rhizobiaceae
Genus-I Rhizobium.
Genus-II Agrobacterium.

ROOT COLONISATION OF ORGANISMS

Manjunath & Bagyaraj (1981) had conducted a time course experiment to study the intensity of root infection & the response of onion. They reported that the mycorrhizal infection with internal hyphae and arbuscules was observed in onion roots 15 days after sowing and infection % progressively increased upto 35 days. Plants inoculated with the mycorrhizal fungus weighed less than non-mycorrhizal plants during initial stages upto 35 days but grew faster later after 38 days.

Umadevi & Sitaramaiah (1991) reported that blackgram plants responded well to the inoculation with four species of endomycorrhizal fungi they have chosen. Plants grown in field soil inoculated with any one of the above species of endomycorrhizal fungi gave increased mycorrhizal root colonisation, had more root volume, fresh & dry shoot & root weights. However, the rhizosphere microbial population was reduced in inoculated plants.

Abbott, *et al.* (1984) reported the formation of hyphae in soil by the VA-mycorrhizal fungus (*Glomus fasciculatum*) with the supplied P varied from severely deficient to adequate for the growth of subterranean clover. After 6 weeks, the alleviation of severe phosphorus deficiency increased both the length of infected root & the length of external hyphae per centimetre of infected root. Further addition of P decreased both of these measurements. However, the level of added P at which the most external hyphae was formed per centimeter of infected root was higher than the level of P which gave the greatest length of infected root. The increase in P supply which gave the greatest increase in the length of external hyphae per centimetre of infected root also decreased the formation of vesicles within the infected roots. At adequate phosphate levels, there was little development of either external hyphae in soil or vesicles within the mycorrhizal roots.

Wilson (1984) reported the effect of inoculum density on the development of infection of *Gigaspora decipiens* (Hall & Abbott), *Glomus fasciculatum* (Thaxter) & *Glomus tenue* (Hall) Among these, *Gigaspora decipiens* was the least and *Glomus fasciculatum* was the most effective *Glomus fasciculatum* produced longer infection seqments and spread within the root more extensively than the other funqi.

Tiwari & Shukla (1991) reported the influence of pesticides on the colonization & population of chlamydospores of *Glomus fasciculatum,* N, P & K content, plant height, branching number of rhizobium nodules, number of pods plant dry weight & seed weight of soybean "Carbendazim" had no inhibitory effect on any of the components of the study. Chlorothalonil didn't inhibit the colonization but showed more inhibitory effect than carbendazim on all the components. All other fungicides, insecticides & herbicides were proved to be inhibitory against all aspects under study. Oxadiazon without exception, drastically inhibited all components of yield by influencing the *Glomus fasciculatum.*

Daniels & Trappe (1980) reported that the soil moisture, temperature & to a lesser degree pH influenced germination of *Glomus epigaeus* spores whereas levels of soil fertility & spore density had little or no effect. Maximum germination occurred in autoclaved forest dune & agricultural

soils or γ-irradiated or steamed chehalis silt loam however, 65-80% germination occurred in most non sterile soils & in both autoclaved & nonsterile chehalis soil containing nonmycorrhizal, ectomycorrhizal or VA-mycorrhizal hosts. Even two other VAM fungi, *Glomus mosseae* & *Gigaaspora gigantea* germinated readily in nonsterile soil as well. Optimum germination of spores seems closely related to conditions that are optimum for growth of many hostplants.

Hayman (1982) reported that the highly fertile soils generally showed less VAM fugal populations & also their population was decreased due to the pesticide treatment.

Cowie *et al* .(1990) reported that increased nitrate concentration reduced nodule number and nodule weight in legume crops. Inhibition of nodulation by increased NO_3^- concentration was greatest in peas, and least in *Lupinus angustifolius*.

Schomberg *et al* (1990) reported that supply of mineral nitrogen to inoculated plants increased dry matter production and N_2 fixation. This mineral N supply to seedlings greately enhanced seedlings vigour, but the amout of N_2 fixation declined at higher N levels. Buttery *et al* (1990) observed in a pot trial that nitrate depressed the growth & N accumulation of both peas & fababeans.

Basu *et al* (1989). observed in a trial with *vigna radiata L.* (Moong) cv-B1, seed inoculation with Rhizobium strains ICA-1 and M-10 increased nodulation & shootdry weight and gave seed yields of 0.92 & 0.87 t/ha respectively compared with 0.80 t/ha without inoculation. Applying 20, 30 and 40 Kg N ha^{-1} gave yields of 0.91, 0.98 and 0.90 t ha^{-1} respectively compared with 0.79 t ha^{-1} without N. Application of N upto 20 kg ha^{-1} increased number of nodules & dry weight of nodules/plant but shoot dry weight increased with application of N upto 40 kg/ha.

Beeker and Gerdemann (1977) developed a colorimetric method to measure the yellow pigmentation of mycorrhizal roots and Hepper (1976) used a colorimetric assay to measure the conversion of fungal chitin to glucosamine.

The percentage colonization by mycorrhizal fungi has been estimated by other researchers, using the presence or absence of fungal structures in roots, in 1 cm segments (Nicholson, 1955; Read, Koucheki and Hodgson, 1976) 150 µm microscope fields (Furlan and Fortin, 1973), 1mm grid sections (Davis, Menge and Erwin, 1979), root length increments (Sutton, 1973). and grid intersection points (Amber and young 1977). Methods of measurement have also used estimates of the proportional colonization of root segments (Hayman, 1970) and entire root segments (Ames & Linderman 1977).

Techniques used to measure colonization by VA-funqi have recently been summarized by Giovannetti & Mosse (1980).

Toth and Toth (1982) employed a technique to quantify the VAM on the basic principle of morphometric cytology on squashed roots offered as a compromise. By employing a symmetrical grid of dots over the image of root squash, counts of invaded cortical cells can be made. This technique is faster to apply the sectioning and yields quantitative information concerning the percentage of the cortical cells containing arbuscules.

RESPONSE OF GREENGRAM TO INOCULATION

Callow *et* al., (1978) reported that mycorrhiza can improve the P nutrition of a host particularly in low fertility due to exploration of the soil by the external hyphae beyond the root hairs of phosphorus depletion zone. The absorbed phosphorus probably converted into polyphosphate granules in the external hyphae and passed to the arbuscules for transfer to the host (White and Brown, 1979).

Gianinazzi *et al.* (1979) reported that the flow of phosphorus occurs in the presence of acid phosphatases during the arbuscule life span (Cox and Tinker, 1976)

Cooper and Tinker (1978) reported that the increased uptake of phosphorus was not only the effect of VAM fungi on the plant growth but they also stimulate plant uptake of Zn, Cu, S,K & Ca although not markedly as phosphorus.

Powell (1979) reported that mycorrhizal fungi had been shown to mobilise organic and inorganic P-sources in soil which are normally unavailable to non-mycorrhizal plants.

Crush,(1974) Smith and Daft, (1977); Carling *et al*, (1978) reported that the increased uptake of phosphorus was not only the effect of VAM fungi on plant growth but also the mycorrhizal inoculation enhance nodulation in legumes.

Carling *et al*., (1978) reported that the mycorrhizal nodulated plants exhibited higher level of nitrogenase and nitrate reductase as compared to non-mycorrhizal plants. This increase in the nitrate reductase system appears to have a role in increasing the symbiotic effectiveness of VAM.

Powell and Daniel (1978) reported that the VAM fungi have been shown to double the efficiency of P fertilisers and help the utilisation of traditional sources of P fertilizers like bonemeal and rock phosphate more efficiently.

Hulton (1970) reported that a combination of legumes rhizobia and mycorrhizal fungus brought significant improvement in the plant growth through increased availability of phosphorus together with higher nitrogen fixation in the soil. Thus, this combination may prove the cheapest way to enrich tropical soil with nitrogen

Ross and Harper (1970), Schenck and Henson (1973), Crush (1974), Sanni (1976), Islam *et al.* (1980); Barea *et al* (1980) Kehri & Chandra (1991a) reported that the tripartite association Viz: Legume-mycorrhizal fungi and nitrogen fixing bacteria has been shown to be effective in increasing the growth, nodulation and yield in legumes.

Heap & New man (1980) conducted six experiments out of which in five experiments, soil sterilization was made & reinoculation was used to obtain two treatments, one having infected with VAM & the other non-mycorrhizal. The shoots of some plants in each pot were injected P^{32} & after a week the shoots were removed and the reaming plants were harvested after a further week & P^{32} content was determined in all experiments & species combination there was some P^{32} transfer to the shoots of both mycorrhizal & non mycorrhizal treatment. The amount of transfer in mycorrhizal treatments was 2-8 folds greater than the nonmycorrhizal ones except in one experiment where mycorrhizal infection was very low.

Levy & Krikun (1980) studied the recovery from water stress on similarly sized VA-mycorrhizal & nonmycorrhizal rough lemon seedlings (*Citrus jambhiri*). VAM affected the stomatal conductance, photosynthesis and proline accumulation but not leaf water potential, suggesting that most of the effect of the mycorrhizal association is on stomatal regulation than on root resistance.

Rachel, *et al.* (1993) reported the influence of application of superphosphate on different VAM fungi in sunflower. *Glomus constrictum* inoculated plants responded equivalent to 50 kg/ha superphosphate fertilized soil while *G.mosseae* inoculation was equivalent to 40 kg /ha. The response was more in *A. morronea* as it was equivalent to 70 kg/ha Mixed inoculation didn't make any positive difference over the individual inoculations. Biomass of both VAM inoculated & P ammended soils has increased.

Islam *et al.* (1980) reported that the growth attributes of the legume, cowpea (*Vigna unguiculata*) was significantly increased when a species of Rhizobium and a VAM fungus were inoculated. This additive effect was demonstrated to a greater degree in P deficient soils as shown in field experiments with cowpea.

Sharma, *et al.* (1986) reported that two legumes viz: pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L). and wheat (*Triticum aestivum* L.) were grown in rabi season, 1977-78 and 1978-79 and two legumes Viz. greengram (*Vigna radiata* L.) & blackgram (*Vigna mungo*) and sorghum (*Sorghum bicolor L*). were grown in Kharif, 1978-79. Total available N content of the soil increased after rabi & kharif legumes as compared to those under their respective cereal crops & follow. The lowest values were recorded in follow soils. The organic carbon content of the soil increased slightly due to cultivation of leguminous crops as compared to the soils under cereals & fallow. There was not much total p content of soil as affected by different crops. The soils under legumes showed more available P as compared to those under cereals which might be ascribed to the development of P solubilizing organisms. Soil under legume harboured higher microbial load except, *Azotobacter* as compared to those under cereals or follow.

Menge (1983) reported the commercial exploitation of VAM fungi in the field of agriculture.

Thiagaranjan & Ahmad (1993) suggested that the competitiveness of rhizobia can be enhanced by co-inoculating with a selected strain of a VAM-fungus taking cowpea in nonsterile soil.

Bagyaraj & Manjunath (1980) studied that the inoculation of three crop plants i.e. cotton, cowpea & finger millet to inoculation with the VA-mycorrhizal fungus *Glomus fasciculatum* on an unsterile Indian soil with low available P significantly increased their root & shoot weights. The P & Zn content of inoculated plants were higher than non-inoculated ones but the Mn content was not significantly different

Kuccy & S. Diab (1984) reported the effects of inoculation with VAM fungi on alfalfa (*Medicago sativa* L.) growth & 'P' uptake in a moderately acidic loess soil with four levels of added lime. Inoculation of alfalfa with VAM fungi from soils of pH 7.2 to 7.5 resulted in increased dry matter production at pH of 6.4-7.5 but not at pH of 5.4-6.1. Addition of lime and/ or P always increased growth of alfalfa, Root colonisation by indigenous mycorrhizal fungi was inhibited at the highest pH levels but root colonisation by added fungi was stimulated at higher pH levels and inhibited at lower pH.

Ojala & Jarrell (1980) reported that the tomato plants were inoculated with *Glomus fasciculatum* mycorrhizal fungi while growing in sand through which recycled nutrient solution was automatically passed several times daily,

concentration of P & N in the solution were maintained at relatively, low levels. Root of inoculated plants became highly infected with mycorrhizal fungi and yield parameters were significantly increased with inoculation over uninoculated control plants.

