EVALUATION OF THE EFFICIENCY OF SOME BACTERIAL INOCULANTS

HARISH KUMAR ACHARAYA

THESIS

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By
HARISH KUMAR ACHARAYA
May, 1977

UNIVERSITY OF UDAIPUR

CERTIFICATE I

MAY, 16 1977

THIS IS TO CERTIFY THAT MR.HARISH KUMAR ACHARAYA HAS SUCCESSFULLY COMPLETED THE PRELIMINARY EXAMINATION HELD ON 8th MAY, 1976 AS REQUIRED UNDER THE REGULATIONS FOR DOCTOR OF PHILOSOPHY.

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DATED MAY, 16 1977

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ADVISOR

HEAD OF THE DEPARTMENT

DEAN
RAJASTHAN COLLEGE OF AGRICULTURE
UDAIPUR

MAJOR ADVISOR

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HKAcharya

Udaipur
May 16, 1977

(HARISH KUMAR ACHARAYA)

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INTRODUCTION

INTRODUCTION

In recent years after energy crisis, attention of agricultural scientists has been diverted towards substitutes for inorganic fertilizers. A number of inoculants are known to improve the overall yields of crops, and for legume particularly Rhizobium inoculation is considered a must. Popularization of the bacterial inoculants has led to problems connected with mass production of the concerned inoculum. Peat is a costly product, and the use of lignite as a base also brings in the question of transport of material from long distances. Comparison of some local organic matter sources for their efficiency as base material appears to be reasonably important. A number of organic matter sources are easily available which include farmyard manure, cowdung cake (Kanda) and leafmould for the purpose of mass production of culture packets. In the present investigation, studies were directed to study build up of number of bacteria under various times of storage. The ability of the rhizobia to survive out side the host is of considerable importance in the context of the maintenance of population on the surface of inoculated seeds and successful establishment in soil (Vincent, 1958; Thompson, 1960; Burton, 1967; Date, 1968; Nair, 1970; Chakrapani and Tilak, 1974 and Dube et al., 1975).

Efficiency of bacterial inoculation under field conditions has also been tested using some of these inoculants with cowpea and mung under pelleted and un-pelletized conditions to fully exploit the benefits from inoculations. It is known that considerable improvement is brought about by pelleting. Radcliffe et al. (1967), Date (1968), Iswaran (1969), Goel (1973), Kanzaria (1974) and Graham et al. (1974) and others have also reported beneficial role of pelleting materials in increasing efficiency of inoculants. The strains tested were those of Rhizobium (mung), Rhizobium (cowpea) and Bacillus megaterium var. phosphaticum. These evaluations have led to some fruitful revealations to provide a realistic basis for carrying out bacterial fertilization.

Efficiency of Azotobacter chroococcum and Bacillus megaterium var. phosphaticum in improving crop yields was also examined in a field study in a bid to evaluate levels of improvement of the yields under field conditions using wheat as a test crop. It has been possible to usefully employ the combinations of Azotobacter chroococcum and Bacillus megaterium var. phosphaticum in a field trial using three levels of nitrogen and three levels of phosphorus. These results have thrown light on the role of bacterial inoculants in improving crop yields. Recently bacterial inoculants like Azotobacter chroococcum in combination with Bacillus megaterium var. phosphaticum have provided a useful improvements in yields in operational research area Chittorgarh (about fifty acres trial). Some useful results have also been reported from Bhilwara. The present investigations were planned with the intention of studying the performance of *Bacillus megaterium* var. *phosphaticum* and *Azotobacter chroococcum* in combination under different fertility gradients. It was intended to provide the best combination of fertilizers level and inoculants.

It is hoped that these findings will provide proper understanding on the use of bacterial inoculants in Udaipur division.

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CHAPTER I

(a) Effect of different inoculum carriers on total bacterial counts.

(b) Effect of various inoculum carriers and pelleting materials on the yield, nitrogen and phosphorus uptake by mung and cowpea.

MATERIAL AND METHODS

In the present studies for evaluation of efficiency of bacterial inoculants *Rhizobium* spp. (mung and cowpea) and *Bacillus megaterium* var. *phosphaticum* were used in two field experiments.

Experiment No.1

Effect of different inoculum carriers on survival of *Rhizobium* spp. (mung and cowpea) and *Bacillus megaterium* var. *phosphaticum*.

Description of carrier sources:

1. Farmyard manure was collected from live stock farm, department of Animal Husbandry and Animal Sciences, Rajasthan College of Agriculture, Udaipur.
2. Ordinary coal was collected from a local depot, crushed and used .
3. Cow dung cake (Kanda) was obtained from local market.
4. Leaf mould was collected from Sajjan Niwas Garden, Udaipur.

All the above mentioned carriers were processed to pass through 300 mesh. Prior to their use the carriers were sterilized for 3 days at 15 lb psi for one hour daily.

Cultures:

Rhizobium strains (for mung as well as for cowpea) were obtained from culture collection, division of Microbiology I.A.R.I., New Delhi-12.

Bacillus megaterium var. *phosphaticum* strain was obtained from U.S.S.R., (all Union Collection of Micro-organisms, Institute of Microbiology, U.S.S.R. Academy Sciences, Moscow, B-133, U.S.S.R.).

The yeast extract Mannitol Agar (YEMA) medium (Fred *et al.*, 1932) was used for maintaining *Rhizobium* spp. (mung and cowpea) having the following composition:

Mannitol	10.0 g
Dipotassium hydrogen phosphate	0.5 g
Sodium chloride	0.1 g
Calcium carbonate	4.0 g
Yeast extract	0.4 g
Agar	15.0 g
Distilled water	1000.0 ml
pH adjusted	7.0

Bacillus megaterium was maintained on Pikovaskaia medium (Pikovaskaia, 1948) having the following composition:

Glucose	10.0 g
Calcium phosphate	5.0 g
Ammonium sulphate	0.5 g
Sodium chloride	0.2 g
Magnesium sulphate	0.1 g

Potassium chloride	0.2 g
Yeast extract	0.5 g
Manganese sulphate	Traces
Ferrous sulphate	Traces
Agar	15.0
Distilled water	1000.0 ml
pH adjusted	7.0

In further text *Rhizobium* spp. for mung has been denoted as *Rhizobium* (mung), *Rhizobium* spp. for cowpea as *Rhizobium* (cowpea) while *Bacillus* *megaterium* var. *phosphaticum* as *B.megaterium*.

Details of treatments:

Each of the four inoculum carriers was inoculated separately with *Rhizobium* (mung), *Rhizobium* (cowpea) and *B.megaterium* and the mixtures were incubated at 28°C for 1,2,3 and 4 weeks.

Total treatments	-	16
Replications	-	3

Inoculation:

In 250 g of each carrier material, 50 ml of sterile water and 50 ml of heavy suspensions of bacterial culture having following population were added:

Rhizobium (mung)	$1X10^9$ per ml
Rhizobium(cowpea)	$1X10^9$ per ml
B.megaterium	$9X10^8$ per ml

These were throughly mixed and the carriers so inoculated were incubated at ± 28°C in plastic containers (which were sterilized using $HgCl_2$ 0.1 per cent followed by several rinsings with sterile water before use) for four weeks. Periodical counts were made by pour plate method.using the serial dilution technique. For Rhizobium (mung) and Rhizobium (cowpea), congored @ 10 ml of 1/400 sterilized aqueous solution per one litre (YEMA) medium were added asceptically before use.

Experiment No.2:

Effect of various inoculum carriers and materials on yield, nitrogen and phosphorus uptake by mung and cowpea fodder crop under field conditions.

To determine the effect of different inoculum carriers and pelleting materials on efficiency and survival of bacterial inoculants; viz., Rhizobium (mung), Rhizobium (cowpea) and B.megaterium in improving the yields, and uptake of nitrogen and phosphorus, mung (*Phaseolus aureus*) and cowpea (*Vigna sinensis*) were taken as test crops. Udaipur is situated at Latitude of 24.3°North and Longitude of 73.4°East at an altitude of 583 metres above mean sea level. Climate of this area is tropical which is characterized by winters which are not severe and summers of average intensity. Relative humidity is comparatively higher during the month of July to September. Details of the experiment carried out are as follows:

Site:

This experiment was carried out at Agronomy Farm, Rajasthan College of Agriculture, Udaipur. Soil characteristics of experimental plot are given in Table 1.

Table 1. Chemical, physical and microbiological analysis of soil

pH	8.2
Electrical conductivity	0.9 mmhos/cm
Organic carbon	0.975 per cent
Olsen's available P	12.1 ppm
$CaCO_3$	3.10 per cent
Sand	43.5 per cent
Silt	24.0 per cent
Clay	32.5 per cent
Actinomycetes counts	5.7×10^6 per g
Bacteria counts	6.9×10^6 per g
Fungi counts	16.1×10^3 per g

Crops:

Mung (*Phaseolus aureus*) var. Pusa Baisakhi

Cowpea (*Vigna sinensis*) var. Local.

Details of treatments:

Main treatments	--	9
Sub-treatments	--	5
Replications	--	3
Number of plots	--	135 (for mung) and 120 (for cowpea)
Plot size	--	4.0 X 1.25 m

Season - Kharif 1975

Main plots were earmarked for eight treatments comprising four carriers inoculated with *Rhizobium* (mung), *Rhizobium*(cowpea) and four carriers inoculated with *Rhizobium* (mung), *Rhizobium* (cowpea) in combination with *B.megaterium* for mung as well as cowpea crops. One main plot per replication was assigned to assess the inoculum buildup in terms of inherent (natural)strains of *Rhizobium* (mung) inhabiting the concerned field. Sub-plots were alloted to different pelleting materials. Treatment schedule was as follows:

1. Farmyard manure + *Rhizobium* (mung)
2. Coal + *Rhizobium* (mung)
3. Cow dung cake (Kanda) + *Rhizobium* (mung)
4. Leafmould + *Rhizobium* (mung)
5. Farmyard manure + *Rhizobium*(mung) + *B.megaterium*
6. Coal + *Rhizobium* (mung) + *B.megaterium*
7. Cow dung cake (Kanda) + Rhizobium (mung)+ *B.megaterium*
8. Leafmould + *Rhizobium* (mung) + *B.megaterium*

9. In addition to these treatments in case of mung an additional treatment of uninoculated seeds was kept.

Sub-treatments:

1. Jaggary 25 per cent solution as sticking material
2. Gum arabic 50 per cent + sodium molybdate
3. Gum arabic 50 per cent + superphosphate
4. Gum arabic 50 per cent + gypsum
5. Gum arabic 50 per cent + mixture of sodium molybdate + superphosphate + gypsum.

Inoculants:

The strains of Rhizobium (mung), Rhizobium (cowpea) and B.megaterium used were the same as in previous experiment.

Carriers:

Farmyard manure, coal powder,'kanda' and leaf mould were used as carriers.

Inoculation of carriers:

Sterilized carriers were inoculated by mixing thoroughly 250 g of each carriers with 50 ml of heavy suspension of 24 hours old Rhizobium (mung) broth ($10X10^9$ per ml), 72 hrs old Rhizobium (cowpea) broth ($1X10^9$ per ml) separately followed by addition of 50 ml of sterilized water. Similarly each carrier was thoroughly mixed with 50 ml of Rhizobium (mung) + 50 ml of B.megaterium, and 50 ml of Rhizobium (cowpea) + 50 ml of B.megaterium culture separately as per treatment schedule. Inoculated carriers were incubated at 28°C for 4 weeks.

Seed inoculation and pelleting:

White pure gum of Acacia arabica was purchased from the market and was powdered properly so as to dissolve easily to from 50 per cent paste. The inoculation of the mung and cowpea seeds with Rhizobium (mung) and Rhizobium (cowpea) was done according to the method received from the I.A.R.I., New Delhi. Accordingly, a slurry was made by adding about 70 gm of each inoculated carrier separately in half litre of

25 per cent jaggery solution, which was prepared by boiling jaggery for half an hour and subsequently cooling. The seeds which were coated with jaggery were mixed in this slurry thoroughly so that all the seeds were uniformly coated with culture. Similarly seeds were inoculated with Rhizobium (mung) + B.megaterium, and Rhizobium (cowpea) + B.megaterium respectively. Inoculated seeds were then dried in shade by spreading on clean newspaper sheets. The remaining seeds which were to be inoculated with Rhizobium (mung) with and without B.megaterium followed by pelleting with 50 per cent gum + sodium molybdate, 50 per cent gum + superphosphate, 50 per cent gum + gypsum and 50 per cent gum + mixture of pelleting materials (1:1:1 , sodium molybdate, superphosphate and gypsum) were mixed with 50 per cent gum arabic solution followed by pelleting according to treatment schedule.

Pelleting was done by the method of Brockwell (1962). The seeds inoculated with inoculum carriers as above (in gum arabic) were placed in very fine powder of pelleting material (passed through 300 mesh and kept as a thick layer). Some more powder was added on them and the seeds were rolled in the powder, until each seed grain was fully coated and became dry and discrete. The pelleted seeds were spread on a clean sheet of paper and allowed to dry in shade for some time for hardening. Excess of pelleting material was removed by sieving. The seeds processed with different treatment materials were then sown in field .

