REPORTS SECTION
(33)

UAS Tech. Series No. 28

Aspects of Biological Nitrogen Fixation

ARY GKVK
UAS(B)
904

1980

UNIVERSITY OF AGRICULTURAL SCIENCES
HEBBAL, BANGALORE-560 024

R-19904
08-12-2022

Please remember this resource is meant for many other users like you, so do not tamper, tear or damage the contents.

We believe that this is an individual social responsibility to take care of the library material.

Please do check before you borrow it as you may have to compensate if the material is returned in a damaged condition.

REPORTS SECTION

JAS Tech. Series *No. 28*

Aspects of Biological Nitrogen Fixation

Proceedings of the Third Southern Regional Conference on Microbial Inoculants in Crop Production, held at University of Agricultural Sciences, College of Agriculture Dharwad Campus, on April 1 and 2, 1977

1980

UNIVERSITY OF AGRICULTURAL SCIENCES
HEBBAL, BANGALORE-560 024

Contents

SESSION I

Crop Response to Inoculation — Legumes

SESSION II

Crop Response to Inoculation — Non Legumes

SESSION III

Ecology of Nitrogen — Fixing Organisms in Soil

SESSION IV

Problems in the Production and Quality Control of Inoculants

The Third Southern Regional Conference on Bacterial Inoculants in Crop Production was held at the University of Agricultural Sciences, Dharwad Campus, Dharwar, on 1st and 2nd April 1977

Dr V P Bhide, Emeritus Professor, I C A R , Maharashtra Association for Cultivation of Sciences, Poona, inaugurated the Conference Dr S V Patil, Director of Instructions, Agricultural College, Dharwad, presided over the function Dr T K R Reddy, Professor of Agricultural Microbiology, Agricultural College, Dharwad welcomed the delegates and Mr A R Madhav Rao proposed the vote of thanks Dr Peter Dart, Microbiologist, ICRISAT, Hyderabad, addressed the gathering on the importance of biological nitrogen fixation in crop production Dr R B Patil, Professor of Agricultural Microbiology, U A S , Bangalore listed the aims and objects of the Conference

Over 40 delegates from the Universities and Institutes of South India including Maharashtra and representatives of the manufacturers of Bacterial Inoculants participated in the Conference Dr G Rangaswami, Vice-Chancellor, Tamil Nadu Agricultural University, was to deliver the key-note address but could not do so on account of the last minute cancellation of the flight to Belgaum However, his address was circulated to the participants

Dr V P Bhide inaugurating the Conference emphasized the need for wider use of Bacterial Inoculants in Crop Production He also pointed out that the research on the use of Bacterial Inoculants has not been satisfactory and is not being carried out in a coordinated manner He urged the Universities and other Agricultural Institutions to take up the studies on the response of various crops under different agro-climatic situations to inoculation He also indicated that the Indian Standards Institute would soon come out with the standards for production and use of Bacterial Inoculants and this would protect the farmers against the spurious claims of some of the inoculant manufacturing firms

Dr Peter Dart pointed out that though India is the home of a wide variety of legumes capable of being nodulated by rhizobia, the Indian soils are improvised in respect of nitrogen, whereas, the soils of United States of America, Australia and some of the European countries are enriched with nitrogen This may be attributed to the extensive use of rhizobial inoculants in production of legumes in these countries He also listed some of the recent findings of the research on Biological Nitrogen Fixation and their application in agricultural

production He emphasized the need to assess the distribution of effective and ineffective strains of native rhizobia in Indian soils and the response of grain legumes to inoculation He noted that the attempts to transfer the 'nif' gene to cereal crops are on the way and by the turn of the century this would be achieved and that these crops like legumes may become largely independent of the use of nitrogenous fertilizers in crop production He also urged the need to explore the possibilities of using blue green algae, *Azolla* and other free-living nitrogen fixing bacteria in production of crops

Dr G Rangaswami, Vice–Chancellor, T N A U , in his key note address dealt with newer aspects of research on nitrogen fixation that are being intensively persued Amongst them he listed genetic engineering of nitrogen fixing microorganisms, enhancing the nitrogen fixing capacity of the rhizobia in legume root nodules and search for new nitrogen fixing organisms and their associations with non-leguminous plants He also noted with satisfaction that the group of Scientists who came together since April 1975 have met for the third successive year This, he stated, in itself was a progress and he hoped this enthusiasm for research in this narrow field of Biological Nitrogen Fixation in crop production would be continued during the coming years Since, a small minority of scientists are involved in this area of research the emphasis has been more on the applied aspect and therefore, he suggested to the group to take up some work on the fundamental aspects of the problem He noted that the farmers though, in the initial stages showed great interest in the use of rhizobial inoculants, that interest has been gradually waning in recent years This he attributed to the inequalities in crop response to inoculation noted by farmers He, therefore, emphasized the need to evolve a technology that will assure at least a minimal level of benefit to the farmers through the use of rhizobial inoculants Likewise, the results on the response of crops to *Azotobacter* inoculation were varying It is therefore, necessary that the cost-benefit ratio with these bacterial cultures should be much higher than that with chemical fertilizers This alone will sustain the interest of the farmers in the use of these inoculants for production of non-leguminous crops The technology of inoculation developed so far has been confined to the grain crops This needs to be extended to fodder legumes and grasses if our Animal Husbandry were also to develop rapidly In conclusion, he noted that the transfer of technology from the research laboratories to farmers has not been uniform and several factors contributed to this He, therefore, urged the Agricultural Microbiologists to take every possible step to counter-balance these factors

The group met in five sessions during two successive days and presented their findings on the response of leguminous and non-leguminous crops to inoculation,

under varying agro-climatic conditions and on the production and quality control of bacterial inoculants At the end of these sessions panel discussion was held and the following resolutions were passed Dr Peter Dart presided over the plenary session and the summary reports presented by the recorders and the Chairmen of the different sessions were discussed

Session I — Crop Response to Inoculation of Legumes

1) Response curves for nitrogen fertilizer application and inoculation in respect of important leguminous crops like groundnut, redgram and bengalgram should be worked out

2) Factors affecting nodulation and nitrogen fixation namely the surface temperature of the soils, moisture stress, depth of sowing and insect damage to nodules have to be critically studied

3) Model experiments on competition between several isolates of rhizobia have to be carried out In these studies, such markers as serology, resistance to antibiotic and metabolite etc , may be used To overcome the competition from native rhizobia the necessity to use very high levels of viable cells per seed has to be examined

4) Effect of residual nitrogen fixed by legumes on succeeding non-leguminous crops has to be assessed

5) In respect of germplasm screening for nodulation, number of nodules produced in the early stages of growth may be an useful criterian The method needs to be standardized

6) In respect of the multistrain inoculants the problem of interaction amongst the strains and with the host plant has to be critically studied.

7) It was agreed that the criteria now being used for the evaluation of nitrogen fixation by various strains of rhizobia were inadequate Hence, it was emphasized that uniform criteria such as dry weight and nitrogen content of tops, grain yield and continuous monitoring of nitrogen fixation in nodules by acetelyne reduction technique or by total nitrogen determination have to be adopted

8) Symptoms of Zn and Mo deficiency in legumes need to be defined on the basis of the experimental results

9) Methods of root washing and recovery of nodules particularly with legumes having flagile nodules have to be worked out

10) Attempts should be made to evolve temperature resistant and moisture stress resistant strains of rhizobia

11) Information on the population of rhizobia in different soils during different seasons and under different crops is not available. It is necessary to assess the native population under the above conditions to obtain a better predictability of response to inoculation

Session – II — Crop Response to Inoculation in Non-legumes

1) Other classes of nitrogen fixing, free-living bacteria in rhizosphere of various non-leguminous crop plants have to be isolated and studied

2) Adaptive and demonstration trials on farmers fields on the response of crops to *Azotobacter* inoculation have to be taken up on a much wider scale

3) The extent of excretion of nitrogen fixed by *Azotobacter* and availability of nitrogen to the crop have to be assessed

4) Trials on *Azotobacter* inoculation of vegetable, tuber crops and cotton need to be conducted

5) Nitrogen uptake by the crops inoculated with *Azotobacter* by acetylene reduction technique needs to be taken up in order to sort out the benefits due to nitrogen fixation and due to production of growth promoting substances by *Azotobacter*

6) Sterilized carrier based inoculants of *Azotobacter* have to be included as an additional control in studies on soil application of *Azotobacter* inoculants to crop plants

7) Role of organic matter in enhancing the efficacy of *Azotobacter* has to be investigated

8) Sub-cultures of *Azotobacter* with claimed capacity for fixation of very high amounts of nitrogen should be exchanged with other laboratories for further verification

9) Those laboratories who have been carrying out phyllosphere and spermosphere fixation of nitrogen may continue those studies

Session III — Ecology of Nitrogen Fixing Organisms

1) Sizeable amount of information on the effect of pesticides on the symbiotic and non-symbiotic nitrogen fixing organisms has accumulated This needs to be pooled and presented as review The Scientists should send whatever information they have on this to Dr A Balasubramanian, Microbiologist, TNAU, Coimbatore-3

2) Research on pelleting with lime, rock phosphate and bauxite should be continued

3) Nodulation in different soil types such as a black soil, red soil, acidic soil and alkaline soils in terms of continuous production of effective and infective nodules by various strains of rhizobia and the growth of the host plant needs to be studied

Session IV — Problems in Production, Quality Control and use of Bacterial Inoculants

1) Wherever convincing data on the response of crops to inoculation are available, the use of Bacterial Inoculants should be included in the recommended package of practices
2) The Bacterial Inoculant manufacturers may form an association to present their problems to the concerned extension agencies and Universities and popularise the use of Bacterial Inoculants in crop production
3) The Indian Standards Institute may be requested to expedite the standards for *Azotobacter* inoculants
4) Research on the carriers, stickers and packaging materials may be continued
5) There is a need to evolve a suitable procedure to facilitate the Inter State Movement of Bacterial Inoculants
6) The State Departments of Agriculture and the Vice-Chancellors of the respective Universities may be requested to set up Inoculant Testing Laboratories

Dr Peter Dart, in his concluding remarks thanked the organizers for the excellent arrangements made for the stay of the delegates and indicated the possibility of holding the next conference at ICRISAT

Dr N N Prasad thanked the host Institute, in particular Dr S V Patil, Dr T K R Reddy and his staff on behalf of the group He expressed great disappointment at the inability of Dr G Rangaswami to be at Dharwad on the occasion He expressed the indebtedness of the group to Dr G Rangaswami for his keen interest it the activities of the group and encouragement

SESSION I

Crop Response to Inoculation – Legumes

Response of Native and Introduced Strains of *Rhizobium* sp. on the Nodulation and Yield of Different Varieties of Blackgram and Greengram

M Rangarajan and P Muthukrishnan
Agricultural College and Research Institute
Tamil Nadu Agricultural University, Madurai-625104

Abstract

Application of composite culture of *Rhizobium* consisting of the introduced and native strains of *Rhizobium* sp, such as IARI-E 9, Coimbatore-BMBS P 47, Bangalore, Jabalpur, Madurai-Mash 64 and Nitrobac (Biocultures) significantly increased the growth, nodulation and yield in Co 2, G 104 and H 21-40-14 varieties of blackgram While the control and various isolates applied singly, gave yields ranging from 8 16 to 10 58 q/ha, the composite culture gave 11 16 q/ha in Co 2 variety thus recording an increased yield ranging from 10 2 to 40 4 per cent over other isolates of *Rhizobium* and control Similar trend was observed with the use of composite culture in G 104 and H 21-40-14 although they recorded lesser yield and nodulation than Co 2

In the case of greengram, application of composite culture consisting of the introduced and native strains of *Rhizobium* sp, such as IARI-D 10, Coimbatore GMBS, Bangalore, Jabalpur, Madurai-PLS 287 and Nitrobac, gave 8 06 q/ha in Co 2 variety which was 11 5 per cent to 54 1 per cent more than those of individual isolates and control that gave the yields ranging from 5 23 q/ha to 7 23 q/ha Similar trend in growth, nodulation and yield was observed with the use of composite culture in S8 and ML 24 varieties of greengram although they recorded lower yields than Co 2