Agnihothrudu & Tripathi (1976) conducted experiment with Rhizobium inoculation at farmers field with bengalgram (6 locations) & ground nut (13 locations) in Andhra Pradesh, Tamil Nadu & Karnataka in 1974-75. The yield increase varied from 10-57 % and the cost benefit ratio observed, varied from 1:9 to 1:39.

Singh (1977) reported the effect of Rhizobium inoculation on nodulation and yield of moong (*Vigna radiata* L.). A small amount of N&P promoted nodulation on inoculated plants. However, inoculated unsterilised plants nodulated profusely during the normal period of branching and flower primordial initiation resulting into highest number of pods. Inoculated crop gave 51 % higher yield than uninoculated. Yield gain of this magnitude couldn't be obtained either from 20 kg ha^{-1} of N or 30 kg ha^{-1} of P_2O_5 or with both.

Patil & Shinde (1980) reported the nodulation pattern & response to rhizobia inoculation in 6 varieties of gram during 1977-78 & revealed that var. Phule G-1 produced maximum number of nodules over all varieties. The differential response to inoculation was reflected in the grain yield & nodulation. The beneficial effect of rhizobium inoculation with regard to the shoot, root and nodule dry weight was clearly observed.

CHAPTER-III

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation included both pot culture experiment and field trials to assess the response of green gram (*Vigna radiata* L.) to the dual inoculation of Rhizobium bacteria and VA-mycorrhizal fungi. Laboratory techniques were used for testing the mycorrhizal spore count in soil and the root infection length percentage with observations of vesicles and arbuscules formed in the infected root cortex of the legumes.

Collection and multiplication of VA-mycorrhiza

Starter culture of vesicular arbuscular mycorrhiza (M) was collected in polythene packet of 1kg in soil-hostroot base carrier from the Dept of Microbiology, University of Agricultural Sciences, GKVK, Bangalore during the month of August-1993. The packet contained chlamydospores of endomycorrhiza (*Glomus fasciculatum*). Being obligative endomycorrhiza, their number was multiplied in soil with association of hostcrop i.e. guinea grass (*Panicum maximum*) grown in earthen pots. Twelve number of pots used for multiplication were washed, air dried and fume-sterilized with 40% formaldehyde. The holes at the bottom of the pots were

kept open for easy drainage. Each pot contained 9kg of air dried sterilized low fertile soil and sand mixture in the proportion of 1:1, the culture packet was opened and about 80 grams of the mycorrhizal soil was spread below 2cms depth & then covered by the soil in each pot. No fertilizer was applied to any of such pot. The seeds of guinea grass about 5-10 in number were sown in each pot on 20.8.93. The grass plants after 7-10 days of germination were thinned to keep 4-5 plants in each pot. Sterile water was given periodically to maintain moisture level approximately at 60-70% of W.H.C. of soil and the grasses were allowed to grow in the pots. The grass plants at 60 days of growth, in their post flowering stage were harvested on 2.10.93. The above-ground plant portions were removed and the under ground portion of soil and root materials were collected. They were mixed, ground, sieved in 2mm sieve and finally the roots collected on the sieve were cut into small pieces and mixed with sieved soil samples. Thus the soil based mycorrhizal cultures were prepared in poly packets containing 500gm weight in each. These were preserved to be used further in field as well as in the potculture experiments. The presence of the spores in the collected cultures were examined in the laboratory and their number per unit gram of soil was counted using the wet-sieving and decanting technique as out lined by Gerdemann and Nicolson (1963).

Collection of Rhizobium bacteria :

Starter culture of *Rhizobium* sp. (Strain M-14) in YEMA broth medium was collected in test tube from the Regional Biofertilizer Development Centre (R.B.D.C.), Sahidnagar, Bhubaneswar and was preserved in refrigerator at low temperature to be used further in the pot culture experiment. But the *Rhizobium* sp. culture (R) (Strain M-14) in lignite base prepared in polythene packets containing 200 gms sample were collected from the Hindustan Biofertizers Ltd., Pubusahi, Khurda and was preserved to be used in field experiment. The number of bacteria per unit gram of carrier material was examined in the laboratory by following the procedure of microbial plate count by dilution technique (Allen, 1959).

Potculture Experiment - I :

The first potculture experiment was conducted inside the wire-net house of the Deptt. of Agriculture Chemistry, Soil Science & Biochemistry to study the response of test crop green gram CV- Nayagarh Local to dual inoculation of Rhizobium bacteria and VA- mycorrhizal fungi. This experiment was conducted in earthen pots of 8" diameter containing six kg mixture of laterite soil and sand prepared in the proportion of 3:1. The soil sample (Ustalf) was collected from the upland paddy field of village Pubusahi, situated at six kilometre away from khurda town.

Collection and processing of soil sample

About 300 kgs of surface soil (0-15 cm depth) was collected and immediately the bulk of the soil was air dried under shade, the root fragments and pebbles were removed, clods were broken to pass through 2 mm sieve. Approximately 1kg of initial soil sample was kept separately for analysis in the laboratory for physico-chemical properties and the rest was sterilized in an autoclave for 30 minutes at a pressure of 15 Psi. About 100 kgs of sand collected in gunny bag was sterilized in the same manner and was mixed thoroughly with the sterilized soil in desired proportion as mentioned above. Then it was ready to be used in the first potculture experiment.

The earthen pots were thoroughly cleared in water, dried and fume sterilized under polythene sheet cover using 40% formaldehyde. The holes at the bottom of the pots were kept opened for easy drainage but pebbles were kept over the holes to retain the soil without causing loss during the time of watering the crop. The pots were numbered to indicate the treatments and were arranged in five rows (each row corresponding to one replication) inside the wire-net house. The experiment consisted of 8 treatments in one replication and the treatments were arranged randomly within the replication. Thus there were altogether 40 number of pots and the experiment was conducted in randomised block design (RBD).

Treatment :

T_1 No inoculant, control (c)

T_2 No inoculant + P_{20}

T_3 Mycorrhiza (M)

T_4 Mycorrhiza + P_{20}

T_5 Rhizobium (R)

T_6 Rhizobium + P_{20}

T_7 Mycorrhiza + Rhizobium (M+R)

T_8 Mycorrhiza + Rhizobium + P_{20}

Phosphorus @ 20kg P/ha as monocalcium-phosphate [$Ca(H_2PO_4)_2 \cdot H_2O$] was applied to particular treatments and common dose of other nutrients like C, N, Mo were applied in general to all pots. Carbon as energy source in the form of dextrose solution @ 0.25%, nitrogen in the form of ammonium nitrate solution @ 20kg N/ha and molybdenum as sodium molybdate @ 0.5 kg salt/ha were applied to soil before sowing seeds in pots. The nutrients and the energy source after soil application were mixed thoroughly by using a sterile glass rod.

The soil-host root carrier of VA-mycorrhiza was inoculated to the designed pots @ 50 gm culture/pot below 2cms of the surface soil. The mycorrhizal spore load was 500-700/pot in each of the mycorrhizal treatment. Then the soil surface in pots was levelled. Healthy green gram seeds were surface sterilized with 1% sodium hypochlorite solution for 5

minutes. Five shallow holes were made in the centre of each pot and seeds @ 2-3 in each hole was sown on 6.1.94. The treatments to receive the rhizobium inoculant were then inoculated with the starter rhizobium culture available in broth @ 1 ml for each hole in the respective pots. The seeds in holes of each pot were covered with the soil of the same pots. The soil was moistened with measured quantity of sterile water to maintain the moisture level at 60-70% of W.H.C. of soil in the pots-during the crop growth period. Seven days after germination plants were thinned and only four number of healthy plants were kept in each pot.

During the growth of crop plants it was observed that frequent watering almost once in every alternate day was necessary other wise crust formation on the surface of the soil due to evaporation was marked. The crop growth was delayed with emergence of less number of leaves. Then the attack of aphids to the crop plants was marked in the pots with certain black spots on the leaf surface. Suspecting the prevalence of anaerobic situation due to accumulation of added water in the rootzone to cause delayed growth of the crop plant hoeings were done periodically i.e. 5 to 6 times during the crop growing period. Ekalux @ 1cc in 1 litre distilled water was applied on 27.1.94 followed by application of monocrotophos @ 2ml mixed with 1gm Bavistin in 1 litre distilled water was made on 7.2.94 as prophylactic measure to

control aphids that were seen in the growing crop after 15-20 days of crop growth. The monocrotophos as applied earlier was repeated on 19.2.94. The crop was harvested on 9.3.94. The pots were watered sufficiently so that the plants could be reported easily without damaging there roots. The nodules were counted, the fresh weight was recorded and also leaf number, shoot and root lengths were measured. At first the plants were sundried and then dried in hotair oven at 70°C to remove moisture & finally at 100°C to record the oven dry weight. Plant-samples of different treatments after harvest of the crop were analysed in the laboratory for nutrient uptake.

Field Experiment :

Preliminary field experiments in farmers' field were conducted simultaneously in two locations one at Baghamari and the other at Pubusahi of Khurda district to study the effect of either single or dual inoculation of symbiotic Rhizobium (Strain M-14) and VA-mycorrhiza (*Glomus fasciculatum*) on the growth and yield of green gram, CV-"Nayagarh Local" during rabiseason, 1993-94. No fertilizer and FYM were applied to any field trials except the inoculants of Rhizobium and VA-mycorrhiza in the treatments & the treatments in the farmers' field were not replicated. The experiment conducted at Pubusahi location included four treatments viz., Control (C), Mycorrhiza (M), Rhizobium (R) and Rhizobium + Mycorrhiza (R+M). But the field trial conducted at Baghamari location

consisted of three treatments viz., Control (C), Rhizobium (R) & Rhizobium + Mycorrhiza (R+M). The plot size of each treatment was 20 cents. The field soil was ploughed twice just before sowing and green gram seeds inoculated with packet culture of Rhizobium were sown behind the plough on 13.12.93 at Baghamari location and on 3.1.94 at Pubusahi location. In respective treatments of the VA-mycorrhiza, the culture was applied at the rate of 15kg/ha behind the plough below 2-3 cms depth. The crops were allowed to grow upto their full maturity & then harvested on 13.3.94 at Baghamari location and on 10.4.94 at Pubusahi location. Crop samples from random $1m^2$ area of each treated plot were harvested, biometric observations like height of plant, length of root, nodule number, dry matter yield etc. were recorded. Five number of plants from each sample after drying were kept for chemical analysis in the laboratory.

Pot culture Experiment - II

The experience of crop growth difference in the pot culture experiment-I and that in the field encouraged to take up the pot culture experiment-II inside the wire-net house of the department.

The second pot culture experiment was conducted in small earthen pots of 5" diameter using the sterile soil (Soil:Sand=3:1) drawn from the same lot from where soil for

the first pot experiment was taken. The potculture experiment-II consisted of only four treatments viz: Control(C), Mycorrhiza (M), Rhizobium (R) & Rhizobium + Mycorrhiza (R+M) and each treatment was replicated five times. The pots after proper fume sterilization using 40% formaldehyde were filled in with 1.5kg sterile soil in each. The drainage facilities were provided by placing pebbles on the holes present on the bottom of the pots. As the soil was acidic, lime @ 2 tons/ha (i.e. 2.5 gm $CaCO_3$/pot) was applied as soil amendment 15 days prior to sowing of seeds. Sterilized FYM @ 100 gm/pot was added and mixed properly with soil in each pot to facilitate aeration. After 15 days of incubation when the soil reaction was found to be neutral the experiment was started. A common dose of phosphorus @ 20kg P/ha as monocalcium phosphate [$Ca(H_2PO_4)_2 H_2O$] in solution form was added to each of the pots but no other fertilizers were added to the pots. Mycorrhizal inoculum @ 25 gm/pot containing a sporeload of 250 to 350 was applied to the respective pots at 2-3 cms depth and covered immediately. On the surface soil of each pot, four holes were made by sterilized glass rod and healthy surface sterilized green gram seeds, CV-"SUJATA" were sown on 5.5.94. Starter culture of Rhizobium (Strain M-14) available in YEMA broth was inoculated @ 1 ml in four holes of each pot & covered immediately with soil. Sprinkling of water was given initially to allow the seeds to germinate. Four plants were kept in each pot & excess plants were uprooted and incorporated in

respective pots. Periodical hoeing for 2-3 times was done and measured amount of sterile water was given to maintain the moisture level at 60-70% of the W.H.C. High evaporation due to high temperature during crop growth period demanded frequent watering of plants The crop was harvested by uprooting the plants on 23.6.94 at about 48 days of crop growth. Growth characters like root length, shoot height, leaf number, nodule number, root volume, nodule weight, leaf area etc. were recorded and the plants were oven dried t take the dry weight & ground. After taking the dry matter yield, the relative dry matter yield response over control of the crop due to treatments was calculated by suing the following formula :

$$Y_R = \frac{Y_t - Y_c}{Y_t} \times 100$$

Where, Y_R = Relative yield response (%);

Y_t = Treatment yield;

Y_c = Control yield.