The following seed treatments were carried out:

1. Farmyard manure + Rhizobium (mung) and coated with 25 % jaggery
2. Farmyard manure + Rhizobium (mung) and pelleted with 50 % gum + sodium molybdate
3. Farmyard manure + Rhizobium (mung) and pelleted with 50 % gum + superphosphate
4. Farmyard manure + Rhizobium (mung) and pelleted with 50 % gum + gypsum
5. Farmyard manure+ Rhizobium (mung) and pelleted with 50 % gum + sodium molybdate + superphosphate + gypsum
6. Coal powder + Rhizobium (mung) and coated with 25 % jaggery
7. Coal powder + Rhizobium (mung) and pelleted with 50 % gum + sodium molybdate
8. Coal powder + Rhizobium (mung) and pelleted with 50 % gum + superphosphate
9. Coal powder + Rhizobium (mung) and pelleted with 50 % gum + gypsum
10. Coal powder + Rhizobium (mung) and pelleted with 50 % gum + sodium molybdate + superphosphate + gypsum
11. Kanda + Rhizobium (mung) and coated with 25 % jaggery
12. Kanda + Rhizobium (mung) and pelleted with 50 % gum + sodium molybdate
13. Kanda + Rhizobium (mung) and pelleted with 50 % gum + superphosphate
14. Kanda + Rhizobium (mung) and pelleted with 50 % gum +gypsum
15. Kanda + Rhizobium (mung) and pelleted with 50 % gum + sodium molybdate + superphosphate + gypsum

16. Leaf mould + Rhizobium (mung) and coated with 25 % jaggery

17. Leaf mould + Rhizobium (mung) and pelleted with 50 % gum + sodium molybdate

18. Leaf mould + Rhizobium (mung) and pelleted with 50 % gum + superphosphate

19. Leaf mould + Rhizobium (mung) and pelleted with 50 % gum + gypsum

20. Leaf mould + Rhizobium (mung) and pelleted with 50 % gum + sodium molybdate + superphosphate + gypsum

21. Farmyard manure + Rhizobium (mung) + B.megaterium and coated with 25 % jaggery

22. Farmyard manure + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + sodium molybdate

23. Farmyard manure + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + superphosphate

24. Farmyard manure + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + gypsum

25. Farmyard manure + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + sodium molybdate + superphosphate+ gypsum

26. Coal powder + Rhizobium (mung) + B.megaterium and coated with 25 % jaggery

27. Coal powder + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + sodium molybdate

28. Coal powder + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + superphosphate

29. Coal powder + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + gypsum

30. Coal powder + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + sodium molybdate + superphosphate+ gypsum

31. Kanda + Rhizobium (mung) + B.megaterium and coated with 25 % jaggery

32. Kanda + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + sodium molybdate.

33. Kanda + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + superphosphate

34. Kanda + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + gypsum

35. Kanda + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + sodium molybdate + superphosphate + gypsum

36. Leaf mould + Rhizobium (mung) + B.megaterium and coated with 25 % jaggery

37. Leaf mould + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + sodium molybdate

38. Leaf mould + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + superphosphate

39. Leaf mould + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + gypsum

40. Leaf mould + Rhizobium (mung) + B.megaterium and pelleted with 50 % gum + sodium molybdate + superphosphate + gypsum

41-45 Surface sterilized seeds(Pelleted)

Similar schedule of seed treatments was carried out of cowpea fodder crop except use of surface sterilized seeds.

Land preparation and sowing:

The plots were laid out after one ploughing by tractor followed by cross-harrowing and planking. The furrows were opened by a country plough and mung seeds treated previously and nontreated seeds were dibbled in same manner.

Fertilizer application:

Basal dose of 20 kg P_2O_5/ha (superphosphate) was applied at the time of sowing by drilling.

Other cultural operations:

Agronomic practices recommended for the area were carried out.

Nodulation studies in mung:

Plants were uprooted randomly with care after light irrigation and number of nodules recorded (3 weeks after sowing.).

Harvesting and thrashing:

Mung: Two pickings of dry pods were done after the maturity of pods, and straw was immediately harvested. The thrashing was done sub-plot wise by mannual labour and grain and straw yields were recorded.

Cowpea: Green fodder was cut down after 75 days. Yields were recorded simultaneously. Representative samples were collected sub-plot wise for determination of dry matter.

Collection of samples: Grain and straw samples were collected and prepared separately for chemical analysis.

METHODS

Soil Analysis:

Soil reaction:

The pH was determined using Beckman's pH meter in 1:2.5 soil water suspension.

Electrical conductivity (EC):

Electrical conductivity was determined by solubridge as described by Richards (1954).

Calcium carbonate:

Determination of $CaCO_3$ was carried out as per method of Puri (1963). Five gm of soil was shaken for one hour with 100 ml acetic acid (buffered with 15.8 g of calcium acetate dissolved in 28.6 C.C. glacial acetic acid) and volume was made up to one litre with distilled water. The aliquot (10 ml) was taken from filterate filtered through filter No.1 and titrated with 0.1 NaOH using phenolpthalein indicator. Blank was run simultaneously. The percentage of $CaCO_3$ was calculated as follows:

$$\frac{A - B \times 0.005 \times x \times 100 \times 100}{x \times Y} - 0.74$$

Where : A = Blank reading
B = Sample reading
x = Weight of soil taken
Y = Aliquot taken

Mechanical analysis:

This carried out as per international pipette method described by Piper (1950).

Organic carbon:

This was determined using Walkley Black's method (Jackson, 1967).

Table 2. Composition of media

S.No.	Chemicals	Quantity
1	Streptomycin Rose Bengal Agar	
	Peptone	5.0 g
	Dextrose	10.0 g
	Potassium dihydrogen orthophosphate	1.0 g
	Magnesium sulphate	0.05 g
	Streptomycin	30.00 ug/ml
	Rose Bengal	33.00 ug/ml
	Agar	20.00 g
	Water	1000.00 ml
	pH adjusted	7.00
2.	Nutrient Agar	
	Agar	20.00 g
	Peptone	1.00 g
	Water	1000.00 ml
	pH adjusted	7.00
3.	Dextrose Nitrate Agar	
	Dextrose	1.00 g
	Potassium chloride	0.10 g
	Magnesium sulphate	0.10 g
	Potassium dihydrogen orthophosphate	0.10 g
	Sodium nitrate	0.10 g

Continued Table 2.

S.No.	Chemicals	Quantity
	Agar	15.00 g
	Water	1000.00 ml
	pH adjusted	7.00

Available phosphorus:

This was determined by Olsen's Method (1954).

Estimation of microbial population:

Bacteria, fungi and actinomycete population in soil was estimated using dilution pour plate technique.

Dilution ranges of the order of 10^{-2} to 10^{-4} for fungi, 10^{-4} to 10^{-6} for actinomycetes and bacteria respectively were used. The media (composition in Table 2) used were as follows:

(i) Streptomycin: Rose Bengal Agar (Martin, 1950) for fungi.

(ii) Nutrient Agar (Martin, 1950) for bacteria

(iii) Dextrose Nitrate Agar (Kenknight and Muneie, 1939) for actinomycetes.

The plates were incubated at 28°C for growth of organisms.

Plant analysis:

Determination of nitrogen:

Digestion of plant material was carried out by taking 100 mg of grain or straw sample in 30 ml Kjeldahl's flasks to which 2 ml of concentrated H_2SO_4 were added and heating was carried out on an electric heater for 3 minutes followed by addition of 0.2 ml of hydrogen peroxide till the contents became colourless. On completion of digestion, the contents of the flask were transferred to 100 ml volumetric flasks and volume made up in each flask. Five ml aliquote in case of grain and 10 ml aliquots in case of straw were pipetted out in 50 ml volumetric flasks and colour developed with Nesseler's reagent as per method of Snell and Snell (1955).

Estimation of phosphorus:

The digestion of 1 g of plant material (either straw or grain) was carried out by triacid digestion mixture preceded by digestion with concentrated nitric acid as described by Jackson (1967). The phosphorus was determined colorimetrically as per Dickman and Bray's (1940) procedure.

Uptake of nutrients:

Total uptake of nutrients, namely nitrogen and phosphorus was calculated by adding up the amount of uptake of each nutrient through grain and straw (based on total yield per hacter).

Statistical analysis:

All the data of different experiments for grain and straw yield as well as nutrients uptake were statistically processed in order to give proper interpertation to the differences obtained under the influence of different treatments. The analysis of variance was conducted (Fisher, 1950) and comparisons among means were made by calculating critical difference. The 'F' test was carried out to see the significance between differences due to the treatments.

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RESULTS AND DISCUSSION

Effect of different carriers and periods of storage on mean survival of Rhizobium (mung), Rhizobium (cowpea) and B.megaterium:

The data given in Table 3 indicates the mean survival of Rhizobium (mung) in four carriers (farmyard manure, coal powder, 'kanda' and leaf mould). Coal powder was found less effective carrier whereas the survival was markedly significant in 'kanda' followed by leaf mould. As regards the influence of different storage period on mean survival of rhizobia, it was found that after fourth week there was decline in population (Table 4). When the data presented in Table 5 (describing the effect of farmyard manure, coal powder, 'kanda' and leaf mould during different intervals of storage period) were examined; highest mean survival of Rhizobium (mung) was found in 'kanda' followed by leaf mould whereas farmyard manure and coal powder were found inferior. More or less similar trends were also obtained for survival of Rhizobium (cowpea) in farmyard manure, coal powder, 'kanda' and leaf mould except 'kanda' which was found to be the best carrier (Table 6). When effect of different intervals of time of storage was examined (Table 7), high mean survival was found during fourth week. Highest counts were obtained for 'kanda' followed by leaf mould after fourth weeks; whereas, decreasing trend were found for farmyard manure

Table 3. Effect of different carriers on mean survival of *Rhizobium* (mung)

Carriers	Mean value of survival ($X\ 10^9/g$)
Farmyard manure	0.43
Coal	0.25
Kanda	0.63
Leaf mould	0.56

S.Ed. ± - 0.0054 ; C.D.at 5% - 0.011

Table 4. Effect of periodsof storage on mean survival of *Rhizobium* (mung)

	Period (weeks)			
	1	2	3	4
Survival ($X\ 10^9/g$)	0.34	0.42	0.54	0.58

S.Ed. ± - 0.0054 ; C.D. at 5 % - 0.011

Table 5. Effect of different carriers and periods of storage on mean survival of *Rhizobium* (mung)

Carriers	Period (weeks)				Mean
	1	2	3	4	
Farmyard manure	0.31	0.40	0.46	0.55	0.43
Coal	0.25	0.29	0.26	0;22	0.25
Kanda	0.40	0.52	0.76	0.82	0.63
Leaf mould	0.36	0.46	0.67	0.56	0.56
Mean	0.34	0.42	0.54	0.58	

S.Ed. ± - 0.0109 ; C.D. at 5% - 0.0218

Table 6. Effect of different carriers on mean survival of *Rhizobium* (cowpea)

Carriers	Population ($X\ 10^9/g$)
Farmyard manure	0.31
Coal	0.18
Kanda	0.56
Leaf mould	0.45

S.Ed. ± - 0.0077 ; C.D. at 5% - 0.0157

Table 7. Effect of periods of storage on mean survival of Rhizobium (cowpea)

	Period (weeks)			
	1	2	3	4
Survival($X10^9/g$)	0.24	0.31	0.42	0.51

S.Ed. ± - 0.0077 ; C.D. at 5% - 0.0157

Table 8. Effect of different carriers and periods of storage on mean survival of *Rhizobium* (cowpea)

Carriers	Period (weeks)				Mean
	1	2	3	4	
Farmyard manure	0.21	0.27	0.32	0.41	0.31
Coal	0.14	0.17	0.19	0.19	0.18
Kanda	0.31	0.44	0.68	0.81	0.56
Leaf mould	0.29	0.39	0.51	0.63	0.45
Mean	0.24	0.31	0.42	0.51	

S.Ed. ± - 0.0154 ; C.D. at 5% - 0.0308

Table 9. Effect of different carriers on mean survival of *Bacillus megaterium*

Carriers	Mean value of survival ($X10^9/g$)
Farmyard manure	0.49
Coal	0.35
Kanda	0.67
Leaf mould	0.59
S.Ed. ± - 0.0067	C.D. at 5% - - 0.0137

Table 10. Effect of different time intervals on mean survival of *Bacillus megaterium*

	Period (weeks)			
	1	2	3	4
Survival ($X10^9/g$)	0.39	0.51	0.59	0.60

S.Ed. ± - 0.0067 ; C.D. at 5% - 0.0137

Table 11. Effect of different carriers and periods of storage on mean survival of *Bacillus megaterium*

Carriers	Period (weeks)				Mean
	1	2	3	4	
Farmyard manure	0.35	0.44	0.56	0.62	0.49
Coal	0.29	0.41	0.43	0.27	0.35
Kanda	0.49	0.64	0.76	0.81	0.67
Leaf mould	0.46	0.57	0.64	0.71	0.59
Mean	0.39	0.51	0.59	0.60	

S.Ed. ± - 0.136 ; C.D. at 5% - 0.0272

and coal powder (Table 8). The data summarized in Table 9 describes the effect of farmyard manure, coal powder, 'kanda' and leaf mould on mean survival of *Bacillus megaterium* var. *phosphaticum* 'kanda'was found best carrier. Mean survival of *B.megaterium* was found highest after fourth week (Table 10). When effects of farmyard manure, coal powder, 'kanda' and leaf mould (during different intervals of storage period on mean survival of *B.megaterium*) were examined, 'kanda' was found to have highest count followed by leaf mould after fourth week (Table 11.)

Though bacterial inoculants have received less attention in the country, it is becoming increasingly clear that shortage of fertilizer can be met by stepping up bacterial fertilization. Some organisms have been known to increase phosphate availability by solubilizing different phosphate compounds from soil. Bacteria and fungi often associated with the rhizosphere of plants, are able to dissolve calcium phosphate and pyrophosphate (Manson and Tomashevska, 1955). Swaby and Sperber (1958) showed that spp. of genera *Arthrobacter*, *Pseudomonas*, *Xanthomonas*, *Achromobacter*, *Flavobacterium*, *Streptomyces*, *Asperigillus*, and *Penicillium* released phosphate from hydroxyapatite by producing organic acids. Numerious bacterial types including *Bacillus subtilis*, *B.cereus* var. *mycoides* (*B.mycoides*), *B.megaterium* and *B.mesenterius* release soluble phosphorus from calcium and iron phosphates, calcium glycerophosphate and lecithin.

Phosphorus uptake by *Pinus radiata* inoculated with *mycorhiza* was greater than without it (Harley, 1952). Cooper (1959), Surman (1960), Szember (1960), Goswami and Sen (1962), Das (1963), Paul and Sundra Rao (1971) have also reported increased availability of phosphate after inoculation with certain organisms. Smith *et al*. (1961,1962) on the basis of their work remarked that experimentation of workers from Russia was not scrutinized statistically and results are open to questions. However, since then a number of workers in India and abroad have reported on the beneficial effects of bacterial fertilizers. Sharma and Singh (1971) while working with phosphobacterin noted significant increase in grain yield and phosphorus uptake by rice. Dhyan Sarup (1969), Panwar (1970), Uarova (1956), Manolache and Iordachescu (1961), Stefan and Boti (1961), Kudzin *et al*. (1970), Kudashev (1956), Mishustin and Naumova (1956), Kanzaria (1974), Paul and Sundara Rao (1971) and number of other workers also reported similar findings on the efficiency of phosphate solubilizing organisms.