Introduction

It is reported that the growth, nodulation and yield of legumes are increased by the treatment of bacterial inoculants consisting of single or multi-strain inoculants of rhizobia Oblisami *et al* (1976) reported that the composite culture of *Rhizobium* increased nodulation and yield of blackgram and greengram varieties Srirama Raju (1976) reported about the nodulation of different rhizobial strains from Australia, Bangalore, IARI, Israel strains in bengalgram Nodulation and yield of legumes can be increased by the combinations of effective strains as against single effective strain (Bhargava *et al*, 1975) Habish and Ishag (1974) reported that inoculation of a local strain of *Rhizobium* significantly increased growth, nodulation and yield of beans Bhargava *et al*, (1975) reported that seed treatment of different indigenous cultures of *Rhizobium* increased the nodulation and yield of soybeans Balasundaram and Subba Rao (1972) stressed the importance of composite cultures and reported that the establishment of soybeans with indigenously available cultures of *Rhizobium*

as against the imported cultures of *Rhizobium* from different agro-claimatic conditions in the country Patil *et al*, (1972) reported the efficiency of certain *Rhizobium* inoculants obtained from different sources in increasing the nodulation and yield of soybean In the present paper, the efficiency of six strains of *Rhizobium* and the composite culture containing these six strains in increasing the growth, nodulation and yield of different varieties of greengram and blackgram is presented

Materials and Methods

Two different field experiments one for blackgram and another for greengram, were conducted in *kharif* season of 1976–77 under irrigated conditions in a spilt plot design with three replications having a plot size of 4×2 m The experiment was laid out with eight treatments including control in which no *Rhizobium* culture was used In the case of blackgram, one native (Madurai-Mash 64) and five introduced strains of *Rhizobium viz*, IARI–E 9, Coimbatore–BMBS P 47, Bangalore, Jabalpur and Nitrobac were used individually and as composite culture The composite culture included all these six different strains Three varieties of blackgram *viz*, CO 2, G 104 and H21–40–14 were used for the studies The seeds were coated thoroughly with the peat soil based cultures of *Rhizobium* The seeds were sown in lines with the spacing of 30×20 cm

Similarly in the case of greengram, one native (Madurai-PLS287) and five introduced strains of rhizobia *viz*, I A R I –D 10, Coimbatore-GMBS 1, Bangalore, Jabalpur, and Nitrobac were used for the studies Three varieties of greengram *viz*, CO 2,S 8 and ML 24 were used in the experiments The crops were cultivated with all recommended package of practices In both experiments, plant samp'es were collected on 45th day 75th day after sowing and observations were recorded for growth and nodulation Dry weights of shoot and root were used to assess the effect on growth Total number of nodules per plant and dry weight of nodular tissue were considered as important parameters for nodulation The seed yield was recorded finally for assessing the effect on yield

Results and Discussion

Blackgram The effect of native and introduced strains of rhizobia on growth, nodulation and yield of three varieties of blackgram are given in Tables 1 and 2

(1) *Growth* In case of dry weight of shoot, composite culture performed significantly superior to individual strains and control Among the individual strains, the strain from IARI performed better than all other strains, followed by strains from Coimbatore and Bangalore Similarly the strains from Jabalpur

TABLE I

*Influence of native and introduced strains of rhizobia on the growth and nodulation in blackgram**

Strain	Dry weight of shoot (g)			Dry weight of root (g)			Total number of nodules			Dry weight of nodular tissus (g)		
	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3
I A R I E 9	15 59	14 23	13 83	0 96	0 99	0 98	55 0	46 0	38 3	0 741	0 657	0 546
Coimbatore-BMBS P47	15 05	13 32	11 97	0 99	0 95	0 94	50 0	36 7	35 0	0 707	0 415	0 410
Bangalore	14 74	13 15	12 02	0 99	0 99	0 98	51 3	39 7	34 3	0 721	0 578	0 415
Jabalpur	14 08	13 22	11 15	0 98	0 98	0 97	46 3	34 7	33 0	0 640	0 417	0 397
Madurai-Mash 64	14 21	13 10	11 96	0 99	0 98	0 98	47 7	35 3	35 0	0 697	0 417	0 427
Nitrobac (Biocultures)	13 44	13 10	11 15	0 96	0 90	0 90	44 3	33 0	30 7	0 606	0 402	0 374
Composite (Mixture of 1 to 6)	17 91	15 32	15 66	1 03	1 00	1 03	76 0	73 07	69 0	1 462	0 493	1 387
Control	11 39	10 47	9 00	0 85	0 76	0 69	33 3	26 3	23 7	0 397	0 353	0 333
Mean	14 55	13 24	12 09	0 97	0 94	0 93	50 51	40 7	37 4	0 746	0 592	0 537

	Strain	Variety	Interaction	Strain	Variety	Interaction	St	Vt	In	St	Vt	In
SE	0 168	0 261	0 291	0 013	0 005	0 023	0 54	0 73	0 93	0 10	0 007	0 017
CD at 5 %	0 480	1 026	NS	0 037	0 018	NS	1 53	2 87	2 67	0 028	0 027	0 049

Note V_1 Co2, V_2 G 104, V_3 H21-40-14

*Observations reeorded on 45th day after sowing

TABLE II

Influence of native and introduced strains of rhizobia on the yield of blackgram

Strain	Seed yield (Q/ha)			Mean
	V_1	V_2	V_3	
I A R I E 9	10 58	9 79	8 33	9 57
Coimbatore-BMBS P 47	9 91	9 62	8 33	9 29
Bangalore	9 75	9 16	7 91	8 94
Jabalpur	9 68	9 47	8 12	9.09
Madurai-Mash 64	9 58	9 43	8 08	9 03
Nitrobac	9 75	9 37	9 95	9 02
Composite	11 66	10 00	9 16	10 27
Control	8 16	7 75	7 04	7 65
Mean	9 88	9 32	8 12	9 17

	Strain	Variety	Interaction
SE	0 12	0 05	0 20
CD at 5 %	0 34	0 21	N S

Note V_1 CO2, V_2 G 104, V_3 H-21-40-14

and Madurai were on par in their performances and superior to Nitrobac and control

(ii) *Nodulation* The composite culture produced highest number of nodules *viz*, 69 0 to 85 0 per plant and maximum dry weight of nodular tissue *viz*, 1 38 to 1 46 g per plant depending upon the variety and duration of sampling than the individual strains Among the individual strains, the strain from IARI was superior to all other strains, followed by the strains from Bangalore and Coimbatore The strains from Madurai and Jabalpur were equally effective in nodulation Nitrobac fared least among the various strains

(iii) *Yield* The composite culture was the best by producing 11 66 q/ha which was significantly superior to that of the single strains Among the individual strains the strain from IARI was the best The performances of all other introduced and native strains were on par

Regarding the response of different varieties to the introduced and native strains of rhizobia, Co 2 variety gave seed yield of 11 66 q/ha which was significantly better that G 104 that gave 10 00 q/ha and H 21-40-14 which gave 9 16 q/ha

Greengram The effects of native and introduced strains of rhizobia on the growth, nodulation and yield of three varieties of greengram are presented in Tables 3 and 4

TABLE III

Influence of native and introduced strains of rhizobia on the growth and nodulation in greengram **

Strain	Dry wt of shoot (g)			Dry wt of root (g)			Total number of nodules			Dry wt of nodular tissues (g)		
	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3
IARI-D 10	17 48	17 28	18 08	1 13	1 37	1 45	63 7	66 0	67 0	0 879	0 879	0 902
Coimbatore-GMBS 1	16 67	16 08	17 25	1 05	1 18	1 18	51 7	54 7	58 7	0 810	0 858	0 870
Bangalore	16 57	15 99	16 48	0 95	0 94	1 03	54 7	56 7	58 7	0 895	0 585	0 898
Jabalpur	15 78	15 91	16 18	0 86	0 93	0 99	46 0	49 7	51 3	0 781	0 812	0 813
Madurai-PLS, 287	16 74	16 07	16 73	0 96	1 03	1 03	57 7	57 7	60 7	0 853	0 851	0 884
Nitrobac	15 71	15 47	15 87	0 87	0 92	0 96	44 0	46 3	44 3	0 819	0 820	0 782
Composite	18 48	18 28	19 50	1 43	1 60	0 75	77 7	80 3	82 3	1 275	1 283	1 285
Control	13 28	13 22	13 96	0 74	0 77	0 85	26 0	27 3	25 7	0 483	0 479	0 477
Mean	16 34	16 04		0 99	1 09	1 15	52 7	54 8	56 9	0 847	0 853	0 864

	Strain	Variety	Interaction	Strain	Variety	Interaction	Strain	Variety	Interaction	Strain	Variety	Interaction
SE	0 181	0 101	0 314	0 023	0 013	0 040	0 641	0 311	1 110	0 009	0 002	0 016
CD at 5%	0 518	0 398	N S	0 065	0 052	N S	1 830	1 223	N S	0 027	0 010	N S

Note V_1 Co 2, V_2 S 8, V_3 ML 24

** Observations recorded on 45th day after sowing

TABLE IV

Influence of native and introduced strains of rhizobia on the yield of greengram

Strains	Seed yield (q/ha)		
	V_1	V_2	V_3
IARI-D 10	7 13	6 86	6 10
Coimbatore-GMBS 1	7 16	6 71	5 58
Bangalore	7 23	6 66	5 31
Jabalpur	6 73	5 90	5 36
Madurai-PLS 287	7 10	6 33	6 10
Nitrobac	6 86	6 13	5 98
Composite	8 06	7 73	6 50
Control	5 23	5 03	4 16
	Strain	Variety	Interaction
SE	0 61	0 17	1 06
CD at 5 %	1 76	0 79	3 05

Note V_1 Co 2, V_2 S8, V_3 ML 24

(i) *Growth* The composite culture produced more dry weight of shoot which was superior to those by all the individual strains Among the individual strains, the strain from IARI was superior to all other strains, followed by the strains from Coimbatore, Bangalore and Madurai with different varieties of greengram Although the composite culture was better than the other strains in increasing the dry weight of root, there is no significant difference between the individual strains

(ii) *Nodulation* The composite culture produced 77 7 to 98 3 nodules per plant while individual strains produced nodules ranging from 44 0 to 77 0 nodules per plant depending upon the variety and duration of sampling Among the individual strains, the strain from IARI was best followed by strains from Madurai, Bangalore, Coimbatore, Jabalpur and Nitrobac,

(iii) *Yield* The performance of composite culture and of all other individual strains was on par in increasing the grain yield of Co 2 and ML 24 varieties In case of S 8 variety, the composite culture was superior to individual strains

REFERENCES

BALASUNDARAM, V R AND SUBBA RAO, N S 1972, Research on the establishment of soybeans in India through indigenous inoculants *Symp Legume Inoculants–Science and Technology*, New Delhi, p 13

BHARGAVA, J K, SAXENA, M C AND TILAK, K V B R 1975, Response of soybean to inoculation with various cultures of *Rhizobium japonicum Proc Indian Natl Sci Acad*, **40** (B) 471–475

HABISH, H A AND ISHAG H M, 1974, Nodulation of legumes in the Sudan III Reponse of haricot bean to inoculation *Experimental Agriculture* **10** 45–50

OBLISAMI, G, BALARAMAN, K AND NATARAJAN, T, 1976, Effect of composite cultures of *Rhizobium* on two pulse crops *Madras agric J*, **63** 587–589

PATIL, R B, GANTOTTI, B R AND BAGYARAJ, D J, 1972, Comparative field trials on the performance of different soybean specific *Rhizobium* inoculants *Symp Legume Inoculants-Technology New Delh*, p 13

SRI RAMA RAJU, K AND SAMUEL, A V, 1976, Response of gram (*Cicer arietinum* L) to different rhizobial inoculants *Madras agric J*, **63** 582–586

Nodulation Pattern in Certain Germplasm Collections of Blackgram and Greengram

G OBLISAMI, K BALARAMAN AND T NATARAJAN
Department of Biology, Tamil Nadu Agricultural University, Coimbatore

ABSTRACT

Preliminary screening of the high yielding germplasm collections of blackgram and greengram was carried out for their interactions to *Rhizobium* Nodulation pattern indicated that the genotypes of blackgram were comparatively more responsive to the *Rhizobium* inoculant than the greengram genotypes, forming more number of nodules per plant In case of greengram, the same genotype contained maximum number of nodules per plant in both the treated as well as untreated plants, so also minimum number of nodules were recorded in the same genotype However, in blackgram, though the same genotype contained minimum number of nodules in both the cases, maximum number of nodules were formed in different genotypes The nodulation in the inoculated genotypes was significantly superior over the uninoculated genotypes of both the plant species Higher number of nodules were formed during the vegetative stage of both the plant species

SUCCESSFUL symbiosis between a legume and its rhizobium involves mutual compatibility all the way from an interaction that prepares the root for and initiales the process of 'invasion to the final stage which secures efficient functioning time for nitrogen fixing tissue to satisfy the plant needs (Vincent 1974) Lot of informations are available on the role of *Rhizobium* in the nodulation of legumes However, very little information is available on the role of plant genotypes in the interaction between the rhizobium and the legume Genetically diverse lines of *Trifolium ambiguum* showed marked differences in the