The plant samples were chemically analysed for their nutrient uptake. Representative soil samples from each treated pot were taken to assess the post harvest nutrient status of the soil.

METHODS OF ANALYSIS :

The laboratory techniques used for analysing the soil samples, plant samples and microbial assessments in the inoculum applied during the course of investigation were as follows :

(A) SOIL ANALYSIS :

i) Mechanical analysis: The soil separates were determined by using the standard hydrometer method as outlined by Piper (1950).

ii) Water holding capacity: This was determined following the Keen-Raczkowski box method, out lined by Piper (1955)

iii) Bulk density : The B.D. of the soil was determined by following the core method as outlined by Black, (1965).

iv) Soil reaction : The pH of the soil was measured in 1:2, soil : Water suspension by means of glass electrode, used in systronics digital pH meter as described by Jackson (1973).

v) Specific conductivity : The electrical conductivity of soil-water suspension at 1:2 proportion was measured by using Elico conductivity bridge type CM 82T as per the method outlined by Jackson (1973).

vi) Cation Exchange Capacity : It was determined by the method outlined in soil chemical analysis by Jackson (1958). 10gm of the soil was leached with neutral normal NH_4OAC solution followed by leaching with 60% alcohol. The soil thus freed from excess NH_4, was distilled with

MgO and NH_4 - nitrogen was absorbed in standard H_2SO_4. The CEC was calculated from the acid consumed which is equivalent to NH_4-N exchanged.

vii) Organic carbon : It was determined by Walkley and Black's rapid titration method as described by Jackson (1973).

viii) Total nitrogen : This was determined by using standard Kjeldahl's distillation method as outlined by Jackson (1973).

ix) Available phosphorus :This was determined by using Bray-I extractant following the procedure as outlined by Jackson (1973).

x) Available Potassium : Available K using normal neutral NH_4OAC extract was determined in a flame photometer, systronics mark-II outlined by Jackson (1973).

(B) PLANT SAMPLE ANALYSIS :

i) Total leaf area measurement : This was determined by graphical method. The area of five representative leaves (randomly collected) was measured graphically and the equivalent dry matter weight of those was recorded. Then the total dry matter weight of all the leaves plucked from the plants of a pot was converted to total leaf area on the basis of five leaves average.

ii) Total nitrogen : Total N was determined in plant samples by using Kjeldahl's distillation method as outlined by A.O.A.C. (1970).

iii) Total phosphorus : Plant samples were digested with diacid mixture of HNO_3 & $HClO_4$. The digestion extract was analysed for total phosphorus by vanado-molybdate yellow colour method as outlined by Jackson (1973).

iv) Chlorophyll content of the leaves : The chlorophyll content of the fresh green leaves of test crop was determined following the procedure of extraction with 80% acetone as outlined by Arnon (1959).

The fresh leaves were cut into small pieces and 500 mg of fresh leaf was weighed and taken in a 50 ml conical flask containing 15 ml of 80% acetone. The mouth of the conical flask was sealed by aluminium foil to check the volatilization loss of acetone and kept as such for 24 hours with intermittent shaking by hand for extraction of chlorophyll. The supernatant coloured solution was decanted off to a cuvette. The absorbance of light in spectronic-20 was measured at 652 nm taking 80% acetone as blank. The amount of chlorophyll in mg/gm of fresh leaf was calculated as follows:

$$C = \frac{D\ 652 \times 1000}{34.5} \times \frac{V}{1000 \times W} .$$

Where,

C = Amount of total chlorophyll in mg/gm of fresh leaf.

D 652 = Optical density at 652 nm.

V = Final volume of the extract (ml).

W = Fresh weight of the leaf tissues (gm).

v) Haemoglobin content of the root nodules : The leg-haemoglobin content of the legume root nodules was determined by using the Drabkin's solution as per the method outlined by Wilson & Reisenauer (1963).

Fresh clean nodules properly washed with distilled water weighing 0.5gm was crushed in a chilled mortar and pestle with 3ml of Drabkin's solution which was prepared by dissolving 52 mg of KCN, 198 mg of potassium ferricyanide and 1gm of sodium bicarbonate ($NaHCO_3$) per litre of distilled water and added to the mortar with an automatic pipette. It was then transferred to the centrifuge tube and the volume was made up to 7ml and the homogenate was centrifuged at 5,000 rpm for 25 minutes. The clear supernatant thus obtained was made to 10ml with Drabkin's reagent. The absorbance of the clear super natant was read at 540nm using Drabkin's reagent as reference blank solution. The amount of leg-haemoglobin present in sample was calculated from calibration curve prepared with known concentration of cyanomethemoglobin and the results were expressed as 'mg' per 'gram' of fresh weight.

(C) MICROBIOLOGICAL ANALYSIS

i) Plate count of Rhizobium bacteria by dilution technique (Allen, 1959) : The Rhizobium population in the carrier based culture packet sample and post harvest soil samples collected from plots of each treatment determined.

10gm of soil or culture sample was suspended in a 250 ml bottle containing 95 ml of sterile water with few glass beads to give 10^{-1} dilution. It was shaken for 5 minutes for removal of bacteria from the adhering surface through the process of agitation by the glass beads. 10 ml of the above suspension was transferred by a sterile pipette from about 1 cm depth to another bottle containing 90 ml sterile water. This was 10^{-2} dilution. In this way dilutions were progressively made serially upto 10^{-7} in a series of bottles each containing 90 ml of sterile water. The pipette used once was discarded and another sterile pipette was used for the next dilution. One ml portions of final dilution were transferred to each of three petridishes containing sterile yeast extract mannitol agar (YEMA) mixed with congored (1%) and they were incubated at 28 ± 2°C and the average number of bacterial colonies per plate were counted after three days and recorded. The moisture content of the soil or carrier material was determined separately to express the number of bacteria present per gram of soil or carrier material.

Composition of YEMA medium

K_2HPO_4	=	0.5 gm.
$MgSO_4 \cdot 7H_2O$	=	0.2 gm.
NaCl	=	0.1 gm
Mannitol	=	10 gm.
Yeast extract	=	1 gm.
Distilled water	=	1000 ml.
Agar	=	20 gm.

Congored (1% solution)= 2.5 ml (only for solid medium during isolation).

Congored was sterilized separately and was added to the agar medium. The broth was adjusted to pH 6.8 by using a solution of IN NaOH or HCl as required.

The characteristic of Rhizobium bacteria was confirmed by following the Gram staining technique (Vincent, 1970).

Smears of bacteria were made by taking a loopful suspension on a microscope slide and allowed to dry in air. The bacteria were fixed on the slide by passing it over the flame of the bunsen burner, then stained with crystal violet solution (A) for 1 minute, followed by eight rinsing with water. Then the slide was flooded with Lugol's iodine solution (B) for 1 minute, after which drained and decolorized with

iodinated alcohol (C) for 5 minutes. The slide was washed in water, drained and counter stained with safranine (D) finally, the slide was washed in water, drained, air dried and observed under microscope with oil immersion.

Gram reagent

(A) Crystal violet solution.

Crystal violet = 10 gm.

Ammonium oxalate = 4 gm.

Ethanol = 100 ml

Distilled water = 400 ml.

(B) Iodine Solution

Iodine = 1gm

Potassium iodide = 2 gm.

Ethanol = 25 ml

Distilled water = 100 ml.

(C) Iodinated alcohol

Iodine solution (B) = 5 ml.

Ethanol = 95 ml.

(D) Counter stain

25% safranine in ethanol = 10 ml.

Distilled water = 100 ml.

ii) VAM-spore counting : This was done in the laboratory by using the wet sieving and decanting method as outlined by Gerdemann & Nicolson (1963).

50 gm of the carrier material containing VA-mycorrhizal spores was mixed in 100 ml of water in a wide aluminium container and the heavier particles were allowed to settle down during a period of few seconds. The soil water suspension was poured off through a coarse soil sieve (500-800 mm) to remove large pieces of organic matter. The suspension that passed through the sieve was collected. The sieve was washed in a stream of water to ensure that all small particles have passed through. The particles were resuspended in water which passed through the coarse sieve and the heavier particles were allowed to settle down for a few seconds. This suspension was passed through a sieve fine enough to obtain the desired spores generally 38-250 m. The materials retained all the colloidal materials passed through the sieve. Small amounts of retaining debris was transferred to a petridish and examined under the microscope to get the spore count.

iii) Root infection length percentage determination: The root infection was determined in the laboratory with the method devised by Phillips and Hayman (1970).

The infected roots were cleaned with water & cut into small pieces of about 1-2 cms length and boiled at 90°C for 1 hour in 10% KOH solution. Then these were rinsed with distilled water. After that the boiled root fragments were acidified with 0.1N HCl for about 5 minutes. The root

fragments were then strained by simmering for 5 minutes in 0.05% trypan blue in lactophenol. The excess stain was removed by using plain lactophenol. Then the root pieces were mounted over slides in lactophenol and the root infection length percentage was assessed. The vesicles and arbuscules formed in the root cortex were observed along with the spores.

Composition of Lactophenol

Phenol (Crystal)	= 20 gm.
Lactic acid	= 20 gm.
Glycerol	= 40 ml.
Distilled water	= 20 ml.

CHAPTER-IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Soil characterisation:-

The analytical data for mechanical composition and physico-chemical properties and chemical characteristics of the soil sample used in the pot culture experiments and that of the nature of the field soils are presented in Table -1. The values indicated that the soil used in the pot culture experiments contained 74.2% sand and was loam in texture with moderate water holding capacity of 28%, having 1.60 gm ml^{-1} bulk density, very low cation exchange capacity, poor organic carbon content, acidic soil reaction and contained low amount of free salts. The fertility status was almost poor due to low total nitrogen and low available potassium content, however, the available phosphorus was medium. This soil sample was collected from upland area of "Pubusahi" location which comes under lateritic soil groups (Ustalf).

One field experiment was also conducted in medium land of lateritic soil at Pubusahi location but the characteristics of the field soil varied from that used for pot culture. The soil contains 48% sand and 21.8% clay. The

Table 1. Physico-chemical properties of the soil

Sl. No	Soil Properties	Potculture experiment (I & II)	Field Experimental locations	
			Pubusahi	Baghamari
1.	**Mechanical composition**			
	(a)sand (%)	74.2	48.2	37.2
	(b) silt (%)	12.0	30.00	35.00
	(c)Clay (%)	13.8	21.80	27.80
	Texture class	Loam	Silty loam	Silty clay loam
2.	Bulk density (g/cc)	1.60	1.50	1.40
3.	W.H.C. (%)	28%	32.5%	34%
4.	C.E.C. c mol (P +) kg^{-1}	4.50	6.90	5.80
5.	pH (1:2)	5.23	6.52	5.10
6.	E.C. (dsm^{-1})	0.07	0.10	0.19
7.	Organic control (%)	0.51	0.77	0.98
8.	Total N (kg ha^{-1})	(0.046%N) = 927.36kg ha^{-1}	(.064%N) = 1280.00 $kg.ha^{-1}$	(0.076%N) = 1520 $kgha^{-1}$
9.	Available P (kg ha^{-1}) (Bray-I).	20.30	12.00	11.40
10	Available K (Kg ha^{-1}) (NH_4OAC. method)	100.00	120.00	260.00
11.	C/N Ratio	11.02	12.03	12.89

texture was silty loam having bulk density of 1.5 gm ml^{-1} medium water holding capacity of 32.5%, low cation exchange capacity of 6.9'Cmol (P+) kg^{-1}, neutral soil reaction and contained low amount of free salts. The soil carbon status of 0.77% considered as medium, available potassium medium but the available phosphorus was low. Relatively this field soil was better than that used for pot culture experiment as seemed from the soil analytical values.