Rhizobium is known to be quite potent in legume and its combination with phosphate solubilizers may provide a good situation (Kanzaria, 1974). The present studies were carried out with this objective in view and mung and cowpea were chosen for the purpose. To overcome ineffective inoculation of seed and unfavourable environments in soil some basis have been used as inoculum carriers and different pelleting materials have also been used along with gum *arabic* (50 % paste) as adhesive coating to screen out the best possible combination of inoculants, inoculant carrier and pelleting material. Yield increases due to seed inoculation of legume seeds with rhizobia have been recorded as high as 53 per cent in case of pea, 49 per cent in gram, 64 per cent in mung, 71 per cent in urd, and 100 per cent in case of cowpea, but at the same time this treatment is reported to have negligible or no effect due to unfavourable conditions existing in the soil for the survival of the organisms (Bains et al.,1970). This difficulty is obviated to a great extent if the seed is pelleted after inoculation because of the ability of rhizobia to survive on seeds and to effectively nodulate their host under this condition. In review of the fact that the leguminous seeds as well as the average soil provides rarely a favourable environment for survival of rhizobia, considerable interest has been focused on rhizobial longivity. These studies had two objectives i.e., (i) developing effective methods of inoculating seeds for sowing under adverse conditions and (ii) improving preinoculation techniques (Burton, 1967). Rhizobia in peat was reported as better equipped to survive on seed than in broth, water, suspension of agar grown cells (Burton, 1964). Thompson (1960) reported inhibition of growth of rhizobia due to toxins (antibiotic) substances of seed coats of legumes. Use of inoculum carriers has been reported beneficial in improving inoculation capabilities (Burton, 1967,Mishustin and Naumova, 1956, Atwal and Sidhu, 1964; Kandasamy and Prasad 1971 ; Burton and Curley, 1965; Iswaran et al.1972; Graham et al. 1974; Chakrapani and Tilak, 1974; Kanzaria, 1974

Nandi and Sinha, 1974; Date, 1968; Dadarwal and Sen, 1971 and Iswaran and Chhonkar, 1971). Certain additives, such as sucrose, dextrin, maltose and gum arabic were also reported as beneficial materials for improvement of rhizobial survival on legume seeds (Burton, 1960, 1964; Hely, 1965; Vincent, 1958) Workers in Australia (Bergersen *et al*., 1958; Brockwell, 1962 and Hely, 1965), in Newzealand (Hasting and Drake, 1960) and California (Holland and Street, 1965) reported good success after incorporating peat base inoculant in a pellet containing pulverized calcite or dolomite lime stone and gum arabic. Pelleting of seeds after inoculation has been reported to be helpful in increasing ability of rhizobia in infecting the host plants (Date, 1968 ; Radcliffe *et al*., 1967; Iswaran and Chhonkar, 1971; Iswaran, 1969; Loneragon *et al*., 1955; Bergersen *et al*., 1958; Hastings and Drake, 1960; Goel, 1973; Brockwell, 1962; Kanzaria, 1974; Graham *et al*., 1974 and Chahal and Rewari, 1975).

Effect of inoculation by Rhizobium (mung) with and without B.megaterium using different carriers on grain yield of mung:

The results presented in Table 12 and Fig.1 described the effect of inoculation with *Rhizobium* (mung) alone and in combination with *B.megaterium* on grain yield using four inoculum carriers viz., farmyard manure, coal powder, cowdung cake 'kanda' and leaf mould. Farmyard manure was found to be the poorest whereas 'kanda' under conditions when *B.megaterium*

Table 12. Effect of inoculation by *Rhizobium* (mung) and *Bacillus megaterium* var. *phosphaticum* through different carriers on grain yield of mung (q/ha)

Cultures	Carriers				Mean
	C_1	C_2	C_3	C_4	
R	6.90	7.34	7.35	7.36	7.24
R B	7.68	7.55	8.65	8.19	8.27
Mean	7.29	7.44	8.00	7.78	
Control	3.93				
S.Ed.±	0.0704				
C.D.at 5%	0.1490				

Table 13. Effect of different pelleting materials on grain yield of mung (q/ha)

	Treatments				
	P_1	P_2	P_3	P_4	P_5
Mean	6.67	7.30	8.26	6.95	7.45
S.Ed. ±	0.045				
C.D.at 5%	0.090				

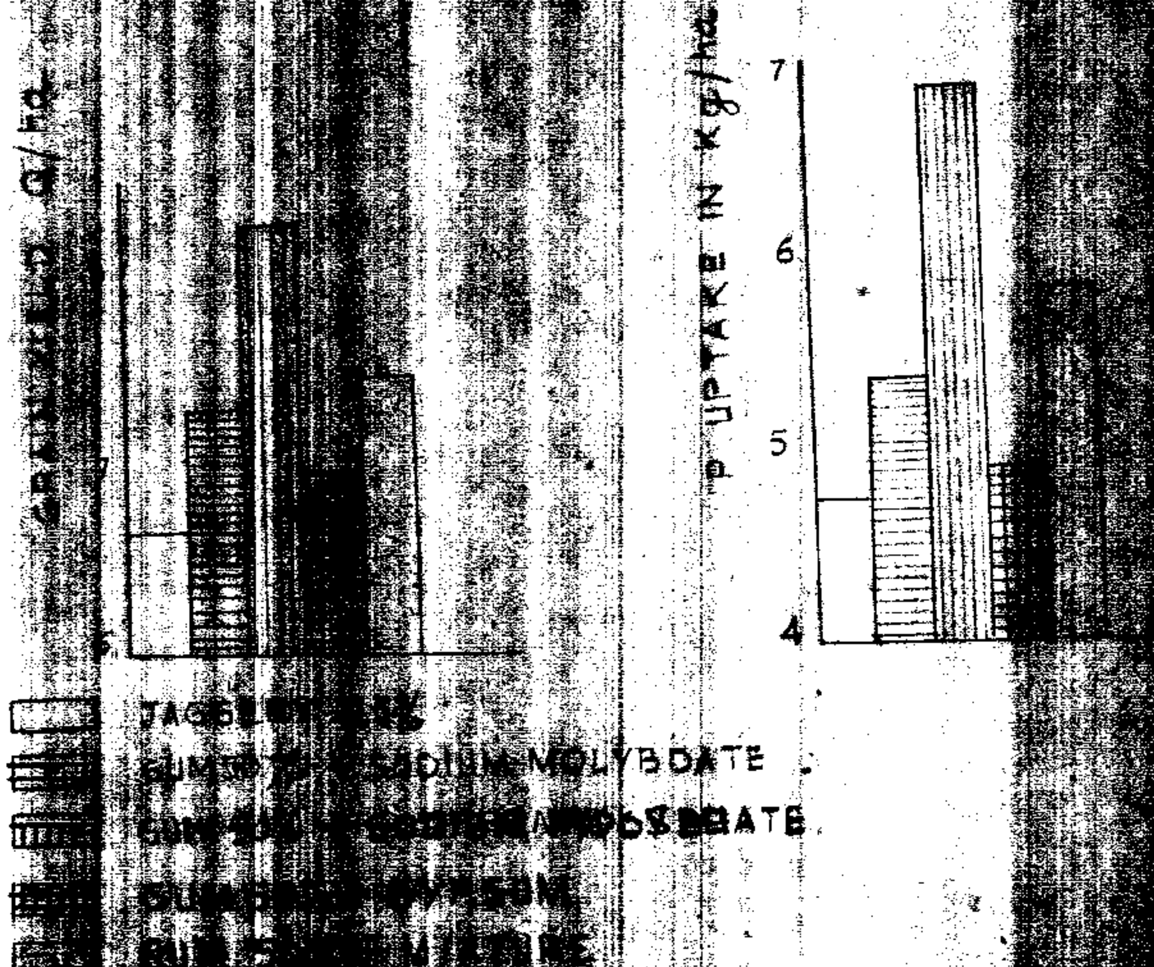
...GATERIUM

... AND B.MEGATERIUM ING...
... YIELD AND PUPTAKE...

was used along with Rhizobium (mung) was found to be the best followed by leaf mould and farmyard manure and coal powder. Thus for legume inoculation using Rhizobium and B.megaterium 'kanda' can be used successfully which is easily available. The utility of 'kanda' lies in the fact that as a result of drying of fresh cowdung and animal dung some organic materials are available which normally decompose during manuring. Bhardwaj and Gaur (1972) have reported growth promoting effect of humic substances on Rhizobium trifolli. The possibility of presence of such substances can not be ruled out and this accounts for superiority of 'kanda' and leaf mould for stimulation of activity of Rhizobium and B.megaterium. Individually farmyard manure, coal, 'kanda' and leaf mould are statistically significant. Also Rhizobium (mung) inoculation is inferior to Rhizobium (mung) when used along with B.megaterium. Thus the results are clear that mixing of B.megaterium with Rhizobium (mung) has a distinct advantage. Bacillus megaterium has been reported to produce vitamin B_{12} which is used as culture on large scale in U.S.A. (Perlman, 1967) In view of the fact that species and strains of rhizobia do not produce enough growth factors, some quantity of these can be desirable for stimulation of rhizobial growth. Chatterjee and Banerjee (1972) have also reported on the influence of B.vitamins on growth and extra cellular accumulation of B.megaterium. Growth promoting substances seem obviously responsible for mutual synergism of rhizobia and B.megaterium.

Effect of pelleting materials on grain yield of mung:

When effects of different pelleting materials are examined, superphosphate along with 50 per cent gum arabic has been found to be the best (Table 13 and Fig.2). It is distinctly superior to the use of jaggery alone, 50 per cent gum + sodium molybdate, 50 per cent gum + superphosphate, 50 per cent gum + gypsum and 50 per cent gum + sodium molybdate + superphosphate + gypsum. These results clearly indicate that addition of superphosphate acts as a booster in improving the activity of micro-organisms. Gum arabic 50 per cent paste has been reported as very much beneficial for its stickness and stimulatory influence for survival of rhizobia (Radcliffe *et al.*, 1967; Graham *et al.*,1974 and Iswaran and Chhonkar, 1971). Thus 'kanda' as the inoculum carrier (inoculated with Rhizobium along with *B.megaterium*) may become effective with 50 per cent gum arabic and superphosphate as pelleting method. These statistically significant results presented in Table 12 and 13 are self explanatory. Work of Rewari *et al.* (1965), Iswaran *et al.* (1971) and Kanzaria (1974) and several other workers has clearly indicated that the addition of phosphate has distinct advantage in improving the activities of *Rhizobium* (mung) and *B.megaterium*. Thus these findings corroborate the work reported here. This has distinct impact upon the technology of usage of bacterial inoculants. If raw cow dung cake after drying is used along with bacterial inoculant like *B.megaterium* and Rhizobium in combination with phosphate (superphosphate) the improvement inyields appears to be possible.



...NG MATERIALS ON ...
... UPTAKE BY MUNG

FIG. 2

Effect of inoculation by Rhizobium (mung) with and without B.megaterium using different carriers on mung straw:yield:

The useful trends obtained in terms of overall increase in straw yield (Table 14) were found to be more or less similar to those of grain yields except that the order of performance was different. Farmyard manure was found to be poorest when cultures Rhizobium (mung) along with B.megaterium were used. It is quite apparent from the result that seeds receiving only Rhizobium (mung) gave about 28.78 per-cent lower yield (i.e., 41.83 q/ha) as compared to the yield (58.74 q/ha) due to treatment of seeds with Rhizobium (mung) along with B.megaterium. The statistically significant difference in yield indicates the potentiality of the entire methodology using B.megaterium as a phosphate solubilizer along with Rhizobium (mung) for inoculating legumes. Similar was the case when surface sterilized seeds were inoculated with Rhizobium (mung) and treated with jaggery 25 per cent or pelleted with 50 per cent gum arabic + sodium molybdate, 50 per cent gum arabic + superphosphate; 50 per cent gum arabic + gypsum ; 50 per cent gum arabic + sodium molybdate + superphosphate + gypsum in as much as significantly higher yields were recorded over treatment involving only surface sterilized seeds. Higher yields are possibly due to the fact that inoculum buildup in field was very poor and infection by native rhizobia was almost absent. In addition to it the better survival of Rhizobium (mung) and B.megaterium

Table 14. Effect of inoculation by Rhizobium (mung) and Bacillus megaterium var. phosphaticum through different carriers on drymatter (straw) yield of mung (q/ha)

Cultures	Carriers				Mean
	C_1	C_2	C_3	C_4	
R	37.44	39.05	41.22	49.58	41.82
R B	55.34	59.27	62.92	57.43	58.73
Mean	46.39	49.16	52.07	53.00	
Control	19.57				
S.Ed.$\pm$	0.227				
C.D.at 5%	0.481				

Table 15. Effect of different pelleting materials on drymatter (straw) yield of mung (q/ha)

	Treatments				
	P_1	P_2	P_3	P_4	P_5
Mean	42.34	43.36	53.94	44.58	50.13
S.Ed.$\pm$	0.108				
C.D.at 5%	0.216				

of carriers could have helped in boosting the yields.

Effects of pelleting materials on mung straw yield:

When effects of pelleting materials on straw yield are examined (Table 15) it seems that treatment involving superphosphate along with 50 per cent gum is superior over other pelleting materials followed by mixture of pellets with 50 per cent gum arabic. When effect of inoculum carriers and pelleting materials (Table 16) is simultaneously examined they prove the efficiency of 'kanda' as the best inoculum carrier when 50 per cent gum and superphosphate were used as pelleting material. It may be due to stimulating effect of 'kanda' in presence of superphosphate. Sahu (1973), observed that bacterization of the seeds by rhizobia or phosphatization increased nodulation, nitrogen content in shoot and root dry-matter content of shoot and root portion and yield. Brockwell *et al.* (1975) reported increase in drymatter of soybean from 484 mg/plant to 705 mg/plant due to rhizobial inoculation. Results described in Table 15 seem to be self explanatory in corroborating the work reported.