UNIVERSITY OF AGRICULTURAL SCIENCES
UNIVERSITY LIBRARY, GKVK,
BANGALORE - 560065

Rs. 19904

invasibility and when nodules are produced in the time taken to nodulate (Hely, 1972) Rao and Viswanthan (1974) reported that among the 10 genotypes of soybeen tested, the cultivars Davis and Bragg recorded the highest number of nodules Sen and Bhaduri (1971) studying the relationship between the number and specific volume of nodules in different species of *Phaseolus* observed significant variations in the nodule volume among different species and varieties of *Phaseolus* The data on nodulation pattern of two important pulse crops, *viz* , blackgram (*Phaseolus mungo*) and greengram (*P aureus*) are presented in this paper

Materials and Methods

Twenty high yielding varieties each of blackgram and greengram from the germplasm collection of the pulses maintained in the Departmant of Agricultural Botany were treated with an efficient strain of *Rhizobium* sp (Ah 6) and sown into the field to study the interaction between the *Rhizobium* and the legumes The uninoculated series of the same genotypes sown separately served to assess the interaction between native rhizobium and the host The design of the lay out of the trial was Latin Square design The number of nodules produced at vegetative, flowering stages of the crops and the response of the genotypes for the *rhizobium* treatment or native rhizobia was assessed

Table I

Nodulation pattern in certain of germplasm collections in blackgram
(No of nodules per plant)

Germplasm Collection	Uninoculated series			Inoculated series		
	Vegetative stage	Flowering stage	Mean	Vegetative stage	Flowering stage	Mean
H 21	117	21	69	147	36	92
P 133	73	14	44	118	29	74
H 21-40/22	43	31	37	148	64	106
V Z M 189	117	114	116	46	76	61
CO 2	52	15	34	72	45	59
H 21-40/28	152	59	106	158	64	111
P 58	122	92	107	104	122	113
H 21-50/4	40	38	39	206	49	128
No 55	137	66	102	202	71	137
PLS-364-30/8	87	54	71	223	135	179
T-9	71	19	45	114	65	90
H-21-40/29	191	49	120	91	40	66
M-3	204	19	112	174	54	114
Musiri	117	61	89	127	49	88
H-21-40/30	166	53	110	149	28	89
Pusa-1	118	29	74	131	40	86
H-21-50/15	181	50	116	161	78	118
CO-3	84	16	50	191	56	126
H-21-40/14	26	13	20	41	18	30
U-5-2	39	14	27	131	26	79

Results and Discussions

The resulats on nodulation pattern in certain of the germplasm collections of blackgram and greengiam are presented in Tables I and II

Table II

Nodulation pattern in certain of germplasm collections in greengram
(No of nodules per plant)

Germplasm Collection	Uninoculated series			Inoculated series		
	Vegetative stage	Flowering stage	Mean	Vegetative stage	Flowering stage	Mean
PLS-334	17	12	15	26	11	19
PLS-265	28	11	20	31	13	22
No 293/1/1	3	9	6	7	4	6
PLS-240	11	12	12	13	6	10
PS 16	64	16	40	65	34	50
CO-1	31	29	30	41	18	30
M 2	93	43	68	104	78	91
PLS 367/2	62	23	43	74	27	51
PS 10	93	56	75	73	32	53
Pusa Baisaki	37	34	36	39	34	37
No 414/1/1	124	65	95	163	51	107
Kopargaon	84	64	74	83	59	71
Madira moong	73	23	48	82	43	63
No 122	45	21	33	48	24	36
No 118/1/1	81	36	59	93	14	49
L 24 2	71	21	46	81	24	53
B 1	36	20	28	70	17	44
CO 2	58	28	43	41	46	44
PLS 307	58	14	36	57	11	34
H 70-16	43	7	25	26	13	20

The number of nodules formed in the blackgram and greengram genotypes varied considerably The nodulation in the inoculated genotypes was superior over the uninoculated series In general, high number of nodules were recorded at the vegetative phase of both the crop plants In the uninoculated series of blackgram the mean number of nodules ranged from 20 to 120 per plant whereas in the inoculated series, it varied from 30 to 179 Maximum number of nodules was recorded in the genotype H-21-40/29 having 120 numbers of nodules in the

uninoculated series whereas in the inoculated series, the genotypes PLS–364–20/18 contained 179 nodules per plant Minimum number of nodules were recorded in both the series in the genotype H–21–40/14 The mean number of nodules formed in the uninoculated series of greengram varied from 6 to 95 per plant whereas in the inoculated series it was from 6 to 107 The same genotypes (No 414/1/1) contained maximum number of nodules in both the series (being 95 and 107, respectively), similarly minimum number of nodules (6/plant) were also observed in the genotype No 293/1/1

The present results indicated that the blackgram genotypes were comparatively more responsive to the *rhizobium* treatment than the greengram genotypes which could be seen by the increased number of nodules in blackgram than in the greengram This is in agreement with the earlier report by Sen and Bhaduri (1971) Similar results were reported by Kumar *et al* (1976) in case of soybean genotypes indicating marked variations in the nodule numbers between spring and winter season cultivars Considering the genotypes lot of variations were observed in the nodulation pattern even though they were put into the same environmental conditions Critical study is needed to establish the environmental factors and their role on the rhizobium-legume symbiosis so as to enable us to derive the maximum benefit by selecting the high yielding varieties and highly responsive varieties to *Rhizobium*, as well as most efficient strain of the *Rhizobium* and such a situation might be an ideal one

References

Hely, F W, 1972, Genetic studies with wild diploid *Trifolium ambiguum* M Bieb with respect to time of nodulation *Aust J Agric Res*, **23** 437–446

Kumar, S, Singh, H P and Tilak, K V B R, 1976, Response of different genotypes of soybean to inoculation with various composite cultures of *Rhizobium japonicum Pantnagar J Res*, **1** 30–32

Rao, J V D K K and Viswanthan, R, 1974, Nodulation interaction between genotypes and *Rhizobium japonicum Curr Res* **2** 163–164

Sen, R and Bhaduri, P N, 1971, Relationship between the number and specific volume of nodules in different species of *Phaseolus* and in *Glycine max Indian Agric* **15** 175–183

Vincent, J M, 1974, Root-nodules symbioses with *Rhizobium* In '*The Biology of Nitrogen Fixation*", (Ed) A Quispel, North-Holland Publishing Co, Amsterdam, pp 265–341

Effect of Composite Cultures of Rhizobium on Important Legumes Grown in Marathwada Region

R S Raut, C P Ghonsikar, and V G Musande
Department of Soil Science and Agricultural Chemistry,
Marathwada Agricultural University, Parbhani (M. S)

Abstract

A greenhouse experiment on performance of single strain and composite culture indicated that composite culture was as good as single strain culture and both had significantly increased the nodulation, dry matter and grain yield/plant over uninoculated plants in greengram and blackgram In groundnut, the performance of both single and composite cultures was similar Athar did not show response with composite or single strain culture in respect of dry matter and grain yield Soybean responded well to single strain or composite culture and produced two fold dry matter and grain yield

Introduction

Use of *Rhizobium* inoculants to legume crops is now a well accepted agronomic practice in legume cultivation Efficient strains of *Rhizobium* increased the yield of legumes (Rangaswami and Oblisami, 1962, Ramaswami and Nair, 1965) Inoculation of composite culture responded better with legume crops like soybean and French-bean over the single strain inoculation (Das and Bhaduri, 1975) At least one of the many strains used in the composite culture might establish well and cause effective nodulation in the given legumes (Rangaswami, 1975)

Rhizobia are specific to their hosts and constitute cross inoculation groups The advantage in composite culture is that it would eliminate the supply of different cultures to different crops separately and also the farmers would be able to use one composite culture to inoculate a good number of leguminous crops The performance of composite cultures of *Rhizobium* on five legumes *i e* greengram, blackgram, groundnut, arhar and soybean is reported here

Material and Methods

A greenhouse experiment was conducted in *kharif* season using earthen pots of 250 mm in diameter and with a capacity for 12 kg soil The soil used was a calcareous medium black soil (pH 8 5, $CaCO_3$ 10 4 per cent, clay 69 14 per cent, CEC 54 33 m e /100 g of soil) *Rhizobium* starins from greengram, blackgram, arhar, groundnut and soybean were employed for the preparation of

composite cultures Composite culture no 1 included rhizobia of greengram, blackgram, arhar and composite culture No 2 included rhizobia of greengram, blackgram, arhar, groundnut and soybean Seeds were inoculated with specified cultures Although five seeds of each crop were sown in pots, only three plants were maintained for studies The uninoculated plants served as control Nodules were counted at flowering and dry matter was determined 15 days before harvesting Final yields were recorded on harvest There were three replications Sterilized soil was used in all the experiments

Results and Discussion

The data on performance of single strain culture and composite culture of *Rhizobium* on greengram are presented in Table I Dry matter yield of greengram

Table I

Effect of pure and composite cultures of Rhizobium *on growth parameters and grain yield*

Treatments	Nodulation (nodule number/plant)	Dry matter (g/plant)	yield (g/plant)
Greengram			
Control	3	13 00	2 5
Pure culture (Single strain culture)	61	25 7	4 7
Composite culture* number one	58	20 4	3 8
Composite culture** number two	56	20 0	4 1
Blackgram			
Control	10	14 0	2 8
Pure culture (Single strain culture)	35	27 0	5 5
Composite culture* number one	36	22 5	4 5
Composite culture number two	42	22 5	5 3
Groundnut			
Control	8	12 0	4 0
Pure culture (single strain culture)	80	26 0	8 0
Composite culture number one	76	23 5	7 0
Composite culture number two	75	26 5	9 0
Arhar			
Control	5	44 0	8 0
Pure culture (single strain culture)	25	50 6	9 6
Composite culture numer one	27	48 7	8 9
Composite culture number two	22	49 0	9 2
Soybean			
Control	30	5 8	2 1
Pure culture (Single strain culture)	367	13 5	4 3
Compositive culture number two	319	11 4	3 8

*Composite culture number one — Contains specific rhizobium strains for greengram, blackgram, arhar crops

*Composite culture number two — Contains specific rhizobium strains for greengram, blackgram, arhar, groundnut and soybean crops

was almost doubled when compared with uninoculated control by employing single strain culture, and inoculation with composite cultures increased dry matter yield by one and a half times over the uninoculated treatment Similar pattern was also observed for grain yield/plant The pattern of results obtained with blackgram in respect of nodule count, dry matter yield and grain yield/plant was also similar for single strain and composite culture inoculations

Inoculation either with single strain of *Rhizobium* or with composite cultures produced two-fold dry matter yield Bhargava *et al*, (1975) observed maximum dry matter accumulation and increase in yield with composite culture irrespective of soil conditions Inoculation of arhar either with single strain or composite cultures resulted in significantly higher nodule count per plant when compared with uninoculated plants But the dry matter yield and grain yield/ plant in arhar did not show response to inoculation either with single strain or composite cultures, suggesting that rhizobium cultures did not develop effective nodules

Nodule count in soybean was found greatly increased by inoculation with *Rhizobium japonicum* either as single strain or in composite culture Dry matter yield and grain yield in inoculated soybean was about two-fold higher that in uninoculated treatment

References

Bhargava, J K, Sexena, M C and Tilak, K V B R, 1975, Response of soybean to inoculation with varions cultures of *Rhizobium japonicum* *Proc Indian Natl Sci Acad*, **40** 478-475

Das, S N and Bhaduri, P N, 1975, Host-rhizobium interaction between *Phaseolus vulgaris* L and *Rhizobium phaseoli* *Proc Indian Natl Sci Acad*, **40** 554-561

Oblisami, G, Balaraman, K and Natarajan, T 1976, Effect of composite cultures of *Rhizobium* on two pulse crops *Madras agric J*, **63** 587-589

Ramaswami, P P and Nair, K S, 1965, Symbiotic variations of the *Rhizobium* from nodules of redgram *Madras agric J*, **52** 239 240

Rangaswami, G and Oblisami, G, 1962, Studies on some legume root nodule bacteria *J, Indian Soc Soil Sci*, **10** 175-186

Rangaswami, G, 1975 Bacterial fertilizer-prospectus and limitations *Proc FAI-FAO Seminar on Optimising Agril Production under Limited Availability of Fertilizers* New Delhi pp 453-458

Effect of Seed Inoculation with *Rhizobium japonicum* on Nodule Formation and Yield of Soybean *(Glycine max* L)