The analytical data of the lateritic soil at Baghamari location (Table-1) selected for another field experiment indicated that the soil contained 37.2% sand, 35% silt, 27.8% clay and possessed silty clay loam texture. The bulk density of 1.4 g ml^{-1}, water holding capacity medium & cation exchange capacity was low. The soil fertility status seemed to be fairly high because of high organic carbon content of 0.78% and medium available potassium of 260kg ha^{-1}. However, the available phosphorus status of that soil was lower in comparison to other two soils as indicated from the table. The C/N ratio in all the soils varied between 11 to 13:1. The soils as developed under lateritic area are generally low in cation exchange capacity, low to medium in water holding capacity with high bulk density subject to change due to change in carbon status and management practices of soil (Sahu, 1993).

Nature and count of microbial inoculant population in starter culture:

Characteristics and mycorrhizal spore count on examination of the sample packet of mycorrhizal spores present in rhizosphere soil with the root pieces of guinea grass (Panicum maximum) grown in the pot after multiplication (Plate-1) of the original sample of VA-mycorrhiza (*Glomus fasciculatum*), it was observed that chlamydospores of globuse shape, light yellow in colour with thick cell wall attached to cylindrical hyphae were present in the sieved and decanted suspension. On counting the number, it was found to be 10-14 gm^{-1} of soil in sample packet. Besides, the infected grass root fragments after digestion with 10% KOH solution and stained with trypan blue in lactophenol were examined under microscope and observed the presence of vesicles and some spores stained bluish inside the cortical region. This confirmed that the material was ready to be inoculated further in the pot culture experiment and field experiments.

Rekha Rani and Mukerji ((1988) reported the taxonomical characteristics of *Glomus fasciculatum* (Thaxter Sensu Gerd.) Chlamydospores borne free in soil in small loose clusters, 35-75 µm diameter, spore colour ranges from light yellow when young to almost black at maturity, sporocarps are seldom observed. Hyphae are frequently thick walled and brown, 4-10µm dia. The species is commonly seen in the soil examined

Plate.1. Inoculum Production of VAM in the rhizosphere soil of Guinea grass.

usually associated with rhizosphere soils of *Eucalyptus* and *Prosopis sp.*

Characteristics and Rhizobial population Count:

The microbial method of serial dilution and plate counting included under present investigation was meant for counting the number of Rhizobium population. The bacterial population was 1.27×10^{9} gm^{-1} of the carrier material which was later on used in the field experiment. The colonies that developed over YEMA medium containing congored looked as small, elevated, white, translucent, glistering with entire margin and the bacteria on gram staining test was found to be gram negative in nature as it retained the red colour of counter stain Safranin. These characteristic observations for the bacteria confirmed to the characteristics of the Rhizobium as per the standards mentioned in Bergey's manual of Determinative Bacteriology.

POT CULTURE EXPERIMENT -I

The effect of inoculation and phosphate fertilization on growth attributing parameters of greengram presented in Table-2 and Plate-2. The general appearance of the plant growth as seen in the photoplate seems to be poor.

The results for mean plant height cited in the table indicate that amongst the unfertilized treatments receiving

Table 2: Effect of inoculation and P-fertilization on growth characteristics of greengram in potculture experiment-I

Sl.No	Treatment	Plant ht. (cm)	Root lenth (cm)	Shoot: Root Ratio	Leaf no. per pot	D.M. (mg/pot)
1.	C(P_0)	5.8	15.10	0.384	26.40	244.48
2.	C($_{20}$)	6.6	14.50	0.395	28.80	303.40
3.	M(P_0)	6.5	14.00	0.464	28.40	272.96
4.	M(P_{20})	7.5	16.70	0.449	31.00	315.56
5.	R(P_0)	6.9	14.00	0.492	29.00	273.64
6.	R(P_{20})	6.9	14.00	0.492	32.60	391.80
7.	R+M (P_0)	6.7	17.00	0.394	29.80	324.84
8.	R+M (P_{20})	7.0	16.40	0.426	33.40	382.76
SEM (±)		0.338	1.484	0.049	1.598	43.513
CD(P=0.05)		0.98	NS	NS	4.629	126.013

only inoculants, the plant height for rhizobial treatment (6.9cm) was significantly higher than that of uninoculated control (5.8cm). The plant height for treatments receiving mycorrhiza and control uninoculated were at par but that due to dual inoculation of Rhizobium and VA-mycorrhiza was increased over control by 15.5% approaching the level of significance. The unfertilized treatments when supplemented with P fertilization, showed improvement in the plant height than the corresponding unfertilized but inoculated plants excepting Rhizobium treatments. Phosphorus application with inoculants of mycorrhiza and Rhizobium inoculated either separately or combinedly showed significant increase in height being 7.5 cm, 6.9cm and 7.0 cm for mycorrhiza, Rhizobium and R+M treatments respectively. This may be due to N_2 fixation through the symbiosis with the legume plants.

The data for dry matter yield and relative yield response cited in Table-3 revealed that the dry matter yield increased significantly and was highest in the pot (391.80mg) due to inoculation of Rhizobium in presence of phosphorus which gave 37.6% higher yield response over uninoculated control without phosphate fertilization. The treatment receiving dual inoculation of Rhizobium and VA-mycorrhiza in presence of phosphorus also produced significantly higher drymatter yield, the relative response over control being 36.12%. The drymatter yield varied from 244.48 mg in control

Table 3: Effect of inoculation and P-fertilization on nutrients uptake by geengram in potculture experiment - I

Sl.NO	Treatment	D.M/pot (mg)	Relative DM yield response (%)	% N	N-uptake (mg N/pot)	% P	P-uptake (mg p/pot
1.	C(P_0)	244.48	-	2.184	5.340	0.139	0.339
2.	C(P_{20})	303.40	19.42	2.468	7.488	0.143	0.433
3.	M(P_0)	272.96	10.43	2.563	6.995	0.173	0.442
4.	M(P_{20})	315.56	22.52	3.734	11.783	0.151	0.476
5.	R (P_0)	273.64	10.65	3.671	10.045	0.169	0.542
6.	R(P_{20})	391.80	37.60	3.481	13.638	0.183	0.716
7.	R+M(P_0)	324.84	24.74	2.943	9.560	0.155	0.503
8.	R+M(P_{20})	382.76	36.12	3.386	12.960	0.179	0.685
SEM (±)		43.515	-	-	-	-	-
CD(P=0.05)		126.013					

without P fertilization to 391.80 mg/pot in treatment receiving Rhizobium with P fertilization. The production of higher yield of green gram due to inoculation of Rhizobium alone or in combination with mycorrhiza may be due to associated symbiosis between the Rhizobium bacteria and the green gram as a result of which there was fixation of N_2 and made available to growing green gram plants. The present findings corroborate with the observation made by Samal (1992) growing green gram in pots inoculated with Rhizobium. Inoculation of mycorrhiza in association with Rhizobium might have improved the plant growth by supplying more of plant nutrient elements especially phosphates through self absorption capacity.

The results of root length of the test plants grown under different treatments indicate higher root length as compared to corresponding plant height in respective treatments (Plate-3). Similarly this has been reflected in shoot: root ratio. The root length was found to be insignificant within the treatments. The reason may be due to the low temperature prevailing initially during the cropping period in the pot experiment-I (Appendix Table-1) Stanfield *et al.*(1966) stated that low temperature during plant growth favoured root more than shoot growth.

Plate.2. Plant growth of greengram in Pot Expt. I.

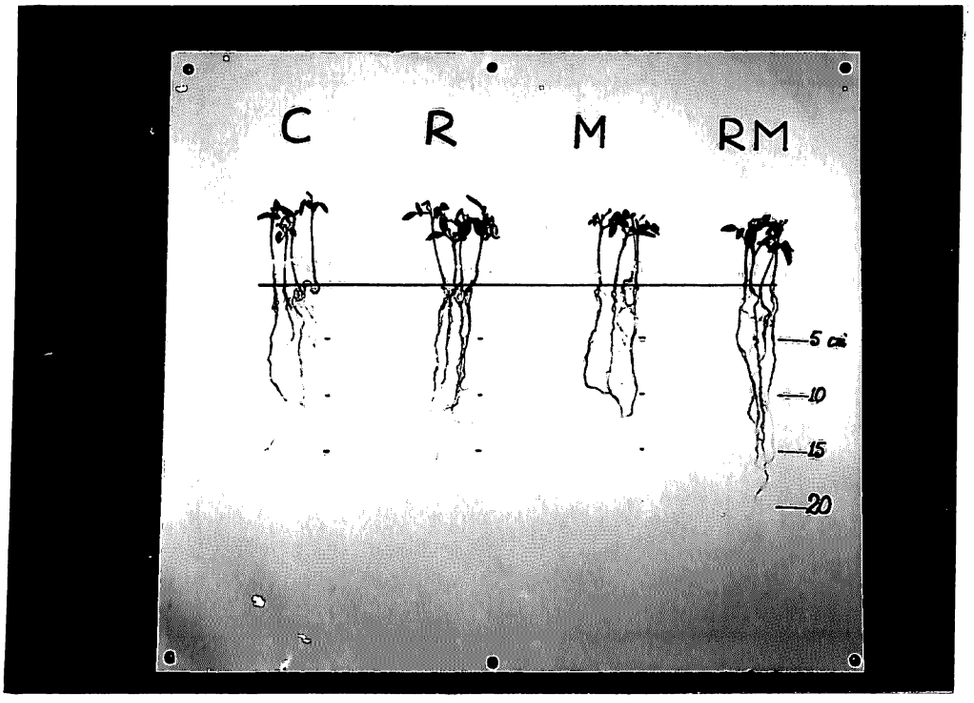

Plate.3. Root growth of greengram in pot. Expt.I.

The data on leaf number per pot show a positive response of inoculants irrespective of P fertilization. Inoculated plants with P fertilization produced significantly higher number of leaves over uninoculated control being 31.0, 32.6 and 33.4 for M, R and R+M treatments respectively.

During the cropping period the growth of plants was observed slow and stunted. This might be due to low soil fertility status, low soil pH and soil compaction created by frequent watering forming an anaerobic situation in the rhizosphere soil as a result of which the growth and activity of inoculants might have been suppressed. Davies and Runge (1967) reported that root growth of legume plant is exponentially and inversely related to soil bulk density.

Islam *et al.*(1980) reported that when species of Rhizobium and VAM fungus are co-inoculated, the growth of the legume is enhanced. The drymatter yield of green gram due to inoculants in absence of phosphate fertilization were all at par and insignificant.

The data for N and P-uptake by green gram plants grown in the first pot culture experiment are presented in Table-3. The results indicated that among the unfertilized treatments the N-uptake was at a minimum of 5.34 mg/pot in the uninoculated control and it was at a maximum of 10.045 mg in

the pot receiving rhizobial inoculation. However, there was an increase in N-uptake in all the treatment when they are fertilized with P @ 20kg ha^{-1}. It was also observed that the mycorrhizal treatment along with Rhizobium didn't show any increase in N-uptake over the treatments receiving Rhizobium alone.

A similar trend in P-uptake was observed in different treatment as revealed from the data in Table-3.

The order of drymatter yield produced under different treatments are related to the nutrient uptake (N and P) by the plant. This relationship has been cited in Figure 1 & 2.

FIELD EXPERIMENT:

The results of field experiments conducted in farmers' field at Pubusahi (Plate-4) and Baghamari locations (Plate-5) are presented in Table 4 and 5. The data (Table-4) reveal that there was an increase in height and root length of green gram plants grown in field experiment at Pubusahi location in the plot inoculated with mycorrhiza and Rhizobium either singly or combinedly over uninoculated control plot. A maximum plant height and root length of 19.7 and 19.5 cms respectively was found in dual inoculation of Rhizobium and mycorrhiza whereas the minimum of that due to uninoculated control was 10.4 and 7.2 cm respectively. The results in the same table for Baghamari location indicates better growth

Plate.4. Crop growth of greengram in the field experiment at pubusahi.