Effect of different inoculum carriers on nodulation in mung:

Data represented in Table 17 revealed that the nodulation pattern was more or less reflected in the order of performance of different carriers and inoculants for grain and straw yield. Here the superiority of leaf mould

Table 16. Effect of different carriers and pelleting materials on efficiency of Rhizobium with and without *B.megaterium* in providing straw yield of mung (q/ha)

Main treatments	Sub-treatments					Mean
	P_1	P_2	P_3	P_4	P_5	
RC_1	34.36	36.00	47.23	30.20	39.40	37.44
RC_2	35.00	30.80	45.93	39.43	44.10	39.05
RC_3	40.20	36.33	47.33	35.03	47.20	41.22
RC_4	48.43	49.53	58.83	41.73	49.36	49.58
RBC_1	51.56	48.73	63.26	54.90	58.23	55.34
RBC_2	49.46	53.53	66.96	61.06	65.33	59.27
RBC_3	53.43	61.70	70.36	64.13	65.00	62.92
RBC_4	49.60	52.20	67.60	55.46	62.30	57.43
Mean	42.34	43.36	53.94	44.58	50.13	

S_0 = 19.570
S.Ed. ± 0.975
C.D.at 5% 1.950

Table 17. Effect of inoculation by <u>Rhizobium</u> (mung) and <u>Bacillus</u> <u>megaterium</u> var. <u>phosphaticum</u> through different carriers on number of nodules in mung

Cultures	Carriers				Mean
	C_1	C_2	C_3	C_4	
R	19.83	26.90	24.06	27.33	24.53
R B	29.70	28.56	23.10	29.93	27.82
Mean	24.76	27.73	23.58	28.63	
Control	6.80				
S.Ed. ±	0.240				
C.D.at 5%	0.509				

Table 18. Effect of different pelleting materials on number of nodules in mung

	Treatments				
	P_1	P_2	P_3	P_4	P_5
Mean	22.75	24.39	28.41	21.83	22.74
S.Ed. ±	0.092				
C.D.at 5%	0.184				

as carrier was discernible in terms of capability of infecting roots in comparision to coal powder and 'kanda'. In combination with B.megaterium the Rhizobium (mung) seemed to be more active in providing more and healthy nodules. Sahu (1973) reported that due to inoculation and phosphate application there was increase in nodule numbers. Ireland and Vincent (1968) reported on the heavy inoculum form earlier nodulation and formation of upto five times as many effective nodules per plant. Iswaran et al. (1972) also reported on plant composit as a substitute for peat for legume inoculants.

Effect of pelleting materials on nodulation in mung:

Persual of data described in Table 18 once again showed superiority of the treatment involving pelleting with 50 per cent gum arabic and superphosphate in increasing the number of nodules over other treatments (comparision of with only jaggery or pelleting with 50 per cent cum + sodium molybdate, 50 per cent gum + gypsum, 50 per cent gum + sodium molybdate + superphosphate + gypsum). Similar trends were observed for 50 per cent gum + superphosphate as pelleting materials when inoculum carrier and pelleting materials interactions were examined (Table 19). Leaf mould as inoculum carrier was found to be the best when interactions with pelleting materials were examined. Scanty pattern of nodulation was observed from plots receiving uninoculated seeds. These results clearly indicate that addition of superphosphate following rhizobial

Table 19. Effect of different carriers and pelleting materials on nodulation in mung (Rhizobium with and without *B.megaterium*)

Main treatments	Sub-treatments P_1	P_2	P_3	P_4	P_5	Mean
RC_1	24.00	18.83	19.33	18.16	18.83	19.83
RC_2	26.66	27.33	37.16	20.66	22.66	26.90
RC_3	20.33	27.16	32.50	19.16	21.16	24.06
RC_4	23.50	26.83	36.83	27.83	21.66	27.33
RBC_1	28.66	31.66	33.83	29.16	25.16	29.70
RBC_2	26.16	28.83	30.66	27.50	29.66	28.56
RBC_3	24.16	21.50	23.66	21.16	25.00	23.10
RBC_4	25.16	28.50	34.66	29.00	32.33	29.93
Mean	22.75	24.39	28.41	21.83	22.74	

S_0 = 6.800
S.Ed. ± 0.836
C.D. at 5% 1.672

inoculation through leaf mould base has beneficial role in improving nodulation pattern. When Rhizobium (mung) along with B.megaterium was used as inoculant (through different carriers viz., farmyard manure, coal powder 'kanda' and leaf mould) leaf mould was found to be the best inoculum carrier. Highest number of nodules (along with more total nitrogen in groundnut plants) were reported by Kandasamy and Prasad (1971), while describing the effect of inoculation using lignite inoculum base. Iswaran *et al*. (1971) also reported on the beneficial role of inoculum carriers like charcoal, peat, humic acids in improving nodulation pattern. Use of inoculum carriers for increasing efficiency of inoculants has been reported by a number of workers (Atwal and Sidhu, 1964; Burton and Curley, 1965; Chhonkar and Iswaran, 1971; Hulmani *et al*.,1972; Vaishya and Sanoria, 1972 Chakrapani and Tilak, 1974 and Dube *et al*.,1975).

Effect of different inoculum carriers on nitrogen uptake by mung:

More or less similar trends were also observed for nitrogen uptake (Table 20). Leaf mould was found to be the best carrier followed by 'kanda' as carrier, whereas farmyard manure was found inferior when Rhizobium (mung) was used with and without B.megaterium. Better returns of grain and straw using leaf mould and 'kanda' as bases for inoculation suggest their utility for substitution for peat which is not

Table 20. Effect of inoculation by <u>Rhizobium</u> (mung) and <u>Bacillus megaterium</u> var. <u>phosphaticum</u> through different carriers on nitrogen uptake by mung (kg/ha)

Cultures	Carriers				Mean
	C_1	C_2	C_3	C_4	
R	45.14	48.61	51.74	62.25	54.43
R B	61.06	70.15	75.91	70.94	69.51
Mean	53.10	59.38	63.82	66.59	
Control	20.31				
S.Ed.±	0.132				
C.D.at 5%	0.264				

Table 21. Effect of different pelleting materials on nitrogen uptake by mung (kg/ha)

	Treatments				
	P_1	P_2	P_3	P_4	P_5
Mean	48.36	54.50	69.39	50.5[illegible]	65.07
S.Ed.±	0.344				
C.D.at 5%	0.729				

available in Udaipur locality. Individually farmyard manure, coal, 'kanda' and leaf mould differ significantly.

Effect of B.megaterium along with Rhizobium (mung) on nitrogen utilization by mung:

Nitrogen uptake as reported in Table 20 reveals that the mixing of *B.megaterium* with *Rhizobium* (mung) has distinct advantage over *Rhizobium* (mung) alone.

Effect of different pelleting materials on nitrogen uptake by mung:

When effects of different pelleting materials are examined, superphosphate along with 50 per cent gum has been found to be superior in terms of nitrogen utilization(Table 21). Mixture of pelleting materials like sodium molybdate, gypsum and superphosphate using 50 per cent gum *arabic* as adhesive are considered inferior to the treatment involving 50 per cent gum + superphosphate. The interactions between inoculum carriers and pelleting material are found statistically significant (Table 22). Leaf mould was found to be superior to other inoculum carriers. It is quite apparent from results that seeds receiving mixture of inoculants have markedly improved nitrogen uptake in comparison to treatments involving inoculation with *Rhizobium* (mung) along with different pelleting materials. Iswaran *et al*. (1971), Kandasamy and Parasad (1971) Sahu (1973), Zhelyuk et al. (1974) and Chahal and Rewari (1975) have also reported on the utility of seed pelleting material

Table 22. Effect of different carriers and pelleting materials on efficiency of Rhizobium with and without *B.megaterium* in improving nitrogen uptake by mung (kg/ha)

Main treatments	Sub-treatments					Mean
	P_1	P_2	P_3	P_4	P_5	
RC_1	36.84	47.08	58.08	36.25	47.48	45.14
RC_2	41.72	42.52	62.07	44.25	52.52	48.61
RC_3	47.08	50.60	63.66	43.19	54.23	51.74
RC_4	58.91	58.76	75.78	56.54	61.25	62.25
RBC_1	49.11	58.70	81.78	51.76	62.95	61.06
RBC_2	57.89	66.68	82.35	67.20	76.63	70.15
RBC_3	64.63	76.36	91.79	68.99	77.81	75.91
RBC_4	58.83	68.79	89.70	65.63	71.74	70.94
Mean	48.36	54.50	69.39	50.54	65.07	

S_0 = 20.310
S.Ed. $\pm$ 1.188
C.D.at 5% 2.376

Table 23. Effect of inoculation by *Rhizobium* (mung) and *Bacillus megaterium* var. *phosphaticum* through different carriers on phosphorus uptake by mung (Kg/ha)

Cultures	Carriers				Mean
	C_1	C_2	C_3	C_4	
R	4.39	4.76	5.11	6.06	5.08
R B	6.04	6.99	7.48	7.08	6.89
Mean	5.21	5.87	6.29	6.57	
Control	1.94				
S.Ed.±	0.0304				
C.D.at 5%	0.0650				

Table 24. Effect of different pelleting materials on phosphorus uptake by mung (kg/ha)

	Treatments				
	P_1	P_2	P_3	P_4	P_5
Mean	4.77	5.39	6.89	4.91	5.80
S.Ed.	0.013				
C.D.at 5%	0.026				

as an aid in improving nitrogen uptake.

Effect of inoculation by Rhizobium (mung) with and without B.megaterium on P uptake by mung :

Data presented in Table 23 and Fig.1 describe the effect of *Rhizobium* (mung) inoculation with and without *B.megaterium*(using farmyard manure, coal, 'kanda' and leaf mould as inoculum carriers) on phosphorus uptake by mung. Farmyard manure was once again found to be inferior inoculum carrier when *Rhizobium* (mung) with and without *B.megaterium* was used whereas leaf mould was found to be superior when inoculated with *Rhizobium* (mung) alone. Kanda was found to be best when *Rhizobium* (mung) was used along with *B.megaterium*. The utility of 'kanda' and leaf mould lies in the fact that some active constituents of organic matter are stimulatory to microbial growth. Individually all the four inoculum carriers differ significantly. *Rhizobium* (mung) inoculation was all along found to be inferior to *Rhizobium* (mung) along with *B.megaterium* in terms of phosphorus uptake. These results are quite clear in describing the utility of *B.megaterium* in improving the phosphorus availability.

Effect of different pelleting materials on P uptake by mung:

When effects of different pelleting materials are examined, 50 per cent gum along with superphosphate was found to be the best (Table 24 and Fig.2). It is distinctly superior over the use of jaggery alone, 50 per cent gum + sodium molybdate, 50 per cent gum + gypsum and 50 per cent gum sodium molybdate, 50 per cent gum + gypsum and 50 per cent gum + mixture of sodium molybdate, superphosphate and gypsum. These results clearly indicate that addition of superphosphate as pelleting material has distinct advantage as pelleting material as also due to the fact that it is easy source of phosphorus for growth of micro-organisms and plant roots. Results presented in Table 25 describe the interactions between inoculum carriers and pelleting materials affecting phosphorus uptake by mung plants.

Farmyard manure was found to be poorest with jaggery alone as coating material. Highest phosphorus utilization has been recorded using leaf mould and 'kanda' as bases, when *Rhizobium* (mung) was used alone and in combination with *B.megaterium*. Significant increase in phosphorus uptake which was observed when *B.megaterium* was used along with *Rhizobium* spp. for (mung) can be due to solubilization of insoluble phosphorus which was not possibly available under other treatments having only *Rhizobium* (mung).

Results described in Table 25 are akin to reported work (Harley, 1952; Cooper, 1959; Goswami and Sen, 1963; Rewari *et al*.,1965; Bajpai *et al*.,1971; Vidhvaskaran *et al*. 1973; Kanzaria, 1974 and Kabesh *et al*.,1975).

Table 25. Effect of different carriers and pelleting materials on efficiency of Rhizobium with and without *B.megaterium* in improving phosphorus uptake by mung (kg/ha)

Main treatments	Sub-treatments					Mean
	P_1	P_2	P_3	P_4	P_5	
RC_1	3.66	4.65	5.83	3.67	4.67	4.39
RC_2	4.15	4.28	5.84	4.41	5.12	4.76
RC_3	4.70	5.05	6.02	4.25	5.55	5.11
RC_4	5.54	5.91	7.65	5.09	6.12	6.06
RBC_1	4.32	5.57	8.16	5.25	6.29	6.04
RBC_2	5.78	6.60	8.27	6.66	7.64	6.99
RBC_3	6.46	7.61	9.18	6.53	7.65	7.48
RBC_4	5.77	6.83	9.20	6.44	7.15	7.08
Mean	4.77	5.39	6.89	4.91	5.80	

S_0 = 1.94

S.Ed. ± 0.118

C.D. at 5% 0.236

The overall useful trends were obtained in terms of increase in grain, straw yield, nodule numbers, nitrogen uptake and phosphorus uptake. Kanda and leaf mould have been found to be best carriers all along.

When different pelleting materials were examined it was found that pelleting with 50 per cent gum + superphosphate was definitely superior to other pelleting materials. Even when interactions between inoculum carriers and pelleting materials were examined, leaf mould and 'kanda' were found better when seeds after inoculation were pelleted with 50 per-cent gum + superphosphate.

Though direct studies relating activities of Rhizobium (mung) and B.megaterium in soil were not possible it is apparent from results that Rhizobium (mung) when combined with B.megaterium provided higher yields along with better phosphorus utilization. Sharma and Singh (1971) while working with phosphobacterin along with bone meal or super-phosphate noted that there were significant increases in grain yield of rice as well as uptake of phosphate. Kudashev (1956), Mishustin and Naumova (1956), Uarova (1956), Kudzin et al.(1970) Manolache and Iordachescu (1961), Stefan and Boti (1961), Dhyansarup (1969), Panwar (1970), Kanzaria (1974) and a number of other workers also reported similar findings on the efficiency of phosphate solubilizing organisms.

Effect of inoculation by Rhizobium (cowpea) with and without B.megaterium using different carriers on cowpea green fodder yield:

More or less similar trends were also obtained in terms of overall increases in cowpea green fodder yields when effects of Rhizobium (cowpea) with and without B.megaterium were examined using different carriers (farmyard manure, coal, 'kanda' and leaf mould) as in Table 26. Individually different carriers did not provide significant differences. Bacillus megaterium when used with Rhizobium (cowpea) was found to be superior to Rhizobium (cowpea).

While the afore mentioned results are enlightening they also indicate certain gaps in available knowledge on rhizobia - B.megaterium beneficial association. Too little is known of combined activities of rhizobia and B.megaterium in rhizosphere. Future experimentation should provide the answers to some of these enigmas.