R S Raut[1], C P Ghonsikar[2] and B G Karle[3]
Department of Soil Science and Agricultural Chemistry
Marathwada Agricultural University, Parbhani (M S)

Abstract

Response of soybean (*Glycine max* L) variety Kalitur to *Rhizobium* inoculation in different row proportions proved superiority over control Complete inoculation of seeds in all rows proved to be best in increasing nodulation and grain yield thus attaining full benefits in soybean production Partial inoculation or some of uninoculated rows enhance yield but does not warrant full potential yield

Introduction

Application of *Rhizobium* culture to seed is an important input in the package of practices for increased pulse production

The air we breathe contains 79 per cent nitrogen gas, but it cannot be absorbed and made use of by plants directly except through the mediation of microorganisms Certain microorganisms like *Rhizobium* living in root nodules of legume plants convert atmospheric nitrogen into ammonia at ordinary soil conditions On a global scale industrial nitrogen fixation is of the order of 30 million tonnes per year while annual fixation of nitrogen by *Rhizobium* in root nodules is about 14 million tonnes per year (Subba Rao, 1973)

In India due to gradual decline in the acreage under legume crops "Nodule nitrogen fixation" has considerably decreased Application of preparations containing specialized living bacteria to seed or soil can enhance soil fertility and yield of crops by rendering unavailable sources of elemental nitrogen, bound phosphate and cellulose containing plant residue into available form as to facilitate root system of plant to absorb the nutrients *Rhizobium* can increase the number and performance of root nodules on a variety of edible pulses and oil yielding crops In recent years much emphasis has been laid to popularise bacterial fertilization to soil

Material and Methods

A field experiment in randomised block design with four replications was conducted at Agronomy Farm, Marathwada Agricultural University, Parbhani, during *kharif* season of 1976 Five treatments were sown in 3 6 × 2 7 m

plot size having spacing 45 × 15 cm Soybean seeds were inoculated with *Rhizobium japonicum* culture Five patterns in sowing followed were as follows (i) one row of non-inoculated seeds and one row of inoculated seeds (1 1), (ii) Two rows of non-inoculated seeds and one row of inoculated seeds (2 1), (iii) All rows of inoculated seeds, (iv) Inoculated and non-inoculated seeds blended in equal proportions for sowing all rows and (v) All rows of non-inoculated seeds (control) Crop was not fertilized with any fertilizer or manure The nodule count was taken at the time of initial stage of pod formation by selecting five random plants from each treatment, yields were recorded on plot basis

Results and Discussion

Number of nodules on soybean roots were significantly increased by inoculation of seed The average count of root nodules per plant was increased from 104 to 388 2 with *Rhizobium* inoculation The maximum nodulation was recorded in the treatment (T_4) where all inoculated seeds were sown This increase in nodulation was 19 per cent more over treatment, 1 1 row of non-inoculated and inoculated, 32 per cent over 2 1 two rows of non-inoculated and and one inoculated 21 per cent over 50 per cent non-inoculated seed blended and 274 per cent over control (Table I) As the rows of inoculated seed or

Table I

Nodule count and yield of soybean as affected by Rhizobium *inoculation*

T	Treatment	Mean No of nodules/plant	Grain yield (g/plot)	Grain yield (kg/ha)
T_1	Control	104 0	446 75	413 65
T_2	1 1 row of noninoculated inoculated	325 0	575 25	532 63
T_3	2 1 rows noninoculated inoculated	281 5	535 00	495 36
T_4	All rows inoculated	388 2	725 75	671 99
T_5	50% non-inoculated seed blended	219 7	523 25	484 49
	S E	47 7	51 43	47 62
	C D at 5%	146 91	158 46	146 72

proportion increased, the average nodule count also increased This may be because of absence of effective strain of rhizobium needed for soybean nodulation in Marathwada soils where soybean crop is recently introduced In this region with semiarid climate where temperatures are high in summer (up to 40°C) and dry conditions during this period perhaps have suppressive effect on *rhizobium* population in soils Saxena and Tilak (1975) observed increased

nodulation in soybean using by *rhizobium* culture to seed. Sahu (1973) reported that increase in root nodules and proteins in seed of legumes by using rhizobium inoculation and phosphorus doses to legumes, while Longeri and Herrara (1972), reported absence of nodulations in non-inoculated seeds and proposed nodulation in inoculated seeds.

Grain yield of soybean was significantly increased by using *rhizobium* culture over control. The maximum yield of 671 kg/ha was recorded in treatment (T_4) where all inoculated seeds were sown in all rows. The yield seems to be relatively low but considering the fact that the crop did not receive any fertilization is above average (430 kg/ha). *Rhizobium* inoculated treatments (1) complete inoculation has increased 28.8 per cent, (2) Two rows of non-inoculated and one row of inoculated has increased 19.8 per cent while 50 per cent non-inoculated seed blended treatment has increased 17.2 per cent grain yield of soybean over control indicating as the dilution of inoculation or proportion in rows decreased the yield of soybean also decreased. The trend of grain yield corresponded to nodulation patterns. Maximum nodulation could be ensured by using full stock of seeds inoculated with *rhizobium*. Partial inoculation or some of un-inoculated rows interspacedmight help to a certain extent enhancing yield but did not warrant full potential yield. The yield responses are reported by different workers at various locations. Subba Rao (1972) in pot culture study got response up to 45 per cent by inoculating the seed with *rhizobium* culture. The yield trials with different soybean varieties in different agro-climatic zones of the country indicated positive response to rhizobium inoculation. Bhatnagar (1971); Sokorenki and Vyvalko (1972) reported a beneficial response to application of *rhizobium* inoculation while testing it for yield, nitrogen content in plant and soils.

References

Bhatnagar, R. S., 1971, *Rhizobium* inoculation effect on selected pulse crops. Ph.D. thesis submitted to I.A.R.I., T-2349.

Longeri, S. L. and Herrera, O. A. 1972, Effect of inoculation of yield, contents of protein and oil per cent in soybean; *Field crop Abst.*, **26** (3):930 (1252).

Sahu, S. K., 1973, Effect of *Rhizobium* inoculation and phosphate application on blackgram and horsegram, *Madras agric J.*, **60**: 989.

Saxena, M. C. and Tilak K. V. B. R., 1975, Response to inoculation in soybean and its residual effect on wheat. *Indian J. Agron.*, **20**: 369.

Sokerenko, M. V. and Vyvalko, I. G., 1972, Effect of nodule bacteria on quality of protein in soybean seed. *Field crop Abst.*, **15** (1): 77 (479).

Subba Rao, N. S., 1972, A case for production of bacterial fertilizer in India. *Fertilizer News*, **17** (6): 37.

Subba Rao, N. S., 1974, Prospectus of bacterial fertilization in India. *Fertilizer News*, **19** (12): 32.

Nodulation of Pigeon Pea in Red and Black Soils at Patancheru

J V D K Kumar Rao and P J Dart*

The aim is to study nodulation and N_2 fixation by pigeon pea in the two contrasting soil types at Patancheru—a deep black soil and a medium depth red soil, and to see if nitrogen supply was limiting growth and yield of pigeon pea During the 1976 *kharif* season treatments included 0, 20, 200 kg N/ha as calcium ammonium nitrate and 20T FYM per ha

Nodulation was rapid, with up to 26 nodules per plant (zero N) in red soil by 15 days after sowing, about half of these were on the primary root 200 N inhibited nodulation only up to 30 days after planting (*e g* in black soil there were 15 nodules per plant for the 200 N treatment *cf* 29 per plant for the zero N and FYM treatments) Nodules continued to form up to 120 days in both soil types, but senescence of nodules was apparent even by 30 days Most nodules formed on the secondary roots and were spread over the root system and up to 50 cm deep in the soil Only 1–2 primary root nodules were present per plant at 60 days when there were 23 nodules on the secondary roots in black soil and 56 in red soil

A greater number and mass of nodules formed in the red soil compared with the black soil, and these nodules had much more nitrogenase activity (acetylene reduction) Most nitrogenase activity per plant occurred at the 30 days harvest in black soil, and at 60 days in red soil Nitrogenase activity was greatest for the FYM treatment, there was no inhibition of activity for the 200 N treatment in red soil at 60 days *cf* either zero N or FYM Virtually no activity was detected at 120 days after sowing when the plants were in the early pod filling stage, and nodules virtually completely senesced in black soil and only 12 pink-red nodules per plant (11 per cent of the total number) present in red soil At earlier harvests the numbers of apparently active (red) nodules per plant did not correlate well with nitrogenase activity

Nodules were severely damaged by insect grubs in both red and black soils, and this eating of the nodules began by 30 days after planting Proportionately more nodules were attacked in black than in the red soil and by 120 days 96 per cent of the nodules were damaged in black soil, with 74 per cent in red soil Such attack seems a major factor limiting nitrogen fixation by

* Microbiology Section, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), 1-11-256 Begumpet, Hyderabad-500 016

UAS LIBRARY GKVK
R-19904

pigeon pea at Patancheru Similar observations were recorded from farmers fields in Mahabubnagar district in A P and Sholapur in Maharashtra

Plant growth was stimulated by the 200 N treatment *cf* zero or 20 N treatments up to 120 days from sowing in red soil and up to 60 days in black soil Plant growth was better in red soil than in black at 30 days, perhaps reflecting the better nodulation, but thereafter there was little overall difference between soil types until 120 days when plants in black soil were heaviest

These results indicate that pigeon pea nodulates poorly in both soil types at Patancheru, in that nodule activity was very small during pod filling N supply from nodules limited early plant growth which was stimulated by N fertiliser addition Nitrogen fertilising had surprisingly little effect on nodule formation and activity from 30 days after planting

Studies on Certain Factors Influencing Legume-Rhizobia Interrelationship VII Total Nitrogen, Organic Carbon and C/N Ratio of the Root Material

D KANDASAMY* AND N N. PRASAD
Microbiology Division, Faculty of Agriculture, Annamalai University, Annamalainagar-608 101

ABSTRACT

The present study on the effect of nitrogen and carbon present in plant tissues on nodulation revealed the absence of any kind of correlation between total nitrogen of the root of three leguminous plant species and nodule number Similarly the effect of organic carbon on nodulation did not support the theory that increase in available carbohydrate would enhance nodulation A comparison of the results of carbon/nitrogen (C/N) ratio and nodulation also indicated no correlation between C/N ratio and nodule number in the three plant species studied

* Present Address Department of Biology, Tamil Nadu Agricultural, University, Coimbatore-640 031

Introduction

The profound influence of the environment on nodule formation and symbiotic nitrogen fixation has been known for a long time A delicate balance prevails between the host plant and the micro-symbiont which can easily be disturbed by environmental stress conditions The response of the symbiosis to the environment is mainly determined by the genetical and physiological condition of the host plant (Lie, 1974) Raggio *et al* (1957) suggested that the nutritional conditions required by the root of legume plants for normal growth and nitrogen fixation are not provided by the external medium while the internal C N ratio of the plant system governs the nodule number (Wilson, 1935) The present study communicates the influence of carbon and nitrogen of the roots of three legume plant species on nodulation

Material and Methods

Greengram (*Phaseolus aureus* Roxb), Blackgram (*P mungo* L) and sunnhemp (*Crotalaria juncea* L) plants grown in earthern pots were sprayed with gibberellic acid (GA 50 and 100 ppm), 2,4-Dichlorophenoxy acetic acid (2,4-D 5 and 10 ppm) and indole acetic acid (IAA 250 and 500 ppm) at periodical interval as per the procedure described by Kandasamy and Prasad (1975) Samples of root material were collected at vegetative flowering and pod-bearing stages of the crop for the estimation of total nitrogen and organic carbon Total nitrogen was estimated as per the procedure described by Bremner (1960) and organic carbon by Walkley and Black (Piper, 1950) The nodules from the plants sprayed with the growth regulators were counted at the three stages of crop growth Assuming total nitrogen, organic carbon and C/N ratio as independent variable, and nodule number as dependant variable, the relationship was worked out by computing the correlation coefficients and dependability following the procedure given by Steel and Torrie (1960)

Results and Discussion

The changes in total nitrogen, organic carbon, C/N ratio of the root materials and nodulation in greengram, blackgram and sunnhemp following foliar spray of growth regulators are given in Tables I to V That the amount of nitrogen present in plant tissues may exert an internal effect on nodulation has been reported by Chailakhyan and Megiabyan (1945) and Mac Connell and Bond (1957) Cartwright and Snow (1962) observed that decrease in nodulation may be due to high level of nitrogen within the plant However, in the present study the foliar spray of IAA–500 ppm at vegetative stage on blackgram, and GA–100 ppm at flowering and pod bearing stages and GA–50 ppm, 2,4–D (5 and 10 ppm) and IAA–250 ppm at pod-bearing stages on sunnhemp increased both the

nodulation and total nitrogen of the root material (Tables I and IV) The statistical analysis of the results also indicated the absence of any kind of correlation between these two factors in any of the three legume species studied