Plate.5. Crop growth of greengram in the field experiment at "Baghamari".

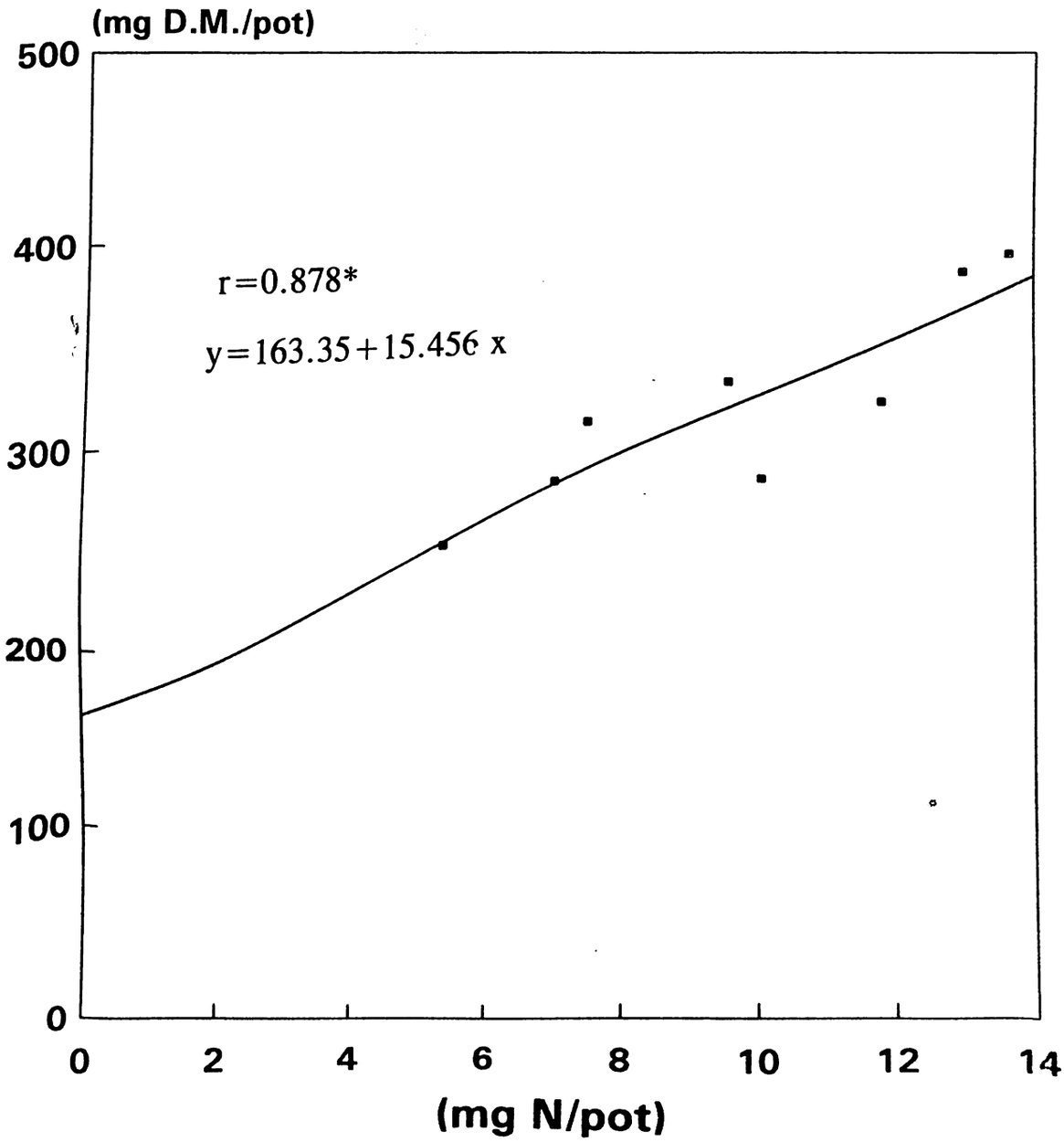

Fig. 1 Relationship between N-uptake and dry matter yield of greengram

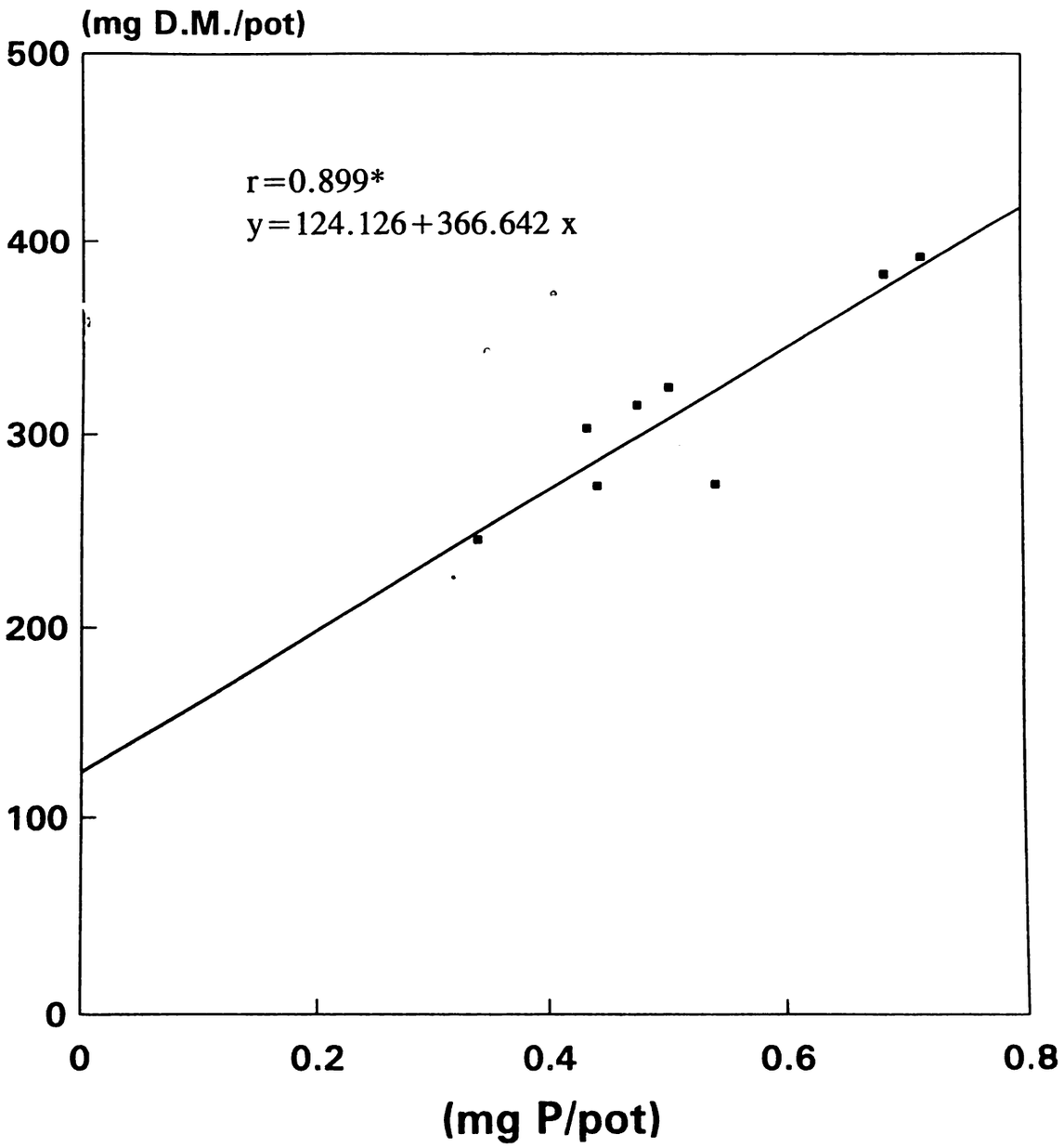

Fig. 2 Relationship between P-uptake and dry matter yield of greengram.

Table 4: Field experiment: Synergistic effect of VAM and Rhizobium inoculation on growth attributes and yield of greengram.

Sl. No	Treatment	Ht. of plant (cm)	Root length (cm)	shoot:root ratio	No. of plant per m^2 at harvest	No. of pods per plant	No. of nodules /plant	Grain yield (Q/ha)
				PUBUSAHI				
1	C	10.4	7.2	1.40	120	2.00* (240)	20	3.19
2	M	15.2	12.4	1.20	125	5.00 (625)	33	8.37
3	R	16.7	16.7	1.00	127	6.00 (762)	42	8.76
4	R+M	19.7	19.5	1.00	162	8.00 (1008)	45	10.40
				BAGHANARI				
1	C	13.3	15.3	0.9	107	2.7 (322)	12	4.32
2	R	31.3	17.6	1.7	119	3.8 (455)	23	5.34
3	R+M	32.6	25.0	1.3	182	4.1 (746)	28	6.31

* Figures in parentheses represent number of pods/m^2 area

performance of the plants, the height being maximum in R+M treatment (32.6cm) and minimum in control (13.3 cm) and the corresponding root growth for these two treatment found to be 25.0 and 15.3 cm respectively. This better growth of plants might be due to high carbon content in Baghamari field soil.

The number of plants per square meter area survived at the time of harvest in the treatment of dual inoculation was highest for both the locations being 162 for Pubusahi and 182 for Baghamri. The survival of plants was 35% and 70% more than that of uninoculated control plots in these two soils respectively. This may be due to the resistance of the mycorrhizal infected plants against stress conditions of field situation.

VAM fungi play an important role in the water economy of plants. The association improves the hydraulic conductivity of the roots and this improvement is one of the factors contributing towards better uptake of water by the plants. It has been suggested that the VAM fungi help the plants in better absorption of water by the roots resulting in a better performance (Kehri and Chandra, 1989, 1990)

The number of nodules per plant as observed from the table 4 increased in the plot receiving Rhizobium treatment by 110% at Pubusahi and by 91.6% at Baghamari location over

control uninoculated plot. The nodule number was further increased to 125% in former and to 133% in the latter field when the Rhizobium was supplemented with mycorrhizal inoculation. Probably in success in nodulation by Rhizobium bacteria in the roots of green gram plant grown at field was encouraged by the neutral reaction (pH 6.52) of the soil and high carbon content (0.77%) at Pubusahi.

The number of pods per plant due to inoculation of green gram with mycorrhiza and Rhizobium separately or combinedly was greater in both the locations over uninoculated control plants. The maximum of 8 pods/plant was observed at Pubusahi and 4.1 pods/plant at Baghamari location in the R+M treatment.

The grain yield increased by 50% at Baghamri soil and the same was about 3 times in R+M treatment (10.4 Q/ha) than that of the control yield (3.10Q/ha) obtained at Pubusahi location.

Effect of microbial inoculants on dry matter yield and nutrient uptake by greengram plant under various treatment of microbial inoculation in the field experiments are presented in Table-5.

Table 5: Drymatter yield and nutrient uptake by greengram crop in field experiment.

Sl. No	Treatment	D.M. yield (kg/ha)	% N in plant	Total N uptake by plants (kg N/ha)	% p in plant	Total P uptake by plants (kgP/ha)
			PURUSHAHI			
1.	C	984	1.676	16.49	0.119	1.170
2.	M	2500	2.360	59.00	0.169	4.225
3.	R	3708	2.562	94.99	0.187	6.933
4.	R+M	5559	2.752	152.98	0.219	12.174
			BAGHAMARI			
1.	C	685	1.898	13.00	0.149	1.02
2.	R	836	2.487	20.79	0.177	1.47
3.	R+M	956	3.066	29.31	0.215	2.05

The results of Pubusahi location showed an increasing trend in the dry matter yield production with the highest of 5559 kg. ha^{-1} in R+M treatment followed by only R (3708 $kg.ha^{-1}$), and only M (2500 $kg.ha^{-1}$) treatment and the lowest of 984 kg. ha^{-1} in the uninoculated control plot. But the field experiment at Baghamari location did not give any spectacular value of dry matter yield as compared to the values at Pubusahi. The highest value of 956 $kg.ha^{-1}$ was observed in R+M treatment which was even less than the control yield of Pubushai site followed by 836 $kg.ha^{-1}$ in R treatment and 685 $kg.ha^{-1}$ in control.