Effects of different pelleting materials on cowpea fodder yield:

When effects of different pelleting materials (summarized in Table 27) are examined treatment 50 per cent gum + superphosphate has been proved to be the best. It is distinctly superior to the use of jaggery, 50per cent gum + sodium molybdate, 50 per cent gum + gypsum and 50 per cent gum + mixture of sodium molybdate, superphosphate and gypsum. This clearly indicates that addition of superphosphate has

Bacillus megaterium var. [illegible]
different carriers on green fodder yield of cowpea

Table 26. Effect of inoculation by Rhizobium (cowpea) and Bacillus megaterium var. phosphaticum through different carriers on green fodder yield of cowpea (q/ha)

Cultures	Carriers				Mean
	C_1	C_2	C_3	C_4	
R	189.5	206.5	201.0	201.0	199.50
R B	218.0	221.0	207.5	217.0	215.90
Mean	203.8	213.8	204.3	209.0	
S.Ed.±	7.57				
C.D. at 5%	16.24				

Table 27. Effect of different pelleting materials on green fodder yield of cowpea (q/ha)

	Treatments				
	P_1	P_2	P_3	P_4	P_5
Mean	174.1	189.1	245.0	190.0	240.3
S.Ed. ±	7.87				
C.D.at 5%	15.74				

Table 28. Effect of different carriers and pelleting materials on efficiency of Rhizobium with and without *B. megaterium* in providing green fodder yield of cowpea (q/ha)

Main treatments	Sub-treatments					Mean
	P_1	P_2	P_3	P_4	P_5	
RC_1	151.5	185.0	227.5	172.5	205.0	189.5
RC_2	162.5	207.5	237.5	180.0	245.0	206.5
RC_3	162.5	170.0	247.5	185.0	240.0	201.0
RC_4	160.0	177.5	247.5	180.0	240.0	201.0
RBC_1	207.5	202.5	252.5	185.0	242.5	218.0
RBC_2	187.5	207.5	257.5	205.0	247.5	221.0
RBC_3	162.5	172.5	245.0	207.5	250.0	207.5
RBC_4	192.5	190.0	245.0	205.0	252.5	217.0
Mean	174.1	189.1	245.0	190.0	240.3	

S.Ed. $\pm$ 23.61

C.D. at 5% 47.22

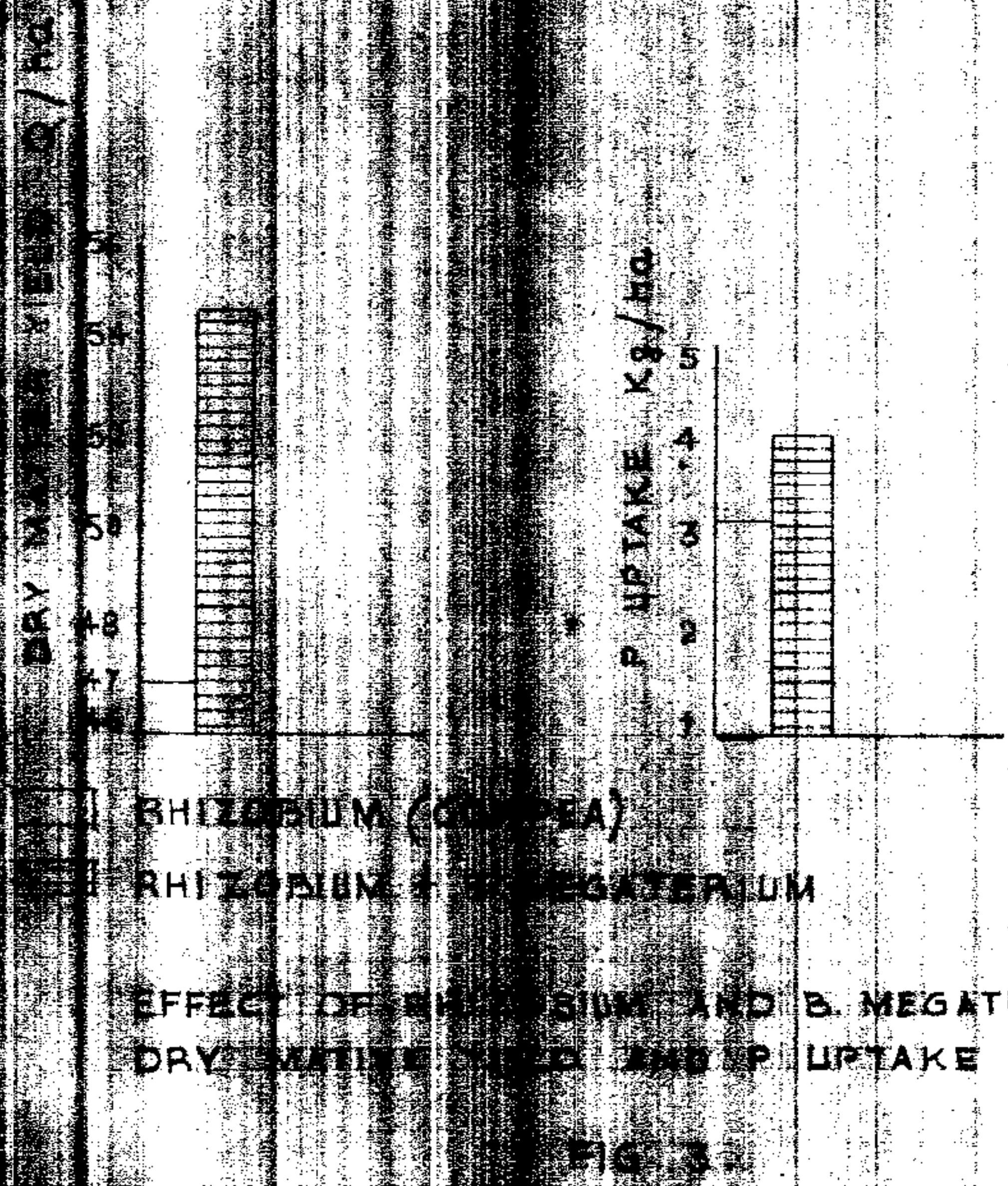
distinct advantage as pelleting material in as much as phosphate acts as booster for stimulation of microbial activities. The results presented in Table 28 describe the effect of different carriers and pelleting materials on efficiency of Rhizobium (cowpea) alone and in combination with B.megaterium in providing green fodder yields of cowpea. Leaf mould and 'kanda' have been found to be superior inoculum carriers along with pelleting materials (50 % gum + superphosphate.

Effect of inoculation by Rhizobium (cowpea) with and without B.megaterium using different carriers on cowpea drymatter yield:

Useful trends were also obtained in terms of overall increases in straw yields (Table 29 and Fig.3). Leaf mould was found to be superior when Rhizobium(cowpea) was used along with B.megaterium, and 'kanda' proved best for Rhizobium (cowpea) alone. Farmyard manure was found to be inferior. Superior performance of 'kanda' and leaf mould as inoculum carriers indicates there utility as base for Rhizobium (cowpea) as well as B.megaterium cultures in improving the efficiency of inoculation.

Effect of pelleting materials on cowpea drymatter yield:

Superphosphate along with 50 per cent gum paste was found superior as pelleting material to all other pelleting materials individually as well as in combination (Table 30 and Fig.4). Thus 'kanda' and leaf mould as inoculum carriers



EFFECT OF RHIZOBIUM AND B. MEGATERIUM ON DRY MATTER YIELD AND P UPTAKE BY COWPEA

FIG. 3.

Table 29. Effect of inoculation by *Rhizobium* (cowpea) and *Bacillus megaterium* var. *phosphaticum* through different carriers on straw yield of cowpea (q/ha)

Cultures	Carriers				Mean
	C_1	C_2	C_3	C_4	
R	41.28	50.43	55.12	41.54	47.10
R B	54.03	55.88	51.52	57.08	54.60
Mean	47.70	53.20	53.30	49.30	
S.Ed. ±	3.53				
C.D.at 5%	7.57				

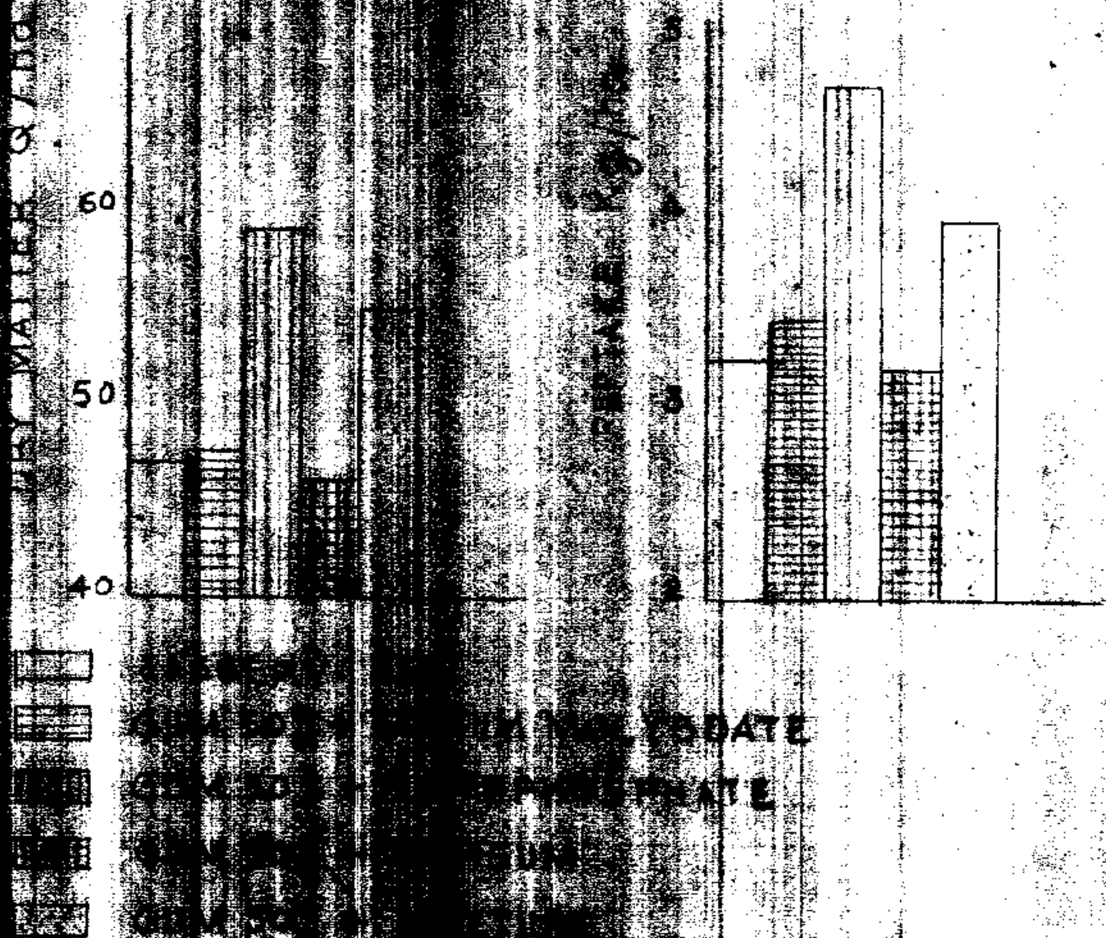
Table 30. Effect of different pelleting materials on straw yield of cowpea (q/ha)

	Treatments				
	P_1	P_2	P_3	P_4	P_5
Mean	47.0	47.5	59.0	45.9	54.9
S.Ed. ±	1.12				
C.D.at 5%	2.24				

Table 31. Effect of different carriers and pelleting materials on efficiency of Rhizobium with and without *B.megaterium* in providing straw (drymatter) yield of cowpea (q/ha)

Main treatments	Sub-treatments					Mean
	P_1	P_2	P_3	P_4	P_5	
RC_1	38.27	44.74	51.41	33.81	38.13	41.28
RC_2	48.26	48.97	51.54	47.52	55.86	50.43
RC_3	40.13	44.20	69.30	50.32	72.00	55.12
RC_4	37.60	37.81	41.33	34.56	56.40	51.54
RBC_1	68.47	57.71	54.79	32.93	56.26	54.03
RBC_2	56.81	46.48	63.09	63.55	49.50	55.88
RBC_3	44.68	50.72	69.82	43.37	49.00	51.22
RBC_4	41.77	49.59	71.03	60.88	62.12	57.08
Mean	47.00	47.50	59.00	45.90	54.90	

S.Ed. ± 3.36
C.D. at 5% 6.72



[illegible] MATERIAL ON DRY MATT[illegible]
YIELD [illegible] BY COWPEA

FIG. 4

(inoculated with Rhizobium and B.megaterium) may become effective with 50 per cent gum and superphosphate as pelleting material. Results described in Table 31 also prove superiority of leaf mould as inoculum carrier and 50 per cent gum + superphosphate as pelleting material.

Effect of inoculation by Rhizobium (cowpea) with and without B.megaterium using different carriers on nitrogen uptake by cowpea:

Similar trends have been obtained in terms of the effect of Rhizobium (cowpea) with and without B.megaterium using four different carriers on nitrogen uptake by cowpea (Table 32). On an average 'kanda' was found best once again when Rhizobium (cowpea) was used individually where as leaf mould was material of choice when Rhizobium (cowpea) was used along with B.megaterium; also inoculation with Rhizobium (cowpea) alone was found less effective as compared to Rhizobium (cowpea) along with B.megaterium. These results suggest improvement in the efficiency of nitrogen utilization when Rhizobium (cowpea) was used along with B.megaterium. Bajpai et al. (1971) have also reported that B.megaterium inoculation improved yield, phosphorus and nitrogen uptake.