The effect of organic carbon on nodulation did not fit in with the theory that increase in available carbohydrate would enhance nodulation (Wilson, 1935, Kanata, 1957) Though foliar spray of GA–100 ppm, IAA (250 and

TABLE I

Influence of foliar treatment with growth regulators on the total nitrogen, organic carbon and C/N ratio of the roots of greengram*

Treatment	Vegetative stage			Flowering stage			Pod-bearing stage		
	Total Nitrogen	Organic Carbon	C/N ratio	Total Nitrogen	Organic Carbon	C/N ratio	Total Nitrogen	Organic Carbon	C/N ratio
GA—50 ppm	1 47	24 0	16 32	1 28	27 0	21 09	1 21	37 5	30 99
GA—100 ppm	1 33	25 5	19 17	1 33	28 5	21 42	1 33	39 0	29 32
2,4—D—5 ppm	1 21	21 0	17 35	1 10	25 5	23 18	1 08	30 0	27 77
2,4—D—10 ppm	1 35	19 5	14 14	1 14	27 0	23 68	1 21	31 5	26 03
IAA—250 ppm	1 35	21 5	15 92	1 28	30 0	23 43	1 28	34 5	26 95
IAA—500 ppm	1 21	24 0	19 83	1 28	28 5	22 26	1 30	31 5	24 23
Control (Dist water spray)	1 21	19 5	16 11	1 35	33 0	24 44	1 21	37 0	30 57

*expressed as percentage

TABLE II

Influence of foliar treatment with growth regulators on the total nitrogen, organic carbon and C/N ratio of the roots of blackgram*

Treatment	Vegetative stage			Flowering stage			Pod-bearing stage		
	Total Nitrogen	Organic Carbon	C/N ratio	Total Nitrogen	Organic Carbon	C/N ratio	Total Nitrogen	Organic Carbon	C/N ratio
GA—50 ppm	1 47	24 0	16 32	1 12	27 0	24 10	1 05	28 5	27 14
GA—100 ppm	1 33	25 5	19 17	1 47	30 0	20 40	1 33	30 0	22 55
2,4—D—5 ppm	1 19	19 5	16 38	1 54	28 5	18 50	1 40	33 0	23 57
2,4—D—10 ppm	1 47	22 5	15 30	1 54	24 0	15 58	1 33	28 5	21 42
IAA—250 ppm	1 19	16 5	13 86	1 26	25 5	20 23	0 98	27 0	27 55
IAA—500 ppm	1 47	22 5	15 30	1 54	27 0	17 53	1 26	27 0	21 42
Control (Dist water spray)	1 19	18 0	15 12	1 54	33 0	21 42	1 40	36 0	25 71

*expressed as percentages

TABLE III

Influence of foliar treatment, with growth regulators on the total nitrogen, organic carbon and C/N ratio of the roots of sunnhemp*

Treatment	Vegetative stage			Flowering stage			Pod-bearing stage		
	Total Nitrogen	Organic Carbon	C/N ratio	Total Nitrogen	Organic Carbon	C/N ratio	Total Nitrogen	Organic Carbon	C/N ratio
GA—50 ppm	1 68	30 0	17 85	1 75	34 5	19 71	1 19	38 0	31 93
GA—100 ppm	1 54	30 0	19 48	1 68	36 0	21 42	1 47	37 5	25 51
2,4—D—5 ppm	1 82	24 0	13 18	1 68	30 5	18 15	1 34	35 5	26 49
2,4—D—10 ppm	1 54	28 5	18 50	1 40	33 0	23 57	1 15	36 0	31 30
IAA—250 ppm	1 54	30 0	19 48	1 61	35 0	21 73	1 26	37 5	29 76
IAA—500 ppm	1 40	28 5	20 35	1 33	35 0	26 31	0 98	38 0	38 77
Control (Dist water spray)	1 40	27 0	19 28	1 33	36 5	27 44	1 12	39 5	35 26

*expressed as percentages

TABLE IV

Changes in the nodule number in greengram, blackgram and sunnhemp as influenced by foliar spray of growth regulators

Treatment	Greengram			Blackgram			Sunnhemp		
	Vegetative stage	Flowering stage	Pod-bearing stage	Vegetative stage	Flowering stage	Pod-bearing stage	Vegetative stage	Flowering stage	Pod-bearing stage
GA-50 ppm	23 6	13 6	14 6	23 0	32 6	35 6	50 0	52 0	62 6
GA-100 ppm	15 0	17 3	22 3	11 3	40 6	29 3	57 1	83 6	58 3
2, 4-D-5 ppm	42 3	62 0	27 3	20 6	32 0	38 6	32 3	39 3	55 6
2, 4-D-10 ppm	14 3	21 3	33 6	43 0	79 0	57 6	51 3	39 0	60 3
IAA-250 ppm	11 0	46 0	31 3	25 6	32 6	40 3	29 3	44 0	52 0
IAA-500 ppm	10 3	51 6	37 6	65 0	38 3	44 6	25 3	30 0	33 6
Control (Dist water spray)	29 0	32 0	17 6	40 0	54 0	46 0	48 3	68 3	35 6

C D (P=0 05) Stage—2 93, Crop=2 93, Treatments=4 48, Control *vs* growth regulators=3 88, Between growth regulators=3 16, Between concentration within growth regulators=4 48

500 ppm) on greengram at vegetative stage 2,4-D-5 ppm on blackgram at vegetative stage, and IAA (250 and 500 ppm) on sunnhemp at vegetative stage increased the organic carbon content of the root material and the treatments did not increase the nodule number correspondingly (Tables II and IV)

TABLE V

Correlation between total nitrogen, organic carbon and C/N ratio of the root and nodule number

Independent Variable	Dependant variable	Coefficient of correlation 'r'	Coefficient of regression 'b'	Prediction equation	Level of significance
Greengram					
Total Nitrogen	Nodule Number	—0 2884	—	—	Not Significant
Organic Carbon	,,	—0 0198	—	—	,,
C/N Ratio	,,	0 0944	—	—	,,
Blackgram					
Total Nitrogen	,,	0 3477	—	—	,
Organic Carbon	,,	0 1598	—	—	,,
C/N Ratio	,,	—0 1105	—	—	,,
Sunnhemp					
Total Nitrogen	,,	0 0653	—	—	,,
Organic Carbon	,,	0 3596	—	—	,,
C/N Ratio	,,	0 0863	—	—	,,

A comparison of the results of Carbon/Nitrogen (C/N) ratio and nodulation also indicated no correlation between C/N ratio and nodule number in the plant species studied For instance, the spray of GA-100 ppm and 2,4-D-10 ppm at pod-bearing stages of greengram, 2, 4-D-10 ppm at flowering and pod-bearing stages of blackgram and IAA-250 ppm spray at pod-bearing stage of sunnhemp though reduced the C/N ratio of the root, there was no corresponding decrease in nodulation (Tables III and IV) These results, besides confirming the view of Raggio *et al* (1957) that C/N ratio hypothesis may be an inadequate explanation for inhibition of nodulation, tends to uphold the observations of Van Schreven (1959) and Tanner and Anderson (1963) that nodulation was not influenced by the C/N ratio within the plant

REFERENCES

BREMNER, J M , 1960, Determination of nitrogen in soil by the Kjeldahl method J *Agric Sci.* **55** 11-33

CARTWRIGRT, P M AND SNOW, D , 1962, The influence of foliar applications of urea on the nodulation pattern of certain leguminous species *Ann Bot , Lond ,* **26** 251-259

CHAILAKHYAN, M K H AND MEGRABYAN, A A , 1945, Effect of soluble nitrogenous compounds upon formation of nodules on roots of leguminous plants *Compt Rend Acad Sci ,* **48** 138-141

KANATA, E , 1957, Morphological and physiological studies on nodulation in soybean I Relationship between nitrogen supply and nodulation *Proc Crop Sci Soc ,* Japan, **25** 145-146

KANDASAMY, D AND PRASAD, N N , 1975, Studies on certain factors influencing legume-rhizobia interrelationship VI Indole acetic acid level in rhizosphere soil *Lab Dev Jour Sci and Tech* (In press)

LIE, T A , 1974, In *The Biology of Nitrogen Fixation* pp 555-582 (Ed) A Quispel, North Holland Publishing Co , Amsterdam, Northerlands

Mac Connell, J T and Bond, G , 1957, A comparison of the effect of combined nitrogen on nodulation in nonlegumes and legumes *Pl Soil*, **8** 378–388

Piper, C S , 1950, *Soil and Plant Analysis* Interscience Publishers, New York, U S A

Raggio, M , Raggio, N and Torrey, J G , 1957, The nodulation of isolated leguminous roots *Am J Bot* , **44** 325–334

Steel, G D and Torrie, J H , 1960, *Principles and Procedures of Statistics* Mc Graw Hill Book Co , Inc New York, U S A

Tanner, J W and Anderson, I C , 1963, An external effect of inorganic nitrogen in root nodulation *Nature*, **198** 303–304

Van Schreven, D A , 1959, Effects of added sugars and nitrogen on nodulation of legumes *Pl Soil* , **11** 93–112

Wilson, P W , 1935, The Carbohydrate nitrogen relation in symbiotic nitrogen fixation *Wisconsin, Agr Expt Sta Bull* , **129** 1–40

Effect of Crop Variety on the Multiplication of *Rhizobium* in the Seed Exudates

K Singaravadivel and N N Prasad
Microbiology Division, Faculty of Agriculture, Annamalai University
Annamalainagar–608 101, Tamil Nadu

Abstract

The multiplication of rhizobia in the seed exudates of different varieties of groundnut, blackgram and greengram was studied The seed exudates of TMV 4, among the different groundnut varieties, that of Co 2 among the different blackgram varieties, and that of Anjugam among the different greengram varieties have been found to support relatively high rate of multiplication of the respective strains of rhizobia

The chemotaxis of *Rhizobium* sp towards the seed exudates of different varieties of groundnut, blackgram and greengram indicated lot of variations when assayed by measuring the number of bacteria attracted into a capillary tube containing the respective seed exudate The seed exudates of TMV 4 groundnut, Co 2 blackgram and Anjugam variety of greengram exerted relatively marked chemotaxis The exudates of these three varieties also contained relatively higher amounts of amino–nitrogen and sugars and also amylase and invertase activity

Introduction

Verona *et al* (1959) reported that due to elaboration of nutrient substances by the germinating seed, the microflora on its surface greatly differs from its surrounding Exudation of sugars (Balaraman and Prasad, 1974) and amino acids (Balaraman and Prasad, 1974, Jhooty and Bains, 1976) by the germinating seeds has been reported earlier The exudation of these nutrient substances may well form the energy source for the establishment of seed inoculated rhizobia (Balaraman and Prasad 1974) That the efficiency of the symbiosis is influenced by the crop variety x *Rhizobium* strain interaction has been stressed by several workers (Gesser *et al*, 1972, Russell and Garethjones, 1975) The effect of seed exudates on the multiplication of *Rhizobium* sp as influenced by crop variety are reported in the present paper

Material and Methods

Groundnut (*Arachis hypogaea* L) varieties *viz*, TMV 2, TMV 4, TMV 7, TMV 8, TMV 9, TMV 10 and Gungapuri, blackgram (*Phaseolus mungo* L) varieties ADT 1, CO 2, CO 3, T 9 and No 55 and greengram (*P aureus* Roxb) varieties ADT 1, CO 2, Anjugam, S-8 and H-70-16 were used in this study Efficient strains of *Rhizobium* sp for groundnut, blackgram and greengram, *viz*, Rh VI, AUB 2 and AUG 5, were obtained from the culture collections of the Microbiology Division, Faculty of Agriculture, Annamalai University

Collection of seed exudates The seeds were surface sterilized with 0 1 per cent mercuric chloride solution and washed in several changes of sterile distilled water Fifteen seeds of groundnut and thirty seeds each of the blackgram and greengram were aseptically transferred to each sterile petri dish containing sterile Whatman No 1 filter paper and moistened with 5 ml of sterile distilled water The exudates were collected from five petri dishes at a time for 5 days at 24 hr intervals 5 ml of sterile distilled water was added to each petri dish after each collection of the seed exudate The filter papers, with small quantity of water with which the petri dishes were rinsed, were finely crushed in a mortar with a pestle, extract centrifuged and the volume was made up to 50 ml The seeds have produced radicles by third day So the exudates from third day onwards also included the exudates from the radicle