The N concentration in the plant samples was increased due to application of mycorrhizal and rhizobial inoculants either singly or combinedly. At Pubusahi, the increase was from 1.676% in control to 2.752% in R+M treatment whereas that in case of Baghamari location is from 1.898% in control to 3.066% in R+M treatment. Though there is not much difference in the N concentration of plant material at the experiment sites, the total N-removal from the field is significantly higher at Pubusahi location (16.49 kgN/ha^{-1}) in control to 152.98 kgN/ha^{-1} in R+M treatment) than that at Baghamari site (13.00 kgN/ha^{-1} in control to 29.31 kgN/ha^{-1} in R+M treatment). This is attributed to the higher drymatter yield at Pubusahi location.

The P-concentration of the plant materials varied between 0.199% and 0.219% in various treatments of both the experiment sites (Table-5). But the total P-removal from the field under various treatments was spectacularly higher at the Pubusahi site than that at Baghamari which was also attributed to relative drymatter yield in the field at the former location. The total P-removal at Pubusahi field was highest (12.174 kgP ha^{-1}) in R+M treatment and lowest (1.170 kgP ha^{-1}) in control plots whereas the same at Baghamari location varied between 2.05 kgP ha^{-1} in R+M treatment and 1.02 kgP ha^{-1} in control.

POT CULTURE EXPERIMENT -II

Biometric observations like plant height, root length, shoot: root ratio and number of days to first flowering of green gram grown in pot experiment -II are cited in Table-6 and the view of plant growth is presented in Plates 6, 7 & 8. The data indicate that there was a significant increase in plant height and root length of green gram when the soil was inoculated with mycorrhiza and or Rhizobium. The increase in root length in inoculated pots was spectacularly higher than that of control pots (17.9 cm in control to 35.5 cm in R+M treated pots). It was also noted from the data that the flowering came nine days earlier in plants grown in pots receiving R & R+M treatments than that appeared in control and mycorrhizal treatments. This may be due to better availability

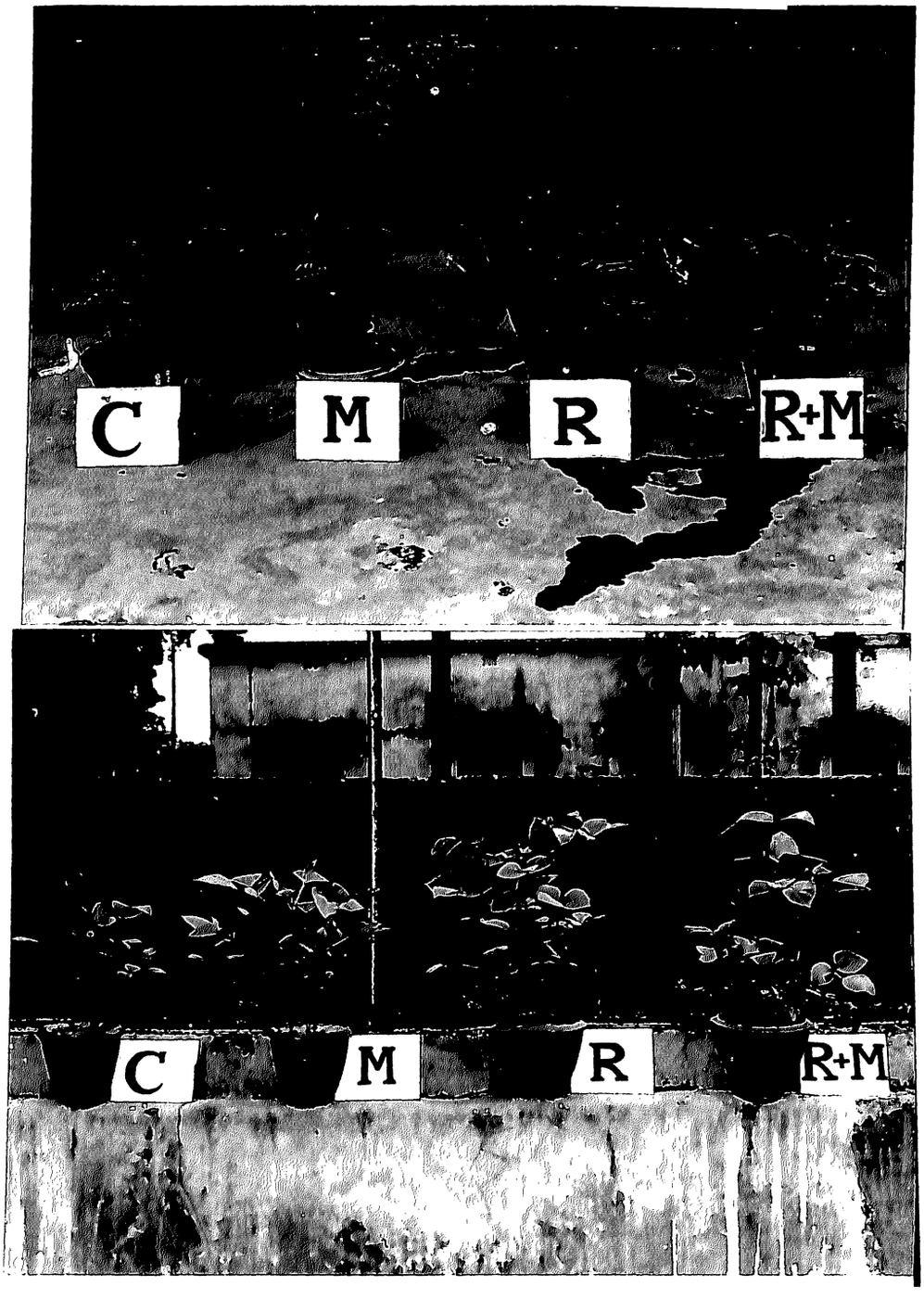

Plate.6,7. Plant growth of greengram in pot culture experiment II.

Table 6 Effect of inoculants on growth attributes of greengram in potculture experiment - II

Sl.No	Treatment	Plant ht. (cm)	Root length (cm)	Total length (cm)	shoot: root ratio	No. of days to first flowering
1	C	29.30	17.90	47.20	1.642	45
2	M	32.40	27.20	59.60	1.201	45
3	R	38.80	33.40	72.20	1.184	36
4	(R+M)	43.58	35.50	79.08	1.255	36
SEM (±)		0.863	1.101	-	0.064	-
CD (P = 0.05)		2.661	3.394		0.199	

of N & P to green gram plants grown in these treated pots of R and R+M.

Effect of inoculants on leaf number and chlorophyll content of greengram grown under the pot experiment-II is presented in Table-7. The data indicate an increase in leaf number per pot varying from 50.8 in control to 74.4 in R+M treatment. This trend is also reflected in the values of total leaf area of the plants. Of course, the only M and only R treatments did not show any significant difference so far as the leaf area was concerned whereas the increase was significantly high (2654.22 cm^2 pot^{-1}) in case of R+M treatment over control.

The chlorophyll content of leaves increased from 0.8258 mg/g fresh wt. of leaves in control of 1.2538 mg/g fresh wt of leaves in R+M treatment having no significant difference between only M and only R treatments (Fig-3).

Effects of inoculants on root volume, nodule number, nodule weight and haemoglobin content in the nodules of greengram is cited in Table-8 and the Plate 8 indicate the root of green gram (Fig 3 & 4).

The data indicate that the root volume of plants grown under different treatment receiving microbial inoculants

Table 7: Effect of inoculants on leaf number, area and chlorophyll content of greengram in pot culture experiment -II

Sl.No	Treatment	Leaf number per pot	Dry wt. of leaves: (mg/pot.)	Av. Total leaf area (cm^2)/pot	Av. chlorphyll content of leaves (mg/gm of fresh weight)
1	C	50.80	2.506	924.849	0.8258
2	M	58.80	3.379	1335.795	0.9782
3	R	68.60	5.486	1609.860	1.0028
4	R +M	74.40	6.758	2654.223	1.2538
SEM (±)		1.481	-	96.847	0.089
CD (P=0.05)		4.563		298.395	0.274

Table 8: Effect of inoculants on root volume, nodule number, nodule weight and haemoglobin content of the nodules in green gram in pot culture experiment II

Sl. No	Treatments	Root volume (cc)	Mean Nodule number/pot	Dry wt. of the nodules/pot (mg)	Mean Haemoglobin content (mg/gm fresh nodule)
1	C	2.60	26.60 (6.65)*	30.90	1.2268
2	M	7.80	28.80 (7.2)	27.00	1.9866
3	R	18.00	163.20 (40.8)	204.96	2.7733
4	R +M	22.80	180.00 (45.0)	179.75	3.0089
SEM (±)		1.676	6.348	5.326	-
CD (P=0.05)		5.163	19.558	16.406	

* Figures in parentheses indicate the number of nodules per plant

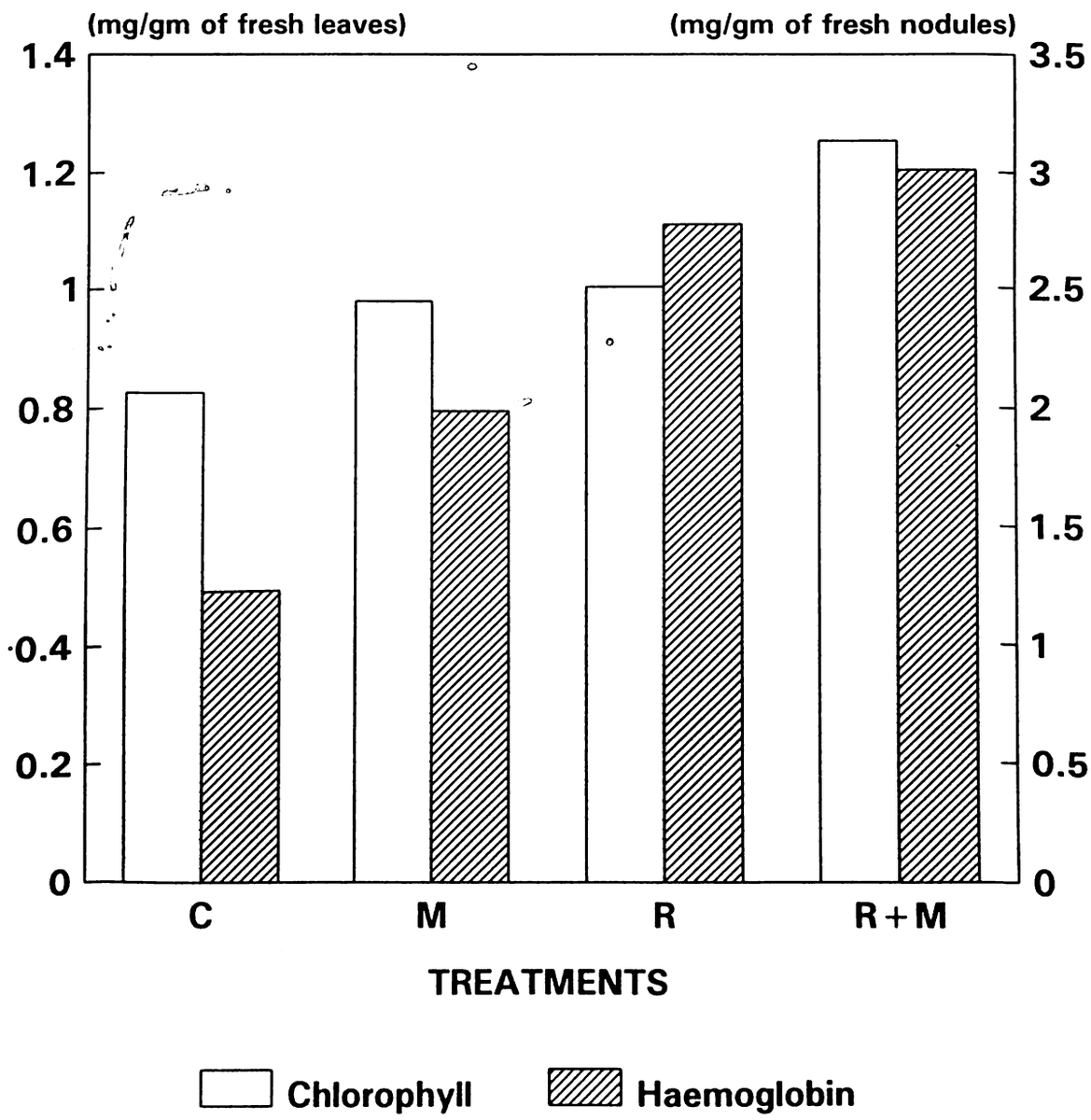

Fig. 3 Contents of Chlorophyll in leaves and haemoglobin in nodules of greengram as influenced by treatments.