Table 32. Effect of inoculation by Rhizobium (cowpea) and Bacillus megaterium var. phosphaticum through different carriers on nitrogen uptake by cowpea (kg/ha)

Cultures	Carriers				Mean
	C_1	C_2	C_3	C_4	
R	27.75	34.68	40.91	31.08	34.61
R B	37.15	39.30	40.75	47.04	41.06
Mean	32.45	36.99	40.83	39.06	
S.Ed. ±	1.27				
C.D.at 5%	2.72				

Table 33. Effect of different pelleting materials on nitrogen uptake by cowpea (kg/ha)

	Treatments				
	P_1	P_2	P_3	P_4	P_5
Mean	32.49	34.40	47.62	31.76	40.40
S.Ed. ±	1.11				
C.D.at 5%	2.22				

Table 34. Effect of different carriers and pelleting materials on efficiency of Rhizobium with and without B.megaterium in improving nitrogen uptake by cowpea (kg/ha)

Main treatments	Sub-treatments					Mean
	P_1	P_2	P_3	P_4	P_5	
RC_1	24.00	29.72	38.04	21.04	25.96	27.75
RC_2	31.25	34.45	40.59	29.58	37.56	34.68
RC_3	28.21	32.12	55.62	35.29	53.31	40.91
RC_4	26.62	27.38	34.06	24.36	42.98	31.08
RBC_1	43.57	39.19	42.67	21.33	38.97	37.15
RBC_2	38.41	31.94	50.73	41.72	37.32	39.30
RBC_3	34.50	39.61	57.77	33.07	38.82	40.75
RBC_4	33.39	40.80	61.49	47.96	51.60	47.04
Mean	32.49	34.40	47.62	31.76	40.40	

S.Ed. ± 3.33
C.D. at 5% 6.66

Effect of pelleting materials on nitrogen uptake by cowpea:

When the effects of different pelleting materials on nitrogen uptake by cowpea are examined (Table 33),, treatment 50 per cent gum and superphosphate was considered good. The interaction between different carriers and pelleting materials indicate that 'kanda' was the best carrier when 50 per cent gum arabic and superphosphate as pelleting material were used along with Rhizobium (cowpea); leaf mould with superphosphate was found superior to other carriers when Rhizobium (cowpea) along with B.megaterium were used (Table 34).

Effect of inoculation by Rhizobium (cowpea) with and without B.megaterium on phosphorus uptake by cowpea:

Utilization of phosphorus was also influenced in a similar manner as that of nitrogen as is evident from data presented in Table 35 and Fig.3. Kanda was found to be the best carrier followed by leaf mould, where as farmyard manure was not considered as suitable inoculum carrier in terms of phosphorus utilization. Higher phosphorus uptake by cowpea was also the pattern when Rhizobium (cowpea) along with B.megaterium was used. These results corroborate the earlier work reported.

Table 35. Effect of inoculation of Rhizobium (cowpea) and Bacillus megaterium var. phosphaticum through different carriers on phosphorus uptake by cowpea (kg/ha)

Cultures	Carriers				Mean
	C_1	C_2	C_3	C_4	
R	2.77	3.30	4.11	3.10	3.32
R B	3.68	3.91	4.01	4.68	4.07
Mean	3.23	3.61	4.06	3.39	
S.Ed.±	0.128				
C.D.at 5%	0.264				

Table 36. Effect of different pelleting materials on phosphorus uptake by cowpea (kg/ha)

	Treatments				
	P_1	P_2	P_3	P_4	P_5
Mean	3.24	3.47	4.66	3.19	3.97
S.Ed.±	0.083				
C.D.at 5%	0.166				

Table 37. Effect of different carriers and pelleting materials on efficiency of Rhizobium with and without *B.megaterium* in improving phosphorus uptake by cowpea (kg/ha)

Main treatments	Sub-treatments					Mean
	P_1	P_2	P_3	P_4	P_5	
RC_1	2.37	3.01	3.79	2.06	2.58	2.77
RC_2	3.15	3.40	3.29	3.28	3.34	3.30
RC_3	2.83	3.19	5.61	3.57	5.35	4.11
RC_4	2.66	2.74	3.40	2.45	4.26	3.10
RBC_1	4.34	3.92	4.21	2.10	3.84	3.68
RBC_2	3.81	3.19	5.04	4.15	3.38	3.91
RBC_3	3.38	3.94	5.72	3.19	3.81	4.01
RBC_4	3.35	4.08	6.19	4.68	5.13	4.68
Mean	3.24	3.47	4.66	3.19	3.97	

S.Ed. ± 0.249

C.D.at 5% 0.498

Effect of pelleting materials on phosphorus uptake by cowpea:

Treatment comprising superphosphate and 50 per cent gum *arabic* was found the best in terms of performance (Table 36). Studies on carriers and pelleting materials (Table 37) indicate that 'kanda' and leaf mould interact with superphosphate (using *Rhizobium* (cowpea) with and without *B.megaterium*).

The overall increase obtained in terms of yield and uptake corroborate the work reported (Chhonkar and Suba Rao, 1967; Chhonkar and Iswaran, 1971; Iswaran *et al.*,1971a; De-Polli *et al.*,1973; Rzaev, 1969; and Rangaswami and Morachan, 1974)

CHAPTER II

Efficiency of inoculation with *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* on the yield and uptake of nitrogen and phosphorus by wheat

MATERIAL AND METHODS

The experiment was carried out to evaluate the efficiency of bacterial inoculants, *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* in improving grain yield, dry matter, nitrogen and phosphorus uptake by wheat crop under different levels of nitrogen and phosphorus fertilization.

Experiment No.1 :

Efficiency of inoculation with *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* on the yield and uptake of nitrogen and phosphorus by wheat.

Location: Agronomy Farm of Rajasthan College of Agriculture, Udaipur. Soil characteristics of experimental plot are given in Table 38.

Season : Rabi, 1974

Crop: Wheat (*Triticum aestivum* L.)
Var. Shera

Design of Experiment: Split - plot

Table 38. Chemical, physical and micro-biological analysis of soil

pH	8.2
Electrical conductivity	0.9 mmhos/cm
Organic carbon	0.975 per cent
Olsen's available phosphorus	11.8 ppm
Calcium carbonate	3.10 per cent
Sand	43.50 per cent
Silt	24.00 per cent
Clay	32.50 per cent
Actinomycets counts	6.30×10^6/g
Bacteria counts	6.21×10^6/g
Fungi counts	15.60×10^3/g

Details of treatments:

Three levels of nitrogen and three levels of phosphorus were assigned to nine main plots. Each main plot was further divided into four sub-plots containing sub-treatments representing no inoculant, *Azotobacter chroococcum*, *Bacillus megaterium* var. *phosphaticum* and *Azotobacter chroococcum* + *Bacillus megaterium* var. *phosphaticum*, respectively. Main treatments were randomised and sub-treatment among each main plot was also randomized.

Main treatments were as follows:

1. N_0P_0	-	No fertilizer
2. N_{60}	-	60 kg nitrogen/ha
3. N_{120}	-	120 kg nitrogen/ha
4. P_{40}	-	40 kg P_2O_5/ha
5. $N_{60}P_{40}$	-	60 kg N + 40 kg P_2O_5/ha
6. $N_{120}P_{40}$	-	120 kg N + 40 kg P_2O_5/ha
7. P_{60}	-	60 kg P_2O_5/ha
8. $N_{60}P_{60}$	-	60 kg N + 60 kg P_2O_5/ha
9. $N_{120}P_{60}$	-	120 kg N+ 60 kg P_2O_5/ha

Size of gross - 5.0 X 1.0 m

Replication - Three

Total number of plots - 108

Details of inoculants:

Bacillus megaterium strain used in this experiment was same as used in previous experiments. Azotobacter chroococcum was obtained from 'Culture collection', division of Microbiology, I.A.R.I., New Delhi-12 on agar slant and was maintained on nitrogen free glucose medium (Norris, 1959) having the following composition.

Glucose	-	10.0 g
K_2HPO_4	-	1.0 g
$MgSO_4$	-	0.2 g
$CaCO_3$	-	1.0 g
NaCl	-	0.2 g
Na_2MoO_4.	-	0.005 g
Distilled water	-	1000.00 ml
pH adjusted	-	7.0

Seed inocuation:

Material used for the inoculation were

(i) Jaggery 25 per cent

(ii) Gum arabic paste 50 per cent

alongwith 5 days old culture (broth) of A.chroococcum containing 1 X 10^{10} cells per ml and 7 days old culture (broth) of B.megaterium having population of 1 X 10^{9} cells per ml.

Seed inoculation was carried out in the manner given below:

Gur slurry (25 %) was prepared, after cooling 50 per cent gum arabic was added and mixed throughly. One hundred ml of lots of heavy suspension of culture were poured to above mixture of gum and slurry with thorough mixing. Seeds of wheat thoroughly washed with distilled water were immersed in inoculated slurry and dried. The seeds so inoculated were used for sowing. Most probable number of A.chroococcum and B.megaterium was determined using five seeds taken randomly for serial dilution just before sowing of seeds.

Following seed treatments were carried out:

1. N_0P_0
2. N_0P_0 + A.chroococcum
3. N_0P_0 + B.megaterium
4. N_0P_0 + A.chroococcum + B.megaterium

5. N_1P_0
6. N_1P_0 + A.chroococcum
7. N_1P_0 + B.megaterium
8. N_1P_0 + A.chroococcum + B.megaterium
9. N_2P_0
10. N_2P_0 + A.chroococcum
11. N_2P_0 + B.megaterium
12. N_2P_0 + A.chroococcum + B.megaterium
13. N_0P_1
14. N_0P_1 + A.chroococcum
15. N_0P_1 + B.megaterium
16. N_0P_1 + A.chroococcum + B.megaterium
17. N_1P_1
18. N_1P_1 + A.chroococcum
19. N_1P_1 + B.megaterium
20. N_1P_1 + A.chroococcum + B.megaterium
21. N_2P_1
22. N_2P_1 + A.chroococcum
23. N_2P_1 + B.megaterium
24. N_2P_1 + A.chroococcum + B.megaterium
25. N_0P_2
26. N_0P_2 + A.chroococcum
27. N_0P_2 + B.megaterium
28. N_0P_2 + A.chroococcum + B.megaterium
29. N_1P_2
30. N_1P_2 + A.chroococcum
31. N_1P_2 + B.megaterium
32. N_1P_2 + A.chroococcum + B.megaterium
33. N_2P_2
34. N_2P_2 + A.chroococcum
35. N_2P_2 + B.megaterium
36. N_2P_2 + A.chroococcum + B.megaterium

Preparation of field:

Field was prepared by ploughing and harrowing followed by planking.

Fertilizer application:

All nitrogen and phosphorus was applied by drilling at the time of sowing.

Sowing:

Seeds were sown by dibbling.

Harvesting and thrashing:

Crop was harvested at maturity plot-wise and thrashed by small thrasher separately. Yield of grain as well as straw were recorded.

Collection of samples:

Samples for grain as well as straw collected at the time of thrashing were processed for chemical analysis

Statistical analysis:

The analysis of variance was conducted (Fisher,1950) and comparisons among means were made by calculating critical difference. The 'F' test was carried out to see the significance between differences due to the treatments.

*****

METHODS

Methods used for soil and plant analysis were same as described in Chapter I.

RESULTS AND DISCUSSION

The data on the effect of nitrogen and phosphorus fertilization on grain yield are presented in Table 39. Grain yield significantly increased as a result of nitrogen fertilization. The yield was significantly higher under N_2P_2 treatment over other treatments. Lower yields from control plot having no fertilization could be due to certain soil factors. Generally wheat crop has shown universal response to nitrogen fertilization throughout the Rajasthan state (Jain *et al*.,1959; Mehta and Jaisinghani, 1962; Gandhi *et al*.,1963; Jain *et al*.,1963; Kala *et al*.,1963; Mathur *et al*., 1964; Gautam *et al*.,1965; Singh and Parsad, 1966; Shrotriya *et al*., 1966; Gandhi, 1967;Singh and Seth,1976; Shrotriya *et al*., 1969; Bhardwaj *et al*.,1969 and Gandhi and Nathawat, 1968). From results presented in Table 39 it is clear that application of urea to wheat (Shera) variety has given significant response upto 120 kg N/ha alone and with 60 kg P_2O_5/ha. Work of Arakeri *et al*. (1961), Parashar *et al*. (1963), Puntamker *et al*. (1965), Sharma *et al*. (1966), Hucklebsy *et al*. (1971) and Singh and Anderson (1973) has clearly indicated that nitrogen fertilization accounted for significant increase in grain yield. Also results from Table 39 indicate that phosphorus fertilization provided significant response. Interaction of nitrogen and phosphorus was also found to be

Table 39. Effect of different levels of nitrogen and phosphorus fertilization on grain yield of wheat q/ha

	P_0	P_1	P_2	Mean
N_0	29.66	37.08	47.37	38.03
N_1	36.16	48.41	50.37	44.98
N_2	51.79	53.85	57.25	54.29
Mean	39.20	46.44	51.66	