Effect of crop variety on the multiplication of rhizobia in the seed exudates Seed exudates of different varieties of groundnut, blackgram and greengram were collected as described earlier Twenty four hour cold cultures of *Rhizobium* sp isolated from respective crop species were suspended in sterile distilled water, shaken well and placed on the laboratory bench for 30 min for the bacterial clumps to settle down The suspension was aseptically

transferred into another sterile test tube, one ml of the suspension was inoculated into 50 ml of seed exudates collected from different crop varieties At 1 hr interval, multiplication of the organisms was estimated by plate count method using congo-red agar medium (Allen, 1953) Inoculation of suspension in sterile distilled water served as control

Assay of chemotaxis of rhizobia towards seed exudates A capillary root model based on the technique described by Royle and Hickman (1964) was used for chemotaxis assay The assay chamber was formed as detailed below A 5 cm length capillary tube was bent into a uniform angular U shape and both ends sealed This tube was placed on a microscope slide and covered with a cover slip This chamber was then filled with about 0 2 ml of the above bacterial suspension

Three cm long capillary tubes were taken and their one end was sealed in a flame, the capillary tube was then quickly passed several times through the flame and immediately plunged open end down into a 10 ml of seed exudate As the capillary cooled, liquid was drawn in After about 5 min, the capillary was then inserted (without rinsing) open end first into the chamber containing the bacterial suspension The capillaries were handled with forceps After incubation for 1 hr, the capillary was removed, its exterior was rinsed with a thin stream of water from a wash bottle and then with ethyl alcohol and the sealed end was broken off over (to catch any droplets) a flask containing distilled water The capillary tube was broken into very small pieces and mixed with distilled water, suitable dilutions were made and plated in petri dishes using congo-red agar medium (Allen, 1953) After incubation period at 28 $\pm$ 2°C the colonies were counted Assay points were replicated for all experiments and average reported

Estimation of amino nitrogen and sugars For the estimation of sugars and amino nitrogen the seed exudate was concentrated in a water bath at 60°C The method of Moore and Stein (1958) was employed to estimate amino nitrogen Reducing and non-reducing sugars present in the exudate were estimated by Nelson's (1944) method

Estimation of amylase, invertase and protease The seed exudates were passed through bacterial filters and subsequently used to estimate the activities of amylase, invertase and protease Amylase and invertase were assayed at pH 5 2 employing Sodium-acetate acetic buffer with 1 per cent soluble starch and sucrose, respectively as substrates (Dawson *et al*, 1959) The release of reducing sugars at the end of 24 hr was determined by employing Nelson's (1944) method

The invertase activity was assayed at pH 5 2 using acetate buffer The method followed by Davis and Smith (1955) was used for the estimation of protease

Results and Discussion

The results on the multiplication of rhizobia in the seed exudates of different varieties of groundnut, blackgram and greengram are presented in Tables I to III

Table I

Multiplication of Rhizobium *sp (Groundnut isolate RH VI) in the seed exudates of different groundnut varieties*

Population in X 10^6/ml

Groundnut Variety	Sampling Time (hr)											
	0	1	2	3	4	5	6	7	8	9	10	Total
TMV 2	1 40	4 88	5 78	6 08	6 14	6 24	7 04	7 37	7 93	8 66	9 24	70 76
TMV 4	1 40	4 94	5 94	6 30	6 50	6 74	7 09	7 54	7 77	8 84	8 97	72 03
TMV 7	1 40	4 10	5 58	6 17	6 56	7 02	7 12	7 36	7 65	7 96	8 65	69 57
TMV 8	1 40	4 70	4 84	4 92	5 16	5 52	6 10	6 70	7 03	7 38	7 68	61 43
TMV 9	1 40	3 92	4 97	5 17	5 40	5 64	5 86	5 94	6 44	7 03	7 24	59 01
TMV 10	1 40	4 12	4 42	4 90	4 99	5 65	5 85	6 50	6 78	7 04	7 43	59 08
Gangapuri	1 40	4 66	4 96	5 10	5 54	5 85	6 10	6 81	7 14	7 38	7 60	62 54
Distilled water Control	1 40	2 78	2 93	3 46	3 58	3 93	4 03	4 19	4 36	4 49	4 90	40 17
Total	11 20	34 10	39 42	42 10	43 84	46 59	49 21	52 41	55 10	58 88	61 71	494 59

CD (P=0 05)=1 08

Table II

Multiplication of Rhizobium *sp (Blackgram isolate AUB 2) in the seed exudates of different blackgram varieties*

Population in X 10^6/ml

Blackgram variety	Sampling Time (hr)											
	0	1	2	3	4	5	6	7	8	9	10	Total
ADT 1	0 86	3 28	3 76	4 36	4 68	5 20	5 60	6 00	6 40	6 88	7 36	54 38
CO 2	0 86	3 52	4 08	4 64	5 12	5 56	6 28	6 72	7 12	7 56	8 12	59 58
CO 3	0 86	2 92	3 28	3 72	4 20	4 64	4 92	5 40	5 92	6 32	6 88	48 86
T 9	0 86	2 48	2 88	3 12	3 32	3 68	4 52	4 84	5 04	5 56	5 88	42 18
No 55	0 86	2 76	3 12	3 44	3 92	4 16	4 68	5 32	5 76	6 00	6 28	46 30
Distilled water Control	0 86	1 76	1 98	2 09	2 23	2 45	2 62	2 87	2 96	3 05	3 52	26 29
Total	5 16	16 72	19 20	21 37	23 47	25 69	28 62	31 05	33 20	35 37	37 74	277 59

CD (P=0 05)=1 32

TABLE III

Multiplication of Rhizobium *sp (Greengram isolate AUG 5) in the seed exudates of different greengram varieties*

Population X 10^6/ml

Greengram variety	Sampling time											
	0	1	2	3	4	5	6	7	8	9	10	Total
Control	0 95	1 58	1 69	1 73	1 78	1 85	1 94	2 07	2 18	2 26	2 35	20 38
ADT 1	0 95	3 08	3 36	3 64	3 90	4 52	4 96	5 18	5 46	5 78	6 06	46 89
CO 2	0 95	2 84	3 04	3 30	3 56	3 86	4 20	4 56	4 80	5 06	5 30	41 47
Anjugam	0 95	2 76	3 30	3 86	4 52	5 08	5 44	5 82	6 04	6 50	6 88	51 15
S 8	0 95	2 50	2 80	3 00	3 16	3 28	3 46	3 80	4 08	4 28	4 66	35 97
H 70-16	0 95	2 46	2 64	2 82	3 02	3 22	3 54	3 64	3 78	3 92	4 06	34 05
Total	5 70	15 22	16 83	18 35	19 94	21 81	23 54	25 07	26 34	27 80	29 31	229 91

CD(P=0 05)=0 91

Rhizobium sp multiplied rapidly in exudates of all the varieties and the population increased with increase in the time of incubation The seed exudates of different groundnut varieties showed variation in supporting the multiplication of *Rhizobium* sp Among the different varieties, exudate of the variety TMV 4 was found to support significantly higher rate of multiplication (Table I)

The multiplication rate varied with different varieties Among the different blackgram varieties, Co 2 was found to support relatively high rate of multiplication of *Rhizobium* The rate of multiplication was in order of Co 2> ADT 1> Co 3> No 55> T 9 (Table II)

The variety Anjugam was found to support relatively high rate of multiplication However, it did not significantly vary with ADT 1 The varieties CO 2, S-8 and H-70-16 did not significantly vary with each other (Table III)

In the seed exudates of all the three crop species the multiplication of *Rhizobium* sp was very rapid during the first hour and later increased steadily with increase in the incubation period That the legume seed exudates form good energy source for the multiplication of rhizobia has been reported earlier by Balaraman and Prasad (1974) and Ramani (1974)

Interestingly, when the chemotactic effect of the seed exudates of different varieties of the three crop species was studied on the respective strain of *Rhizobium* sp (Table IV to VI) these three varieties showed maximum chemotactic effect The chemotactic effect of seed exudates was in the order TMV 4> TMV 7> TMV 2> Gungapuri > TMV 8 > TMV 9 for groundnut varieties,

TABLE IV

Chemotactic effect of seed exudates of different varieties of groundnut on Rhizobium *sp (Isolate-RH VI)*

Variety	Population* (X 10^4)
Control	0 68
TMV 9	2 20
TMV 4	2 68
TMV 7	2 32
TMV 8	2 04
TMV 9	1 52
TMV 10	1 68
Gungapuri	2 08

TABLE V

Chemotactic effect of seed exudates of different varieties of blackgram on Rhizobium *sp (isolate-AUB 2)*

Variety	Population* (X 10^4)
Control	2 02
ADT 1	7 68
CO 2	8 98
CO 3	6 66
T 9	6 28
No 55	6 40

TABLE VI

Chemotactic effect of seed exudates of different varieties of greengram on Rhizobium *sp (Isolate-AUG 5)*

Variety	Population (X 10^4)
Control	0 70
ADT 1	7 15
CO 2	5 30
Anjugum	8 64
S 8	1 78
H-70 16	2 74

CO 2 > ADT 1 > CO 3 > No 55 > T 9 for blackgram varieties and Anjugam > ADT 1 > CO 2 > H-70-16 > S-8 for greengram varieties Adler (1973) reported the chemotaxis of *Escherichia coli* to L-aspartate Balaraman and Prasad (1974) reported the occurrence of fourteen amino acids in the seed exudates of groundnut variety TMV 2 Jhooty and Bains (1976) reported the occurrence of fifteen amino acids and three sugars in the seed exudates of

greengram Further studies on the qualitative nature of the seed exudates of different varieties will reveal the substance(s) that are eliciting the chemotactic response

The exudates of the varieties that supported maximum growth rate *viz*, TMV 4 (groundnut), CO 2 (blackgram) and Anjugam (greengram) also contained relatively high amounts of amino nitrogen and sugars, and the exudates of varieties with low levels of nutrients supported less growth (Tables VII to IX) The exudates of these three varieties also revealed a relatively pronounced activity of protease, amylase and invertase among the varieties of the respective crop species (Tables VII–IX) The high activity of these enzymes might influence the cleavage of macromolecules in the vicinity of germinating seed which, in turn, influences the rhizobial nutrition therein

TABLE VII

Sugars, amino nitrogen and certain enzymes in the seed exudation of different groundnut varieties

Variety	Amino* nitrogen	Reducing* sugar	Non-reducing** sugar	Invertase	**Amylase**	Protease***
TMV 2	4 459	6 471	1 522	6 798	7 137	0 056
TMV 4	4 642	6 851	1 646	7 763	8 235	0 058
TMV 7	2 504	6 379	1 641	6 610	4 187	0 044
TMV 8	3 054	5 708	1 418	6 031	3 284	0 047
TMV 9	1 282	3 140	1 414	4 765	3 140	0 037
TMV 10	2 932	4 320	1 237	5 023	3 521	0 042
Gangapuri	2 137	5 864	1 566	6 094	3 387	0 052

* Glutamic acid equivalents in mg/50 ml of seed exudate
** Glucose equivalents in mg/50 ml of seed exudate
*** Tryptophan equivalents in mg/50 ml of seed exudates

TABLE VIII

Sugars, amino nitrogen and certain enzymes in the seed exudation of different blackgram varieties

Variety	Amino* nitrogen	Reducing** sugar	Non reducing** sugar	Invertase**	Amylase**	Protease***
ADT 1	3 420	6 187	1 237	2 36	4 874	0 023
CO 2	4 347	6 471	1 951	2 760	5 233	0 028
CO 3	2 748	6 043	0 856	1 903	4 58	0 021
T 9	3 176	5 088	0 946	1 954	4 061	0 013
No 55	2 565	5 076	0 570	1 856	4 044	0 019

* Glutamic acid equivalents in mg/50 ml of seed exudate
** Glucose equivalents in mg/50 ml of seed exudate
*** Tryptophan equivalents in mg/50 ml of seed exudates

TABLE IX

Sugars, amino nitrogen and certain enzymes in the seed exudation of different greengram variety

Variety	Amino* nitrogen	Reducing** sugar	Non-reducing** sugar	Invertase**	Amylase**	Protease***
ADT 1	3 290	5 090	0 570	1 637	1 427	0 013
CO 2	2 443	4 377	0 666	1 532	1 521	0 022
Anjugam	6 720	4 948	0 761	1 998	2 372	0 024
S 8	1 710	4 162	0 390	1 523	1 712	0 011
H 70-16	2 443	1 237	0 380	1 427	1 423	0 013