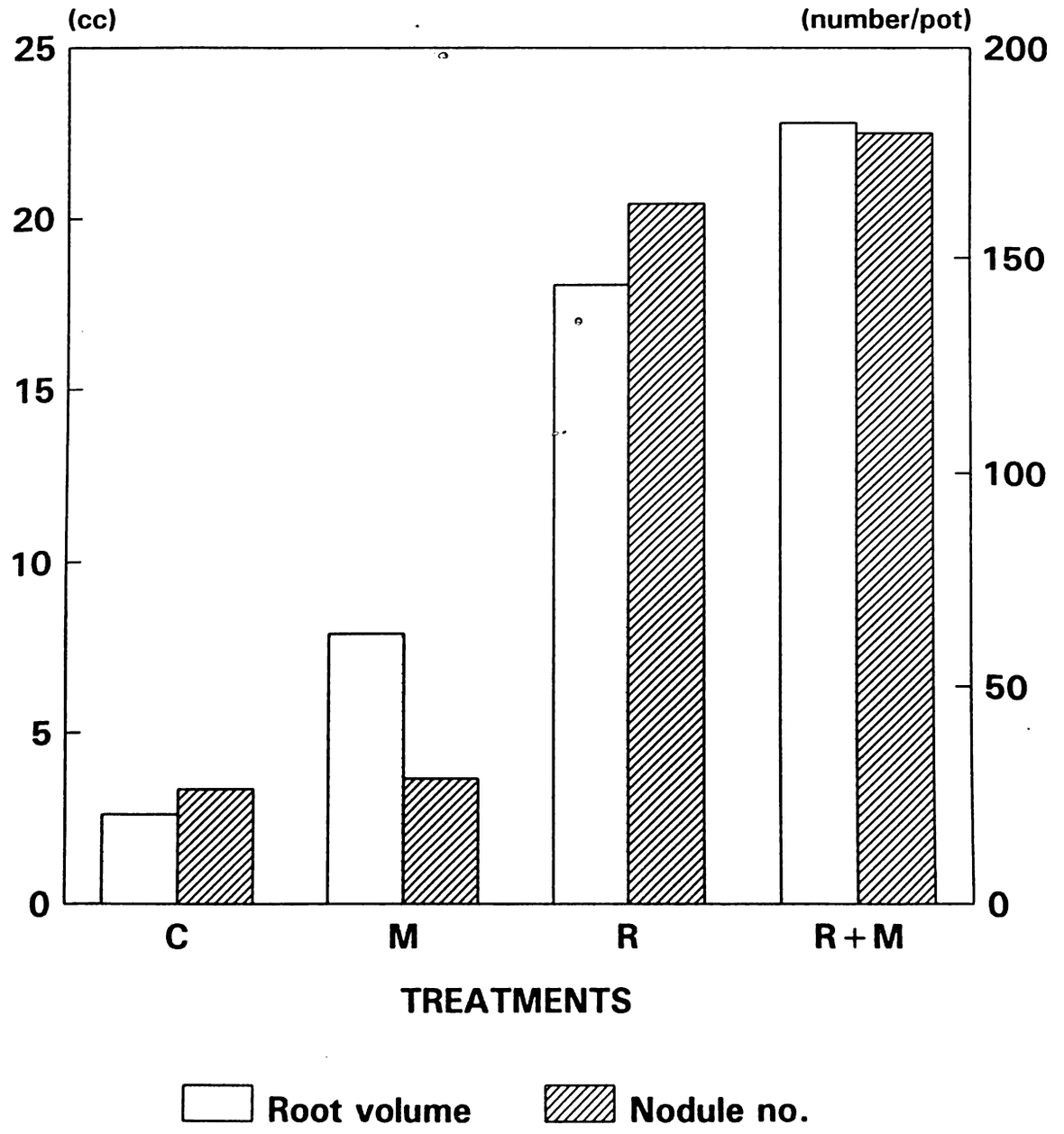

Fig. 4 Root volume and nodule number of greengram as influenced by treatments.

increased from 2.60 cc per pot in control to 22.80 cc/pot in R+M treatment with 7.80 cc/pot in only M and 18.00 cc in only R treatment (Fig-4).

The nodules (Plate-9) and dry weight of nodules (Table-8) reveal that the nodule number increased marvellously in the treatment receiving rhizobial inoculation which was applied either singly or in combination with mycorrhiza. Mycorrhiza applied alone did not show any increase in nodule number or nodule dry weight as compared to those of control pots. The highest nodule number of 180/pot was observed in R+M treatment as against a lowest of 26/pot in control. The nodules appeared in the mycorrhizal treated pot as well as in uninoculated control pot might be due to partial sterilization of the very small amount of FYM added to the soil.

It is also interesting to note from the data that the haemoglobin content of fresh nodules increased from 1.227 $mg.g^{-1}$ in control to 3.009 $mg.g^{-1}$ in R+M treated pots which may be attributed to the formation of more percentage of effective nodules in the treated pots. It was observed during the period of experiment that nodules were begger in size, red in colour and spread more in the tap root and secondary roots of legume plant which indicated their effectiveness for successful symbiosis by the organism and host plant.

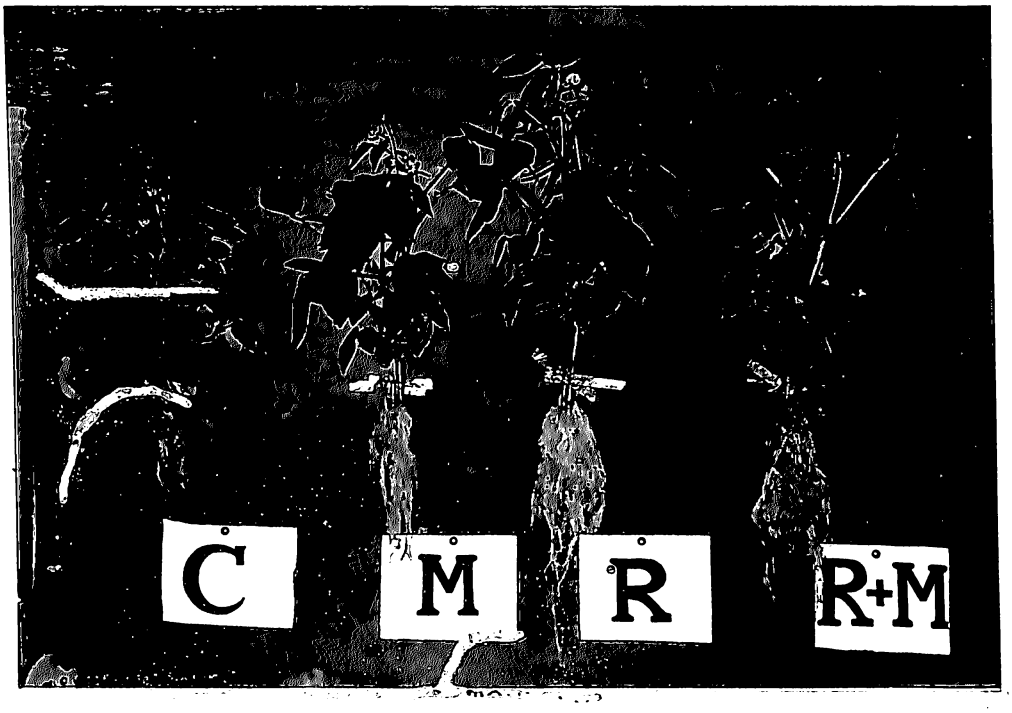

Plate.8. Root growth of greengram in pot.Expt.II.

Plate.9. Root growth showing nodules of greengram in pot. Expt. II.

It may be mentioned here that the soil for pot culture experiment-II was taken from the same lot of sterile soil from where the soil sample for pot experiment I was drawn and used. The soil was ammended with lime and common dose FYM @ of 100 g/pot and P@ 20 kg ha^{-1} was added. Probably this caused to show high response of green gram to inoculation of Rhizobium and mycorrhizal fungi in such ammended soil in pot experiment II. Banasri *et al.* (1989) observed that application of P along with molybdenum significantly increased the number, dry weight and N-content of nodules in green gram under limed condition. The increase in yield and nodulation due to Rhizobium, inoculation of legumes (Blackgram, Pea, Bengalgram and Berseem) along with phosphate and molybdenum application has also been reported by Tiwari *et al.*(1989). Increase in root growth due to Rhizobium inoculation was also observed by Mes (1959) and Ramaswami and Nair (1965). This agrees with the findings of the present investigations.

The increase in nodulation in greengram under dual inoculation of Rhizobium and mycorrhiza agrees with the findings of Bagyaraj *et al.*,(1979) who reported that Rhizobium treatment and mycorrhiza + Rhizobium dual inoculation resulted in good nodulation in soybean and mungbean. Similarly, Adholeya *et al.*,(1988) reported that infection rating, nodule number, nodule dry weight, nigrogenase activity and grain yield in *Vigna radiata* were maximum under dual inoculated (M+R) plants.

Effect of inoculation on drymatter yield and nutrient uptake by greengram cv-Sujata is recorded in Table-9.

The results on dry matter yield of green gram plant with various microbial inoculations in pot experiment-II indicate that the dry matter yield of greengram varied from as low as 4.477 g. to as high as 12.90g/pot in the treatment receiving dual inoculation of Rhizobium and mycorrhiza. The dry matter yield response over control was 65.29% for latter followed by 58.00% for only Rhizobium and 35.88% for only mycorrhiza treatments and all produced significantly higher drymatter yield over uninoculated control. Such high response in dry matter yield obtained in the treatments receiving inoculants was possible by removing N and P in significantly greater quantity from the soil over control and the quantity was 0.2700g N and 0.032 g P uptake per pot receiving dual inoculation followed by 0.2058 g N and 0.026g P for only Rhizobium and 0.1368 g N for only mycorrhizal treatment with 0.020 g P per pot. The dry matter yield production under various treatment and the uptake of N and P by the plant grown under the pot culture experiment II are also graphically presented (Fig -5).