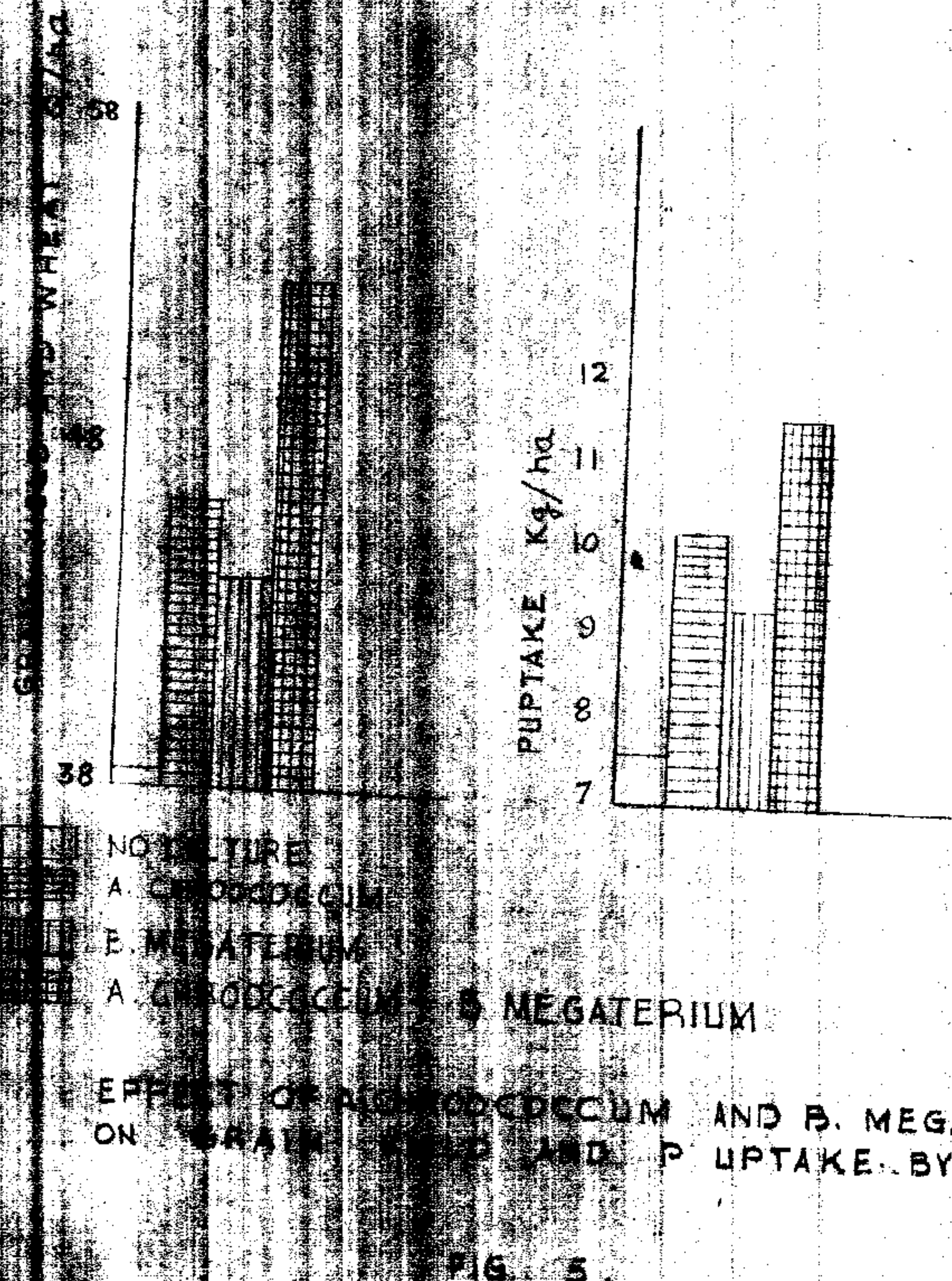
S.Ed.$\pm$ 0.307
C.D.at 5% 0.651

Table 40. Effect of inoculation by A.chroococcum with and without B.megaterium on grain yield of wheat (q/ha)

	B_0	B_1	B_2	B_3
Mean	38.53	46.85	44.48	53.24

S.Ed.$\pm$ 0.065
C.D.at 5% 0.130

significant (Table 39). It seems obvious that N_2P_2 level of fertilization is adequate. The results presented in Table 40 reveal that inoculation by Azotobacter chroococcum with and without B.megaterium improved grain yields of wheat. Inoculation with A.chroococcum along with B.megaterium proved to be the best combination followed by inoculation with A.chroococcum. The highly significant difference between inoculated and noninoculated seeds could be assigned to better environments created by A.chroococcum and B.megaterium in rhizosphere. The beneficial role of micro-organisms in rhizosphere in improving crop yields has also been reported by Sundara Rao and Venkataraman (1963), Rovira and Bowen (1968) and several other workers. Russians who were pioneers in the field of bacterial fertilization (as reviewed by Cooper, 1959) observed that Azotobacterin not only improved yields to the extent of 50-70 per cent for many field crops (with average increase in yield upto 10 per cent), but also produced IAA a growth promoter and antibiotics (acting against phytopathogens). Lack of statistical scrutiny of Russian work led the Western workers to question the validity of such results. A number of workers in India and abroad have reported on the beneficial effects of bacterial fertilizers. Sundara Rao et al. (1963) reported increase of 10-15 per cent in crop yields in field trials although higher increase upto 37 per cent in pot culture were recorded. Also Ridge and Rovira (1968), Brown and Burlingham (1968), Patel (1969), Ridge (1970), Thakre and Saxena (1972) Dolgova (1972), Srivastava and Mishra (1973),



EFFECT OF A. CHROOCOCCUM AND B. MEGATERIUM ON GRAIN YIELD AND P UPTAKE BY WHEAT

FIG. 5.

Thompson (1974) and Tatarova et al. (1975) have reported beneficial role of Azotobacter in increasing yields. Increase in plant growth can be ascribed to the action of Azotobacter in producing growth hormones in microbial composts which are then embibed by the seed or in the rhizosphere after the seeds have germinated. Rasnizina(1938) showed that Azotobacter could form auxins. Brown et al.(1968) detected 0.15-1.0 micrograms of indole acetic acid per ml of culture fluid. Vancura and Macura (1960) found that the effect of adding 1 ml of Azotobacter culture fluid to germinating seeds on agar was equivalent to the addition of 6 micrograms of indole acetic acid, but such quantities were never found in pure culture. Under natural soil conditions it is possible that indole acetic acid is synthesised in greater quantities due to presence of some metabolic products (Smalii and Breshova, 1957). The persual of data presented in Table 41 revealed that the interaction between fertilizers and inoculant was statistically significant. Highest yield was recorded when A.chroococcum along with B.megaterium was used under N_2P_2 fertilizer treatment whereas minimum yield was recorded for control plot receiving no fertilizer. Sen et al. (1958) also reported increase in the availability of phosphorus due to presence of nitrogenous fertilizers and phosphobacterin. Similarly Sharma and Singh (1971) have reported significant effect of nitrogen and phosphorus fertilization on grain yield and uptake of nitrogen and phosphorus. It is known that heavy fertilization of nitrogen decreases gram positive and spore

Table 41. Effect of inoculation by A.chroococcum with and without B.megaterium on grain yield of wheat under different levels of nitrogen and phosphorus fertilization (q/ha)

Main treatments	Sub-treatments				Mean
	B_0	B_1	B_2	B_3	
N_0P_0	24.50	31.16	27.00	36.00	29.66
N_1	30.00	37.50	33.00	44.16	36.16
N_2	44.50	48.00	55.16	59.50	51.79
P_1	28.80	39.50	34.50	45.50	37.08
N_1P_1	39.50	50.00	44.33	56.50	48.41
N_2P_1	46.00	52.00	58.00	59.50	53.85
P_2	38.50	54.00	41.50	55.50	47.37
N_1P_2	42.50	52.50	48.00	58.50	50.37
N_2P_2	52.50	57.00	55.50	64.00	57.25
Mean	38.53	46.85	44.48	53.24	

S.Ed. ± 0.586

C.D. at 5% 1.172

Table 42. Effect of different levels of nitrogen and phosphorus fertilization on straw yield of wheat (q/ha)

	P_0	P_1	P_2	Mean
N_0	44.87	48.86	64.25	52.66
N_1	52.96	66.37	73.62	64.32
N_2	71.75	70.62	84.25	75.54
Mean	59.86	61.95	74.04	

S.Ed. ±	0.261
C.D.at 5%	0.553

Table 43. Effect of inoculation by A.chroococcum with and without B.megaterium on straw yield of wheat(q/ha)

	Treatments			
	B_0	B_1	B_2	B_3
Mean	55.44	67.55	60.00	73.70

S.Ed. ±	0.161
C.D.at 5%	0.322

Table 44. Effect of inoculation by <u>A.chroococcum</u> with and without <u>B.megaterium</u> on straw yield of wheat under different levels of nitrogen and phosphorus fertilization (q/ha)

Main treatments	Sub-treatments				Mean
	B_0	B_1	B_2	B_3	
N_0P_0	40.00	47.00	44.50	48.00	44.87
N_1	46.00	56.50	49.00	60.33	52.96
N_2	64.50	67.50	71.50	83.50	71.75
P_1	38.00	54.00	44.00	59.50	48.86
N_1P_1	56.00	70.00	63.00	76.50	66.37
N_2P_1	59.00	77.50	64.50	81.50	70.62
P_2	52.00	74.00	55.50	75.50	64.25
N_1P_2	65.50	76.50	70.00	82.50	73.62
N_2P_2	78.00	85.00	78.00	96.00	84.25
Mean	55.44	67.55	60.00	73.70	

S.Ed. ± 1.451

C.D. at 5% 2.902

forming bacteria whereas actinomycetes and Azotobacter population increased due to phosphorus and potassium application in rhizosphere (Emmimath and Rangaswami, 1972). High soil organic carbon content along with low available phosphorus could be the reason for higher efficiency of inoculants under N_2P_2 fertilizer treatment.

Useful trends were also obtained in terms of overall increases in straw yields (Table 42). The trends found were more or less similar to those for grain. Lowest yields were found for N_0P_0 fertilizer treatment, whereas N_2P_2 fertilizer treatment resulted in higher yields. Combination of Azotobacter chroococcum with B.megaterium was found to be the best (Table 43). A.chroococcum alone was found better than B.megaterium. These statistically significant differences in straw yield indicate the potentiality of the entire operation using bacterial inoculants viz., A.chroococcum with and without B.megaterium. It is interesting to note that B.megaterium and A.chroococcum, when used alone proved superior to control from statistical point of view (5 per cent significant level). Similar trends as of grain yield were observed for interaction between fertilizer and bacterial inoculants viz., A.chroococcum and B.megaterium (Table 44). Lower levels of fertilizer were not helpful in increasing efficiency of bacterial inoculants.

Data presented in Table 45 revealed that the uptake of nitrogen was more or less reflected in the order of

Table 45. Effect of different levels of nitrogen and phosphorus fertilization on nitrogen uptake by wheat (kg/ha)

	P_0	P_1	P_2	Mean
N_0	50.74	62.78	86.12	66.54
N_1	66.10	94.70	105.43	88.74
N_2	102.78	115.75	142.51	120.34
Mean	73.20	91.07	111.353	

S Ed. ±	0.466
C.D. at 5%	0.946

Table 46. Effect of inoculation by *A. chroococcum* with and without *B. megaterium* on nitrogen uptake by wheat (kg/ha)

	Treatments			
	B_0	B_1	B_2	B_3
Mean	73.89	97.02	85.77	111.20

S.Ed. ±	0.176
C.D. at 5%	0.352

performance of different treatments for grain and straw. Here again N_2P_2 treatment was found superior (in terms of utilization of nitrogen) to other treatments. When effect of bacterial inoculants A.chroococcum and B.megaterium were examined (Table 46), higher nitrogen uptake was found under treatment involving bacterial inoculations over and above the control treatment. Here again the superiority of B.megaterium along with A.chroococcum was discernible in terms of their efficiency of utilization of nitrogen. The treatment involving A.chroococcum with B.megaterium was found to be highly significant as compared to control. This combination of A.chroococcum and B.megaterium was found to the best treatment for yields of grain and straw as well as the utilization of nitrogen by crop. This overall superiority of these inoculants can be attributed to the fact that soil of the experimental plot was having 0.957 per cent organic carbon. Interactions between fertilizers and inoculants were found significant (at 5 per cent level of significance) in terms of nitrogen utilization(Table 47). When seeds were inoculated with A.chroococcum along with B.megaterium under different fertilizer levels, tremendous increase in nitrogen utilization was recorded upto N_2P_2 treatment. Higher yields and nutrient uptake was also reported due to inoculations with A.chroococcum and B.megaterium Similar trends were recorded by other workers (Menkina, 1956; Kudashev, 1956; Uarova, 1956; Sen et al.,1958; Naumova,1962; Sundara Rao and Sinha, 1963; Shestakova, 1963; Sundara Rao et al., 1963;Sen Gupta and Cornfield, 1963; Paul,1966; Chhonkar and

Table 47. Effect of inoculation by A.chroococcum with and without B.megaterium on nitrogen uptake by wheat, under different levels of nitrogen and phosphorus fertilization (kg/ha)

Main treatments	Sub-treatments				Mean
	B_0	B_1	B_2	B_3	
N_0P_0	40.36	54.27	46.26	62.05	50.94
N_1	52.56	70.26	59.54	82.04	66.10
N_2	85.40	96.98	108.33	124.01	102.78
P_1	46.03	69.79	55.25	79.64	62.78
N_1P_1	71.98	97.15	94.28	115.39	94.70
N_2P_1	92.19	117.81	118.85	134.50	115.75
P_2	66.01	102.69	70.56	105.23	86.12
N_1P_2	84.24	119.84	85.39	132.25	105.43
N_2P_2	126.25	144.34	133.80	165.68	142.51
Mean	73.89	97.02	85.77	111.20	

S.Ed. ± 1.584

C.D.at 5% 3.168

Table 48. Effect of different levels of nitrogen and phosphorus fertilization on phosphorus uptake by wheat (kg/ha)

	P_0	P_1	P_2	Mean
N_0	4.92	6.91	10.52	7.78
N_1	6.66	10.36	11.60	9.54
N_2	10.81	11.98	14.39	12.42
Mean	7.46	9.75	12.17	
S.Ed. ±	0.069			
C.D. at 5%	0.148			

Table 49. Effect of inoculation by *A. chroococcum* with and without *B. megaterium* on phosphorus uptake by wheat (kg/ha)

	Treatments			
	B_0	B_1	B_2	B_3
Mean	7.67	10.21	9.26	11.82
S.Ed. ±	0.0215			
C.D. at 5%	0.0430			

Suba Rao 1967; Bhurat and Sen, 1968; Taha *et al*.,1969; Bajpai *et al*.,1971; Mehrotra and Lehri, 1971; Kudzin *et al*., 1971; Sharma and Singh 1971; Jain *et al*.,1973; Azcon *et al*., 1973; Andriyuk *et al*.,1973; Srivastava and Misra, 1973; Thompson, 1974; Kanzaria, 1974 and Zalaback *et al*.,1975).

When effect of different fertilizer treatments were examined for phosphorus utilization it was found that highest phosphorus uptake was recorded under N_2P_2 fertilizer treatment (Table 48). Data presented in Table 49 reveal that higher phosphorus utilization was ascribed to seed inoculation with *A.chroococcum* along with *B.megaterium*; whereas, seeds receiving no inoculant were found inferior in terms of phosphorus utilization. Encouraging performance of *B.megaterium* and *A.chroococcum* seems to be due to the fact that wheat rhizosphere under field conditions provides a better environment for activities of these micro-organisms at all stages of growth. This favourable situation might have provided enough nutrient supply to the crop due to activities of *A.chroococcum* and *B.megaterium*.

Similar trends were observed for interaction between fertilizers and inoculants in terms of phosphorus utilization (Table 50). Fertilization with N_2P_2 treatment was found superior along with inoculation with *A.chroococcum* and *B.megaterium* over and above other treatments.