* Glutamic acid equivalents in mg/50 ml of seed exudate

** Glucose equivalents in mg/50 ml of seed exudate

*** Tryptophan equivalents in mg/50 ml of seed exudate

REFERENCES

ADLER, J , 1973, A method for measuring chemotaxis and use of the method to determine *optimum condition* for Chemotaxis by *Escherichia coli J Gen Microbiol* , **74** 77–91

ALLEN, O N 1953, *Experiments in Soil Bacteriology* John Wiley and Sons, Inc , NewYork and London, p 472

BALARAMAN, K AND PRASAD, N N , 1974, Influence of sugars and nitrogenous substances in the seed exudate of germinating groundnut on the growth of *Rhizobium* sp *Madras agric J* , **60** 1479–1480

DAVIS, N C AND SMITH, E L , 1955, Assay of Proteolytic enzymes *Meth Biochem Analysis* **2** 215–257

DAWSON, R M C , ELLIOTT, D C , ELLIOTT, W H AND JONES, K M , 1959, *Data for Biochemical Research* Oxford Univ Press London, p 183

GASSER, H , GUY, P , OBATON, M AND SIKORA, I , 1972, Efficiency of *Rhizobium meliloti* strains and their effect on alfalfa cultivars *Can J Plant Sci* , **52** 441–448

JHOOTY, J S AND BAINS, S S , 1976, Modification of toxicity of benomyl by seed exudates of mung *Indian J Microbiol* , **16** 73–75

MOORE, S AND STEIN W H , 1958, A modified ninhydrin reagent for the photometric determination of amino acids and related compounds *J Biol Chem* , **211** 907–913

NELSON, N , 1944, A Photometric adaptation of Somogyi method for the determination of glucose *J Biol Chem* , **153** 375–380

RAMANI, C E , 1974, *Studies on the effect of certain fungicides, insecticides and herbicides and boron and molybdenum on the symbiotic nitrogen fixation of* Rhizobium sp *in groundnut* M Sc (Agri) Thesis, Annamalai Univ

ROYLE, D J AND HICKMAN, C J , 1946, Analysis of factors governing *in vitro* accumulation of Zoospores of *Pythium aphanidermatum* on roots I Behaviour of Zoospores *Can J Microbiol* , **10** 151–162-

RUSSELL, P E AND GARETHJONES, D , 1975, Variation in the selection of *Rhizobium trifolii* by varieties of red and white clover *Soil Biol Biochem* , **7** 15–18

VERONA, O , PICCI, G AND GAMBOGI, P , 1959, Influenza esercitata sui microorganismi dalle soostamze che i semi germinati rivosono mel mezzo durante la fuse di imbibizione *Agric Ital* , **59** 62–71

Effect of Crop Variety on the Multiplication of Inoculated Rhizobia in the Spermosphare Region and their Entry into Rhizosphere

K Singaravadivel and N N Prasad
Microbiology Division, Faculty of Agriculture, Annamalai University
Annamalainagar-608 101, Tamil Nadu

Abstract

The *Rhizobium* sp inoculated on the seeds colonized rapidly in the spermosphere and rhizosphere regions of groundnut, blackgram and greengram However, the extent of such proliferation varied with crop variety

Introduction

The success of seed inoculated rhizobia depends on the rate with which they multiply in the spermosphere region and their subsequent entry into the rhizosphere One of the important factors which influences the multiplication of rhizobia in the spermosphere is elaboration of nutrients (Balaraman and Prasad, 1974) as well as inhibitory substances (Kandasamy *et al*, 1974) by the germinating seeds The crop variety is likely to influence the nature of the substances elaborated by the germinating seed The effect of variety of groundnut (*Arachis hypogaea* L), blackgram (*Phaseolus mungo* L) and greengram (*Phaseolus aureus* Roxb) on the multiplication of inoculated rhizobia in the spermosphere and their subsequent entry into the rhizosphere is presented in this paper

Matfrials and Methods

Different varieties of groundnut *viz* , TMV 2, TMV 4 TMV 7, TMV 8, TMV 9, TMV 10 and Gangapuri, blackgram varieties *viz* , ADT 1, CO2, CO3, T9 and No 55 and greengram *viz* , ADT 1, CO2, Anjugam, S-8 and H 70-16 were surface sterilized with 0 1 per cent mercuric chloride and washed in several changes of sterile distilled water They were then treated separately for 12 hr with the respective rhizobial strain Efficient rhizobial strains for groundnut blackgram and greengram *viz* , Rh VI, AUB 2 and AUG 5, respectively, were obtained from the culture collection of the Microbiology Division of the Faculty of Agriculture, Annamalai University and used in this study The treated seeds were dried in shade on filter paper

Circular mud pots of the size 15 cm diameter were filled with soil and sterilized The seeds treated with *Rhizobium* sp were sown in these sterilized soil as well as in unsterilized soil The soil moisture was maintained at 60 per cent level in the pots

Enumeration of Rhizobium sp in the spermosphere and rhizosphere soils

1 *Collection of spermosphere soil sample* Germinating seeds along with the adhering spermosphere soil were collected and 1 g of the sample was transferred to 100 ml sterile water blank in a 250 ml Erlenmeyer flask

2 *Collection of rhizosphere soil sample* The rhizosphere soil samples were collected by pulling out the young seedlings carefully with the root system The roots after gentle tapping to remove as much of adhering soil as possible, were cut with sterilized scissors and transferred to a 100 ml sterile water blank in 250 ml Erlenmeyer flask (Timonin, 1940)

The dry weight of the samples from each of the flasks were determined by transferring the contents to petridishes of known weight, evaporating over a water bath and drying to a constant weight in a hot air oven at 150°C The total dry weight of the sample was calculated, taking into consideration the quantity utilized for microbial estimation

3 *Estimation of Rhizobial numbers* For estimating the rhizobial numbers in the spermosphere and rhizosphere soil, samples collected in the sterile water blanks in Erlenmeyer flasks were shaken thoroughly on a rotary shaker for 5 min, and serial dilutions of the suspensions made using sterile water blanks One ml of the diluted suspension were transferred aseptically to petridishes and melted congo-red agar medium (Allen, 1953) added The petridishes were incubated at room temperature (28±2°C) for 5 days and the colonies conducted

Results and Discussion

The seed inoculated *Rhizobium* sp has established and multiplied in the spermosphere and rhizosphere regions of different groundnut varieties However, the extent of such proliferation varied with different varieties In both sterile and nonsterile soils rhizobial multiplication and establishment in the spermosphere region was relatively higher in the variety TMV 4 Variety TMV 9 was found to support relatively less number of rhizobia in the spermosphere and rhizosphere compared to other varieties The rhizobial numbers in the spermosphere and rhizosphere regions of these varieties were statistically on par excepting for TMV 9 (Table I)

Among blackgram varieties Co 2 and ADT 1 were found to support relatively higher multiplication of rhizobia However, these varieties were on par with CO3 and No 55 varieties (Table II)

TABLE I

Effect of crop variety on the multiplication of Rhizobia in the Spermosphere *and* Rhizosphere *regions of groundnut (Population in 10^6/g of moisture free soil)*

Treatment sampling time (in days)	Variety						
	TMV 2	TMV 4	TMV 7	TMV 8	TMV 9	TMV 10	Gangapuri
Sterile soil							
Spermosphere							
1	7 86	9 54	5 10	3 90	1 10	4 30	5 04
3	8 40	10 30	7 40	6 66	2 54	5 90	7 22
Rhizosphere							
7	8 72	7 74	6 54	6 76	4 54	6 43	6 34
9	4 56	5 44	6 33	4 64	4 88	4 56	62 3
Non-sterile soil							
Spermosphere							
1	4 56	3 70	21 6	2 50	1 14	1 91	3 06
2	4 84	7 20	6 24	4 70	3 80	4 92	5 04
Rhizosphere							
3	7 40	6 90	5 78	5 60	4 20	4 64	5 32
7	2 58	2 66	3 72	3 10	2 84	2 78	3 54

Sterile Soil C D (P=0 05)=2 46
Unsterile soil C D (P=0 05)=1 17

TABLE II

Effect of crop varieties on the multiplication of Rhizobium *in the Spermosphere and Rhizosphere regions of blackgram*

(Population in 10^6/g moisture free soil)

Treatment sampling time (in days)	Variety				
	ADT1	CO 2	CO 3	T 9	No 55
Sterile soil					
Spermosphere					
1	4 28	4 80	2 60	1 80	2 48
3	4 60	5 40	5 20	2 72	3 68
Rhizosphere					
7	6 60	6 80	5 52	3 68	4 33
9	7 04	7 24	5 68	3 32	5 12
Non-sterile soil					
Spermosphere					
1	2 75	3 40	2 68	1 48	2 20
3	3 28	3 80	2 80	3 20	2 80
Rhizosphere					
7	5 12	5.56	3 88	3 28	3 60
9	5 68	5 92	4 48	3 70	4 02

Sterile C D (P=0 05) =1 03
Unsterile C D (P=0 05)=1 93

Among the greengram varieties, Anjugam was found to support a relatively higher rate of multiplication of Rhizobia compared to other varieties used in both sterile and nonsterile soils However, it was on par with ADT 1 and CO 2 varieties in sterile soil and ADT 1, CO 2 and S-8 in nonsterile soil (Table III)

TABLE III

Effect of crop varieties on the multiplication of Rhizobium *in the spermosphere and rhizosphere regions of greengram*

(Population in 10^6/g of moisture free soil)

Treatment Sampling time (in days)	Variety				
	ADT 1	CO 2	Anjugam	S 8	H 70-16
Sterile soil					
Spermosphere					
1	3 85	2 70	4 21	1 96	1 62
3	4 13	2 92	4 44	2 92	1 71
Rhizosphere					
7	5 00	3 26	5 50	3 66	3 52
9	5 35	4 80	6 48	2 80	3 35
Non-sterile soil					
Spermosphere					
1	3 68	2 60	3 76	1 92	1 22
3	3 88	3 17	4 67	2 88	1 48
Rhizosphere					
7	5 60	4 36	5 34	3 06	3 66
9	5 88	6 80	6 62	5 22	4 12

Sterile soil C D (P=0 05) =2 26
Unsterile soil C D (P=0 05) =2 39

Thus, the different varieties of the three crop species varied in supporting the multiplication of respective strains of inoculated rhizobia in the spermosphere and rhizosphere regions Gasser *et al* (1972) reported a significant variety × *R meliloti* strain interaction among alfalfa cultivars Russell and Garethjones (1975) reported a considerable variation among the cultivars of red and white clover in their selection of rhizobial strain The results of the present investigation clearly point to the need for an intensive work on the legume variety × *Rhizobium* strain interaction in the cowpea cross inoculation group

The multiplication of *Rhizobium* was higher in the rhizosphere region than in the spermosphere region both in sterile and unsterile soils with respect to the three crop species Similar result was reported by Balaraman (1970) and Kandasamy (1974) This might be due to the differences in the chemical nature of the seed and root exudates Balaraman (1970) and Ramani (1974) reported that the reducing sugars, non-reducing sugars and amino nitrogen

level in the seed exudates increased with increase in time during germination and Kandasamy (1974) reported that the total phenolics in the root exudates of blackgram, greengram and sunnhemp decreased with increase in the seedling age

The multiplication of rhizobia was in general higher in sterile soil than in unsterile soil Van Schreven (1970) and Danso and Alexander (1974) reported that microorganisms are responsible for the changes in rhizobial numbers in the non-sterile habitat Danso *et al* (1975) reported the appearance of many protozoa in normal soil to which the rhizobia are added which feed on these bacteria and cause decline in multiplication

References

Allen, N , 1953, *Experiments in Soil Bacteriology* Burgees Publishing Company, Minneapolis, Minn , U S A , pp 69–70

Balaraman, K , 1970, *Studies on certain aspects of pre-treatment of groundnut seeds with Chemicals and Rhizobium* sp M Sc (Agri) Thesis, Annamalai Univ

Balaraman, K and Prasad N N , 1974, Influence of sugars and nitrogenous substances in the seed of germinating groundnut on the growth of *Rhizobium* sp *Madras agric J* , **60** 1479–1480

Danso S K A and Alexander, M , 1974, Survival of two strains of *Rhizobium* in soil *Soil Sci Soc Amer Proc* , **38** 86–89

Danso, S K A , Keya, S O and Alexander, M , 1975 Protozoa and the decline of *Rhizobium* Populations added to soil *Can J Microbiol* , **21** 884–895

Gasser, H , Guy, P , Obaton, M and Sikora, I , 1972, Efficiency of *Rhizobium meliloti* strains and their effects on alfalfa cultivars *Can J Plant Sci* , **52** 441–448