The results showed that increase in N and P uptake has increased the drymatter yield of greengram in dual inoculation (M+R) treatment. Plants are capable of absorbing

Table 9. Dry matter yield, N-uptake and P-uptake by the greengram crop in pot culture experiment-II

Sl.No	Treatment	Mean D.M. yield (gm/pot)	Relative DM yield response	N-uptake (gm/pot)	P-uptake (gm/pot)
1	C	4.477	-	0.0718	0.010
2	M	6.982	35.878	0.1368	0.020
3	R	10.660	58.002	0.2058	0.26
4	(R+M)	12.900	35.294	0.2700	0.032
SEM(±)		0.571	-	0.0165	0.00164
CD(P=0.05)		1.759		0.051	0.0051

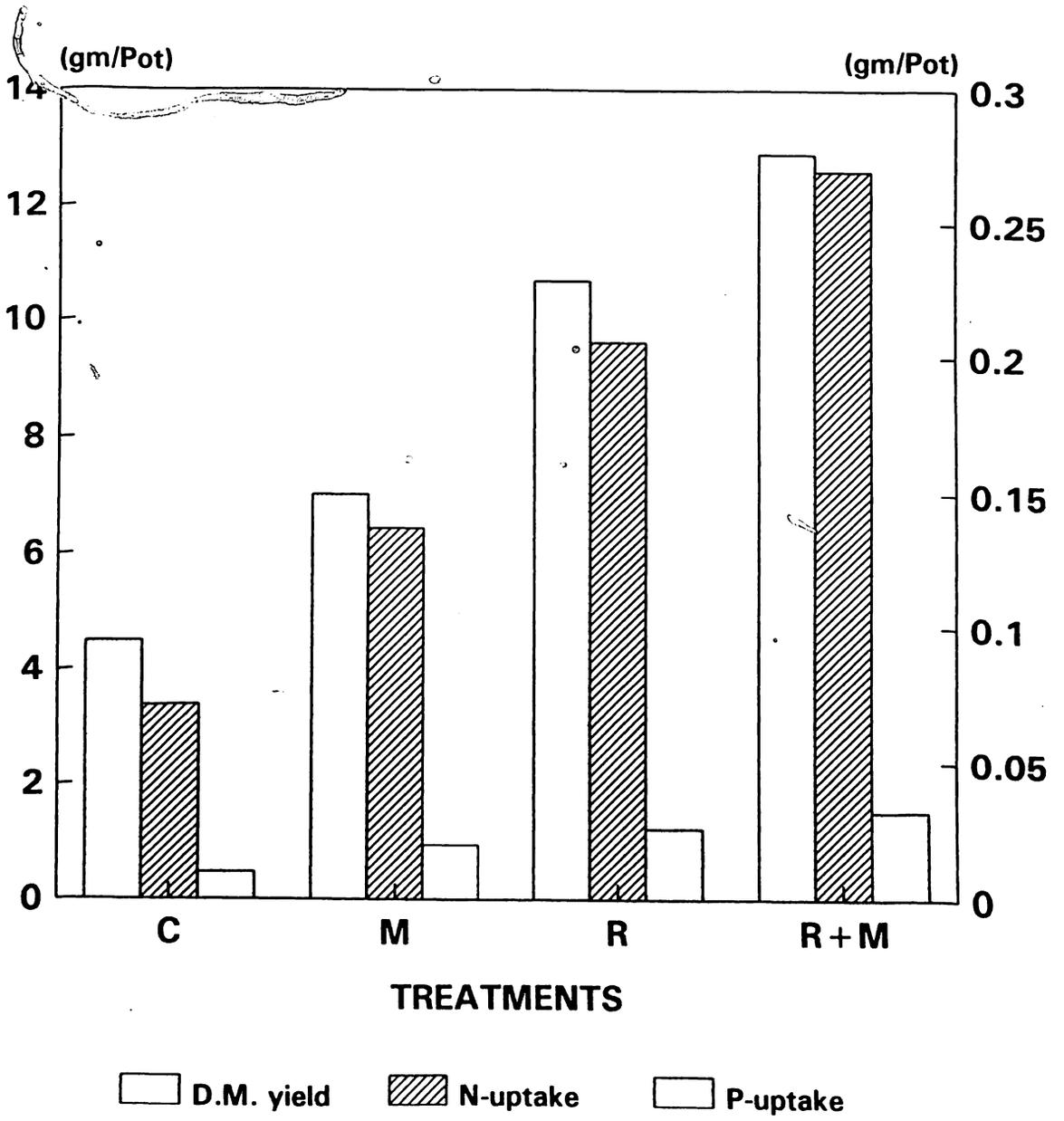

Fig. 5 Dry matter yield and N-P uptake by greengram as influenced by various treatments.

nutrients through their roots depending on the total absorption surface offered by the roots. It is now well established that mycorrhizae can improve the P-nutrition of a host particularly in low fertility due to exploration of soil by the external hyphae beyond the root hairs and P-depletion zone. Increase uptake of P is not the only effect of VA-mycorrhizal fungi on plant growth but they also stimulate the plant uptake of Zn, Cu, S, K and Ca although not as markedly as P (Cooper and Tinker, 1978). In some experiments mycorrhizal fungi have been shown to trap organic and inorganic P sources in soil which are normally unavailable to non-mycorrhizal plant (Powell, 1979). In the present investigation the roots of green gram under dual inoculation was highly infected (Plate- 10 & 11). Probably such infection might have mobilised nutrients in more quantity under the treatments. Vesicles are produced by the internal mycellium in the root cortex but mostly inter cellularly. They are regarded as structures of storage. The arbuscules are formed inter cellularly and ensure an extremely wide contact between the two symbionts but are short lived and digested by host few days after their formation (Sudhir Chandra, 1992).

The findings of the present investigations focused a beam of light regarding the extent to which dual inoculation of Rhizobium and VA-mycorrhiza becomes highly beneficial in the cultivation of greengram and suggested that an effective

Plate.10. Endophytic spores of *Glomus fasciculatum* showing oil globules and hyphal attachment in infected greengram roots.

Plate.11. Root segment of greengram showing typical VAM infection.

strain of Rhizobium and VA-mycorrhiza should be co-inoculated in the cultivation of green gram crop in low fertile light textured lateritic soil ammended with lime. It is hoped that the present study would assist in popularizing legume bacteria and introducing VA-mycorrhiza for co-inoculation in a large scale among the poor farmers of the state to get high return from the cultivation of legumes.

CHAPTER-V

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

During the present investigation, pot culture studies and field experiment were made to evaluate the sole effect of inoculants over uninoculated control and VAM-Rhizobium interaction in greengram (*Vigna radiata* L.) for productivity, N- uptake, dry weight of plants, root volume, nodulation, content of haemoglobin in nodules, content of chlorophyll in leaves and laboratory techniques were used to acertain the infection in infected roots. The soil of field experiments and that used for potculture experiments belong to low fertile light textured acid lateritic group (Ustalf ochrept). No fertilizer was added to field experiments conducted during the Rabi season of 1993-94 in two locations viz: Baghamari and Pubusahi in Khurda district. But phosphate @ 20kg P/ha was applied in pot experiments conducted in the wirenet house of the Deptt. of Agril chemistry, Soil Sc. and Biochemistry, College of Agriculture, Bhubaneswar. Unammended lateritic soil was used in the first pot culture experiment whereas the soil was amended with lime and FYM in second pot experiment.

VA-mycorrhiza (*Glomus fasciculatum*) and *Rhizobium sp.*(M-14 strain) were used as test inoculants. One packet of VAM mother culture was procured from the Deptt. of microbiology, University of Agricultural science, G.K.V.K. campus, Bangalore and subsequently it was multiplied in the rhizosphere zone of guinea grass (*Panicum maximum*) grown in pots containing light textured lateritic soil. Soilbased-root associated VA-mycorrhiza was collected in polythene packets for further use in pot culture experiments and field experiments. The spore load was examined in the laboratory and found to be 10-14 g^{-1} of soil. Broth culture of Rhizobiu (M-14 strain) was obtained from Regional Bio-fertilizer Development centre, Bhubaneswar was inoculated to the plants in pot experiments and the lignite based Rhizobium culture available in polythene packet obtained from Hindustan Biofertilizers Ltd., Pubusahi, Khurda was used in the field experiment at both the locations.

Treatments included uninoculated control (c), Mycorrhiza (M), Rhizobium (R) and (R+M).

Experimental findings under field test showed a positive response of greengram cv: Nayagarh local to either sole or dual inoculation of VAM and Rhizobium. Treatment

receiving dual inoculation of VAM and Rhizobium increased the plant height in both the locations showing maximum of 19.7cm at Pubusahi location and 32.6cm at Baghamari against the corresponding lowest plant height in uninoculated control being 10.4cm at former and 13.3 cm at latter. The number of plants per m^2 area survived in the treatment of dual inoculation was highest for both the location being 35% and 70% more than that of uninoculated control plots at Pubusahi and Baghamari location respectively indicating resistance of inoculated and infected plants to overcome the stress conditions in the field. The nodule number increased by 110% over control at Pubusahi and by 91.6% at Baghamari location in R treatment but when supplemented with mycorrhiza the nodule number increased to 125% in former and 133% in the latter. The grain yield increased by 50% at Baghamari soil and the same was about 3 times in R+M treatment (10.4 Q/ha) than that due to control (3.19 Q/ha) obtained at Pubusahi location.

The overall growth of plants in potculture experiment-I was poor. However, the data for dry matter yield and relative yield response indicated that the drymatter yield increased significantly over control and was highest (391.80 mg) in the pot receiving rhizobial inoculation in the presence of phosphorus. With respect to the yield response, this

increase was by 37.6% over uninoculated control without phosphate fertilization.

In the pot culture experiment-II, the dual inoculation of Rhizobium and VA-mycorrhiza to greengram cv. sujata grown in the soil amended with lime and FYM was found to increase over the control, the dry matter yield (4.4 to 12.9 gm per pot), root volume (2.6 to 22.8cc per pot), the nodule number (2.6 to 45 per plant), N-uptake (0.072 to 0.27 gm N per pot), P-uptake (0.01 to 0.03 mg p per pot), content of haemoglobin in nodule (1.227 to 3.008 mg per fresh weight), Chlorophyll in leaves (0.826 to 1.254 mg per gm fresh weight) during the period of seven weeks growth under study. The mycorrhizal inoculated greengram roots were found heavily infected as evidenced from the vesicles formed in the cortical cells. The response of greengram in the cv Sujata with respect to yield and yield attributes to inoculants under various treatments was in order of (R+M) > R>M>C.

CONCLUSION:

1. Inoculation of Rhizobium culture increased the nodule number in the greengram roots.
2. Dual inoculation of Rhizobium and mycorrhiza further improved the nodulation and especially the number of effective nodules in greengram.

3. Inoculation of VA-mycorrhiza (*Glomus fasciculatum*) and Rhizobium inoculated either separately or in combination increased the root volume and drymatter yield of greengram.

4. The N-P uptake by green gram improved due to inoculation of VA-mycorrhiza and Rhizobium.

5. The content of haemoglobin in nodule and chlorophyll in leaves of greengram increased due to inoculation.

6. Dual inoculation of greengram with VA-mycorrhiza and Rhizobium increased the survival percentage of greengram plants in the field.

7. The soil ammended with lime and FYM improved the response of greengram to inoculation of VA-mycorrhiza and Rhizobium.

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*Original not seen

APPENDICES

APPENDIX - I

Mean weekly temperature and relative humidity during growing period of greengram in potculture Experiment-I

Sl.No (weeks)	Period (week)	Temperature (°C)			Relative humidity (%)
		Maximum	Minimum	Mean	
1st	6.1.94-8.1.94	30.26	16.96	23.61	67.17
2nd	9.1.94-15.194	29.65	14.385	22.01	60.57
3rd	16.1.94-22.1.94	27.31	14.557	20.93	62.50
4th	23.1.94-29.1.94	30.42	15.28	22.34	61.35
5th	30.1.94-5.2.94	29.31	17.31	23.34	68.06
6th	6.2.94-12.2.94	29.50	19.02	24.26	70.07
7th	13.2.94-19.2.94	29.47	20.42	24.94	77.35
8th	20.2.94-26.2.94	30.91	19.62	25.26	69.21
9th	27.2.94-5.3.94	32.78	18.54	25.78	61.20
10th	6.3.94-9.3.94	35.45	21.35	28.40	61.57

APPENDIX - II

Mean weekly temperature and relative humidity during growing period of greengram in potculture Experiment-II

Sl.No (weeks)	Period (week)	Temperature (°C)			Relative humidity (%)
		Maximum	Minimum	Mean	
1st	5.5.94-7.5.94	43.03	27.1	35.06	57.66
2nd	8.5.94-14.5.94	40.85	27.81	34.33	67.06
3rd	15.5.94-21.5.94	38.4	26.57	32.48	69.57
4th	22.5.94-28.5.94	37.05	26.22	31.63	72.92
5th	29.5.94-4.6.94	37.40	28.07	32.73	70.99
6th	5.6.94-11.6.94	37.27	27.58	32.40	72.24
7th	12.6.94-18.6.94	31.60	24.91	28.25	87.63
8th	19.6.94-23.6.94	29.96	23.94	26.95	83.20