Table 50. Effect of inoculation by A.chroococcum with and without B.megaterium on phosphorus uptake by wheat under different levels of nitrogen and phosphorus fertilization (kg/ha)

Main treatments	Sub-treatments				Mean
	B_0	B_1	B_2	B_3	
N_0P_0	3.78	5.17	4.66	6.06	4.92
N_1	5.08	6.80	6.30	8.46	6.66
N_2	8.61	9.56	11.55	13.27	10.81
P_1	4.86	7.58	6.37	8.84	6.91
N_1P_1	7.67	11.06	9.84	12.86	10.36
N_2P_1	8.93	12.45	12.29	14.23	11.98
P_2	7.61	12.27	8.05	12.26	10.52
N_1P_2	9.62	12.21	10.67	13.89	11.60
N_2P_2	12.91	14.50	13.59	16.55	14.39
Mean	7.67	10.21	9.26	11.82	

S.Ed. ± 0.194
C.D. at 5% 0.388

The overall increase in yield and nutrient uptake by wheat under fertilization corroborate the work reported (Eck *et al*., 1963; Parashar and Singh, 1963; Hera *et al*., 1971 and Johnson *et al*., 1963). Efficiency of bacterial inoculants was also found significant statistically and results obtained seem akin to reported work (Sundara Rao, 1963b; Menkina, 1950; Bhattacharya, 1958; Sen *et al*., 1966; Irusalimskii and Egoreva, 1960; Sperber, 1957, 1958; Bhurat and Sen, 1963; Pavlova, 1973; Andriyuk and Mal'Tseva, 1973).

GENERAL DISCUSSION

GENERAL DISCUSSION

The technology behind the use of bacterial inoculants needs to be developed in view of the fact that some useful increases in the yield have been recorded by their usage(Cooper 1959 ;Sundara Rao *et al*.,1963; Sharma and Singh,1971 and Kanzaria, 1974). The low cost improvements need examination specially in the context of high cost of fertilizers. The farmers in the poor income group often seek substitutes to fertilizers,and bacterial inoculants fit in well in this. Thus a case exists for the improvement of methodology of bacterial fertilization with a view to provide a realistic and scientific back ground for mass scale operation.

Use of Rhizobium was considered quite useful in the past in the pre-independance era but subsequently with the manufacture of inorganic fertilizers Rhizobium inoculation was not practised. It is known that very useful improvement in yields can be brought out merely by the use of Rhizobium. Mass scale production of Rhizobium has serious problems. Choice of proper strain for particular crop is often coupled with problems connected with production. This is particularly true when a substitute for peat base is to be found from various organic sources easily available. Further the problem connected with survival of rhizobia in bacterial inoculants is very important in providing the proper cultures for purposes of inoculation. Reasons behind non popularization of cultures in the past lies in the fact that poor quality cultures were supplied to the farmers which led to improper inoculation and consequently caused little or no improvement in yields. Keeping these problems in view the present investigations were started. The four sources used were farmyard manure, coal powder, 'kanda' and leaf mould. It is apparent from Tables 3,5,6, 8,9 and 11 that 'kanda' or leaf mould can be used successfully in maintaining good bacterial counts for mass scale production. Results presented in Tables 3,5,6,8,9 and 11 show highest counts for inoculants in 'kanda' followed by leaf mould. However, counts were very low in farmyard manure and coal powder based preparations and thus farmyard manure and coal powder are very much less effective in terms of survival of inoculants. The effect of time is very clear on population counts. Sizable improvement could be brought about over period of four weeks. These findings are in conformity with those of Burton (1967).

There are ample reasons to believe that mass scale production of culture packets of Rhizobium spp. for mung as well as cowpea and B.megaterium could easily be carried out by using these inoculum carriers (the transport of peat from faraway places can be avoided). Some field trials have been carried out in Udaipur region to demonstrate the utility of 'kanda' as a base for commercial purpose. Use of A.chroococcum has also been suggested for improving crop yields. By and large in soil areas rich in organic matter improvement has been recorded. In the Udaipur region and adjoining Madhya Pradesh and Maharashtra useful increase in yield has been recorded by the application of Azotobacter. In states like Punjab, Haryana and other places where organic matter levels are low, Azotobacter, performance has not been found upto the mark (Goswami, 1976). It appears reasonable to assume that in soils above 0.7 per cent organic carbon (as in the present case in Udaipur) improvement in yields is possible by the use of *Azotobacter chroococcum*. In about 50 acres under operational research project Chittor, increase in yields of the order of 2 q/ha appears possible by the use of *Azotobacter chroococcum* (Saxena, 1976).

The improvements in the yields are often most remarkable when *B.megaterium* as phosphate solubilizer is used along with Rhizobium or *A.chroococcum*. This useful association appears to be the out come of production of Vitamin B_{12}, IAA and Cytokinin by the organisms like *B.megaterium* and *A.chroococcum* (Coppola *et al*.,1971; Vancura, 1961 and Brown, 1974). The results presented in Tables 12,14,26, 29,40 and 32, clearly indicate that inoculation with *B.megaterium* along with *Azotobacter chroococcum* or *Rhizobium* (mung) and *Rhizobium* (cowpea) as provided increase in yield of the order of 14-38 per cent over by inoculation with Rhizobium. This indicates the technology is well in sight for improvement of crop yield using *B.megaterium* (phosphate solubilizer). The limited work has no doubt tried to place the use of *B.megaterium* at a better level but proper selection of strains may furtheraccentuate the useful role of *B.megaterium* as phosphate solubilizer in association with *Rhizobium* (cowpea) as well as *Rhizobium* (mung) and *Azotobacter chroococcum*. There appears a need for continuing research in isolation of effective strains of *Azotobacter chroococcum*, Rhizobium and *B.megaterium*.

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SUMMARY

SUMMARY

A number of experiments were carried out to evaluate the effect of some bases, pelleting materials and bacterial inoculants on the over all yield and uptake patterns for the crops viz., mung (Phaseolus aureus), cowpea (Vigna sinensis) and wheat (Triticum aestivum L.). In all one laboratory experiment and two field experiments have been under taken. The relevant findings are listed below:

Survival of inoculants:

1. Mean survival of Rhizobium (mung), Rhizobium (cowpea) and B.megaterium was found significantly greater in 'kanda' upto period of four weeks.

2. Farmyard manure and coal powder were found inferior.

Mung crop:

1. The seed inoculation with Rhizobium (mung) with and without B.megaterium resulted in significant increase in yield over plots receiving no culture.

2. Inoculation with Rhizobium (mung) along with B.megaterium was found superior over Rhizobium (mung) inoculation in terms of yields, and nutrient uptake by mung.

3. Kanda and leaf mould were found suitable bases in terms of nitrogen and phosphorus utilization by mung.

4. Leaf mould was found quite effective carrier in terms of nodulation.

5. Superphosphate along with 50 per cent gum was found very promising pelleting material in providing better nodulation, yields and utilization of nitrogen as well as phosphorus by mung.

6. The use of 25 per cent jaggery as coating material was not found better than the pelleting materials.

Cowpea crop:

1. Kanda and leaf mould were found superior over farmyard manure and coal powder in providing better yields of cowpea.

2. Farmyard manure and coal powder were found inferior as inoculum carriers in affecting yields and nutrient utilization by cowpea.

3. Pelleting with 50 per cent gum and superphosphate were found the best coating agents in terms of yields, nitrogen and phosphorus uptake by cowpea.

4. The combination of B.megaterium with Rhizobium (cowpea) brought good returns in terms of yields and uptake of nitrogen and phosphorus by cowpea.

5. Kanda and leaf mould were found suitable carriers when 50 per cent gum with superphosphate was used as pelleting material and provided better yields and nutrient uptake by cowpea.

6. The use of gypsum or sodium molybdate in presence of 50 per cent gum was not helpful in affecting yields as well as nitrogen and phosphorus uptake.

Wheat crop:

1. The highly significant differences were found between inoculated and uninoculated seeds in terms of yield, nitrogen and phosphorus uptake by wheat.

2. Seed treatment with A.chroococcum and B.megaterium brought significantly higher yields, nitrogen and phosphorus utilization by wheat.

3. Increases of 21.5 per cent, 15.4 per cent and 38 per cent of grain yields of wheat over control were recorded as a result of a inoculation with A.chroococcum, B.megaterium and A.chroococcum along with B.megaterium, respectively in the field which is very rich in organic matter content(1.68 %).

4. The maximum yield were recorded for 120 kg N/ha + 60 kg P_2O_5 treatment whereas minimum yields were found under N_0P_0 (no fertilizer) treatment.

5. Bacillus megaterium inoculation resulted in higher phosphorus uptake by wheat.

6. Mixing of A.chroococcum and B.megaterium proved most promising situation for boosting yields of wheat.

7. Overall better response of bacterial inoculants in amiliorating yields as well as nitrogen and phosphorus uptake, was observed.

Based on the findings it is suggested that bacterial fertilization can be practised using B.megaterium and A.chroococcum for wheat and Rhizobium spp. and B.megaterium for mung and cowpea crops.

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APPENDIX

Appendix I

Effect of different carriers and time intervals on survival of *Rhizobium* spp.(mung as well as cowpea) and *B.megaterium*(x 10^9/g)

Source of variation	d.f.	M.S.S.		
		Rhizobium (mung)	*Rhizobium* (cowpea)	*B.megaterium*
Replications	2	0.0007*	0.0023*	0.00005
Carriers	3	0.03201*	0.3366*	0.2367*
Weeks	3	0.1545*	0.1723*	0.1152*
Carriers X weeks	9	0.0294*	0.0232*	0.0157*
Error	30	0.00018	0.00036	0.00028

*Significant at 5 percent level

Appendix II

Effect of Rhizobium (mung) with and without B.megaterium on grain, straw yields, nodulation, nitrogen and phosphorus uptake, using different carriers and pelleting materials

Source of variation	d.f.	M.S.S.				
		Grain yield	Straw yield	Nodule Number	Nitrogen uptake	Phosphorus uptake
Replications	2	0.81	2.18	26.33	6.90	0.24
Main Treatments (A)	8	29.70*	2867.59*	791.39	4402.77*	44.22*
Control v/s Rest	1	194.72*	12576.72*	5007.36*	21779.90*	219.68*
Carriers	3	3.16*	300.08*	108.35*	1040.13*	9.67*
Inoculum	1	31.81*	8583.50*	515.15*	9365.72*	95.14*
Carrier X inoculum	3	0.526	291.75*	161.21*	318.72*	3.30*
Error 'a'	16	1.118	11.62	21.00	26.74	0.21
Sub-treatments	4	9.92*	665.64*	184.90*	1857.61*	134.37*
Jaggery v/s Pelletilization	1	14.56*	691.56*	54.15*	2093.80*	20.77*
Mixtur of Pellets v/s Av.of individual pellets	1	0.07	216.58*	123.14*	1.83	0.135
Between individual pellets	2	12.53*	877.21*	281.16*	2666.91*	28.68*
A x B	32	0.075	42.20*	31.79*	57.25*	5.05*
Error 'b'	72	0.735	8.55	6.30	12.71	0.13

*Significant at 5 per cent level

Appendix III

Effect of Rhizobium (cowpea) with and without B.megaterium on yield and nitrogen and phosphorus uptake by cowpea using different carriers and pelleting materials

Source of variation	d.f.	M.S.S.			
		Green fodder	Drymatter	Nitrogen uptake	Phosphorus uptake
Replications	2	281.72	41.12	21.13	0.29
Main treatments (A)	7	1697.07*	582.93*	560.52*	5.63*
Inoculum	1	8036.03*	1696.44*	1631.88*	16.29*
Carrier	3	659.12	241.71	391.43*	3.98*
Carrier X Inoculum	3	622.12	552.99*	355.82*	3.71*
Error 'a'	14	430.74	93.86	12.12	0.114
Sub-treatment(B)	4	24539.27*	802.77*	1095.03*	0.12*
Jaggery v/s Rest	1	42399.02*	561.20*	877.88*	8.20*
Mix.pellets v/s av.of individual pellets	1	25026.00*	402.55*	146.70*	0.89*
Between pellets	2	15366.03*	1123.07*	1677.77*	13.69*
A x B	28	527.34	236.93*	112.92*	1.363*
Error 'b'	64	638.24	17.16	16.70	0.18

*Significant at 5 per cent level

Effect of inoculation by *A. chroococcum* with and without *B. megatherium* on phosphorus uptake by wheat under different levels of nitrogen and phosphorus fertilization

Source of variation	d.f.	M.S.S.			
		Grain yield	Straw yield	Nitrogen uptake	Phosphorus uptake
Replications	2	15.02	12.84	14.29	0.066
Main treatments (A)	8	1039.29*	1782.79*	10062.20*	108 .670*
Nitrogen (N)	2	2397.55*	4645.64*	266559.42*	235.72*
Phosphorus (P)	2	1409.38*	2331.46*	12917.81*	185.89*
N x P	4	171.11*	247.04*	335.79*	6.470*
Error 'a'	16	21.29	15.35	48.98	0.550
Sub-treatment(B)	3	998.62*	1762.89*	6844 .62*	81.680*
b_0 v/s $b_1+b_2+b_3$	1	1887.41*	2744.59*	11765.50*	154.17*
b_1 v/s b_2	1	75.85	770.67	1707.02	12.38
b_3 c;s b_1+b_2	1	1032.59	1773.43	7061.34	77.81
A x B	24	21.98*	37.54	152.40*	43.66*
Error 'b'	54	3.10	18.96	18.22	0.14

*Significant at 5 per cent level

Appendix V

Abbreviations used in this thesis

R	=	*Rhizobium* spp.
RB	=	*Rhizobium* + *B.megaterium*
C	=	Carrier
C_1	=	Farmyard manure
C_2	=	Coal powder
C_3	=	Cow dung cake (kanda)
C_4	=	Leaf mould
P_1	=	Seed coating with 25 % jaggery
P_2	=	Seed pelleting with gum 50 % and sodium molybdate
P_3	=	Seed pelleting with gum 50 % and superphosphate
P_4	=	Seed pelleting with gum 50 % and gypsum
P_5	=	Seed pelleting with gum 50 % and mixture of (1:1:1) sodium molybdate, superphosphate and gypsum.
S_0	=	Surface sterilized seeds.
B_0	=	Seeds treated with no bacterial culture
B_1	=	Seeds treated with *A.chroococcum*
B_2	=	Seeds treated with *B.megaterium*
B_3	=	Seeds treated with *A.chroococcum* and *B.megaterium*

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