Kandasamy, D , 1974, *Studies on certain factors influencing legume-rhizobia interrelationship in greengram* (*Phaseolus aureus* Roxb), *Blackgram* (*P mungo* L) and *sunnhemp* (*Crotolaria juncea* L) Ph D thesis Annamalai Univ

Kandasamy, D , Kesavan, R , Ramaswamy, K and Prasad, N N , 1974, Occurrence of microbial inhibitors in certain seed coats of leguminous seeds *Indian J Microbiol* , **14** 25–27

Russell, P E and Garethjones, D , 1975, Variation in the selection of *Rhizobium trifolii* by varieties of red and white clover *Soil Biol Biochem* **7** 15–18

Timonin, M I , 1940, The interaction of higher plants and soil microorganisms II Study of the microbiol population of the rhizosphere in relation to resistance of plants to soil-borne diseases *Can J Res* , **18B** 444–456

Van Schreven, D A , 1970, Some factors affecting growth and survival of *Rhizobium* spp in soil-plot cultures *Pl Soil*, **32** 113–130

Effect of Soil Moisture on the Establishment of *Rhizobium* in the Spermosphere and Rhizosphere Regions of Leguminous Crops

K Singaravadivel and N N Prasad
Microbiology Division, Faculty of Agriculture, Annamalai University
Annamalainagar-608 101, Tamil Nadu,

Abstract

The rhizobial colonization in spermosphere and rhizosphere regions was high at 70 per cent moisture level, in three legumes *viz* , groundnut, blackgram and greengram

Introduction

Survival and multiplication of inoculant rhizobia in the spermosphere and rhizosphere regions is a deciding phase for the success of nodule formation Many workers (Vincent and Waters, 1954 , Rovira, 1961 , Marshal 1964 , Chowdhury *et al* , 1968 , Kandasamy, 1971 , Balaraman and Prasad, 1972 , Ramani, 1974) studied the factors influencing the survival and multiplication of rhizobia on the seed surface The effect of different soil moisture levels on the establishment and multiplication of rhizobia in the spermosphere and rhizosphere regions of groundnut (*Arachis hypogaea* L), blackgram (*Phaseolus mungo* L) and greengram (*P aureus* Roxb) are presented in this paper

Material and Methods

The seeds of groundnut (TMV 4), blackgram (Co 2) and greengram (Anjugam) were surface sterilized with 0 1 per cent mercuric chloride solution and washed with several changes of sterile water They were then treated with respective rhizobial suspensions for 12 hr The treated seeds were dried in shade on filter paper

Circular mud pots with 15 cm diameter were filled with soil and sterilized The *Rhizobium* treated seeds were sown in the sterilized pots at different moisture levels *viz* , 30, 50 70 and 90 per cent and submerged condition

The *Rhizobium* population in the spermosphere soils was estimated at the end of 24 hr and 72 hr and that of rhizosphere soil on 7th day and 9th day, following the dilution plate technique using congo-red agar medium (Allen, 1953) The populations were counted after 5 days of incubation using Arnold colony counter

Results and Discussion

The *Rhizobium* colonization in spermosphere and rhizosphere regions of groundnut was high at 70 per cent moisture level There was no marked variation in the colonization of *Rhizobium* between 50 and 90 per cent moisture levels The colonization was in order of 70> 50> 90 > submerged> 30 per cent moisture levels The rhizobial numbers declined rapidly after 7th day in the rhizosphere (Table I)

Table I

Effect of soil moisture on the establishment and multiplication of Rhizobium *in spermosphere and rhizosphere regions of groundnut*

(Population in $\times 10^6$/g of moisture free soil)

Treatment	Spermosphere		Rhizosphere	
	1st day	3rd day	7th day	9th day
30 per cent	2 92	3 92	2 40	2 14
50 per cent	4 40	8 12	7 80	4 60
70 per cent	6 60	10 20	11 20	5 80
90 per cent	3 20	5 23	9 23	5 68
Submerged	1 80	4 40	4 60	3 09

C D (P=0 05)=2 03

Table II

Effect of soil moisture on the establishment and multiplication of Rhizobium *in the spermosphere and rhizosphere regions of blackgram*

(Population in $\times 10^6$/g of moisture free soil)

Treatment	Spermosphere		Rhizosphere	
	1st day	3rd day	7th day	9th day
30 per cent	1 52	2 28	2 80	2 08
50 per cent	2 53	8 59	9 20	5 00
70 per cent	4 21	8 92	9 03	6 68
90 per cent	2 18	2 48	3 40	2 64
Submerged	1 60	3 20	2 60	2 00

C D (P=0 05) =0 23

The colonization of *Rhizobium* both in spermosphere and rhizosphere regions of blackgram was high at 70 per cent moisture levels The results revealed that the submerged condition does not favour the rhizobia in the rhizosphere

TABLE III

Effect of soil moisture on the establishment and multiplication of Rhizobium *in the spermosphere and rhizosphere regions of greengram*

(Population in $\times 10^6$/g of moisture free soil)

Treatment	Spermosphere		Rhizosphere	
	1st day	3rd day	7th day	9th day
30 per cent	6 05	8 54	7 51	6 08
50 per cent	7 52	8 65	10 80	9 25
70 per cent	8 20	12 81	15 50	10 97
90 per cent	7 21	8 03	9 03	8 56
Submerged	6 22	6 52	8 05	7 02

C D (P=0 05) = 1 81

The colonization of *Rhizobium* in the spermosphere and rhizosphere regions of greengram was high at 70 per cent moisture level At different moisture levels the colonization was in order of 70> 50> 90> 30 per cent> submerged moisture levels The rhizobial numbers were higher in 7th day sampling than on 9th day At 30 per cent level the spermosphere soil recorded more numbers compared to rhizosphere soil De-polli *et al* (1974) reported that survival of native and inoculated rhizobia decreased with increasing moisture levels

REFERENCES

BALARAMAN, K AND PRASAD, N N , 1972, Compatibility of certain seed dressing chemicals with *Rhizobium Curr Sci* , **41** 344

CHOWDHURY M S , MARSHALL, K C AND PARKER, C A , 1968, Growth rates of *Rhizobium trifolii* and *Rhizobium lupini* in sterilized soils *Aust J Agric Res* , **19** 919-925

DE-POLLI, AVILIO, A F AND DOBEREINER, J , 1974, Survival of *Rhizobium* in flooded soils. *Pesaqut Agropea Braz Ser Agron* , **8** 133-138

KANDASAMY, R 1971, *Studies on certain aspects of legume root nodule bacteria* M Sc (Ag) thesis, Annamalai Univ

MARSHALL, K C , 1964 Survival of (Clover) root-nodule bacteria in dry soil exposed to high temperatures *Aust J Agric Res* , **15** 273-287

RAMANI, C E , 1974, *Studies on the effect of certain fungicides, insecticides and boron and molybdenum on the symbiotic nitrogen fixation of Rhizobium* sp *in groundnut* M Sc (Agri) thesis Annamalai Univ

ROVIRA, A D 1961, *Rhizobium* numbers in the rhizospheres of red clover and *Paspalum* in relation to soil treatment and number of bacteria and fungi *Aust J Agric Res* , **12** 77-83

VINCENT, J M AND WATERS, L M , 1954, The root nodule bacteria as factors in clover establishment in the red basaltic soils of the Lismore Dt New South Wales 11 Survival and Success of inoculant in laboratory trials *Aust J Agric Res* , **5** 61-76

Effect of Soil Compaction and Moisture on Nodulation and Yield Components in Groundnut

B G KARLE, C P GHONSIKAR AND R S RAUT

Department of Soil Science and Agriculturl Chemistry, Marathwada Agricultural University, Parbhani (M S)

ABSTRACT

Soil compaction in a range from 0 9 to 1 2 B D tended to increase flower and gynophore production in groundnut grown when with restricted moisture supply at 20 and 60 per cent available moisture in soil Pod bearing pegs per stand decreased with increasing bulk density at 40 and 60 per cent available moisture levels Soil compaction delayed total flower production Increasing soil compaction reduced the development of nodules per stand Soil bulk density of 1 0 at 20 per cent available moisture produced 120 nodules per stand as against 31 33 nodules per stand for bulk density of 1 2 at 60 per cent available moisture Pod and dry matter production was affected correspondingly with increasing soil compaction and soil moisture stress

INTRODUCTION

SOIL compaction is in important problem in areas where soils contain large proportion of clay fraction and poor drainage conditions Soil compaction developed by way of repeated operations of equipments over a soil greatly affect the germination of seeds and emergence of seedlings Crop responses to soil compaction have received attention in recent years in an endeavour to identify the factors affecting the plant responses Bertrand and Kohnke (1957) found that sub soil compaction slowed diffusion of soil atmosphere which in turn reduced the growth of corn Veihmeyer and Hendrickson (1948) indicated that restricted root growth is a function of bulk density Adams *et al* (1960) reported that increasing surface soil bulk density lowered the yields of potato, sugar beet, wheat and corn Flocker *et al* (1958) observed that compaction of fine sandy loam soil to a bulk density of 1 58 g/cm^3 reduced the yield of number of winter cover crops and legumes

Compaction and hardness at the soil surface likewise affect greatly gynophore penetration and pod development in groundnut Moisture stress in soil further hardens the soil suiface The present study is aimed to investigate the effect of compactness and soil moisture levels on nodulation and yield components in groundnut

TABLE I

Effect of soil compactness and moisture on nodulation and yield components in groundnut

Moisture levels / Soil bulk densities	M 1 (20 % available moisture) D0 (0 9)	D1 (1 0)	D2 (1 1)	D3 (1 2)	M 2 (40 % available moisture) D0 (0 9)	D1 (1 0)	D2 (1 1)	D3 (1 2)	M 3 (60 % available moisture) D0 (0 9)	D1 (1 0)	D2 (1 1)	D3 (1 2)
Per cent germination	60 00	80 00	80 00	60 00	60 00	80 00	80 00	73 73	60 00	66 66	46 66	60 00
Days to first flower	48 00	50 00	39 33	39 66	48 00	39 00	46 00	37 00	40 00	39 00	38 33	39 66
Total flowers produced/plant	15	21	32	36	23	56	18	63	27	49	57	48
Pegs (Gynophores)/plant	10	17	25	26	16	43	15	53	21	39	41	35
Pegs as per cent of total flowers	66 66	80 91	77 99	72 11	69 64	77 26	83 59	84 74	77 80	80 85	72 13	72 75
Pod bearing pegs/plant	4	4	6	5	7	6	4	3	12	3	5	2
Pod bearing pegs as per cent of total flowers	26 66	19 07	18 66	14 07	30 40	10 65	21 97	4 81	44 57	6 24	8 76	4 26
Pegs without pods	2	4	10	15	5	21	7	35	6	25	17	19
Pegs without pods as per cent of total flowers	39 99	61 83	59 32	58 03	39 23	66 60	61 62	79 42	33 22	74 47	63 48	68 48
Per cent flowers not producing pegs	33 33	19 08	22 00	27 88	30 34	22 73	16 39	15 74	22 19	19 13	27 85	27 24
Days after sowing to produce 50 per cent of total flowers	49 00	54 33	48 00	52 33	47 33	49 00	45 00	54 00	44 00	45 66	49 66	52 00
Days after start of flowering to produce 50 per cent of total flowers	14 00	19 33	13 00	17 33	12 66	14 00	10 00	19 00	13 00	10 66	14 66	17 00
Total pods/plant	3	4	6	5	5	6	4	3	9	3	5	2
Pod yield in g/plant	00 75	00 83	2 10	2 16	1 75	3 00	1 83	1 75	3 50	1 26	3 00	2 25
No of undeveloped pods/plant	3	2	2	2	1	2	1	2	3	2	3	1 3
No of developed pods/plant	3	2	4	3	4	4	3	2	6	1	2	1
Dry weight of plant at harvest	6 00	3 30	5 80	7 30	6 80	7 80	6 00	7 00	9 60	10 30	6 50	7 30
No of nodules/plant	64 33	120 00	85 00	69 33	77 33	88 66	41 66	34 33	93 00	75 00	42 66	31 33

Materials and Methods

A green house experiment was conducted using metal pots of 27 cm diameter A medium black cotton soil, previously passed through 2 mm sieve, was filled in the pots The soil was pressed uniformly to desired bulk density *i e* , 0 9, 1 0, 1 1 and 1 2 g/cm^3 Soil moisture levels were adjusted to 20 per cent, 40 per cent and 60 per cent available moisture levels Five bold groundnut seeds of variety SB XI inoculated with rhizobium culture were sown in the pots at the surface and loose soil was spread over uniformly Only one healthy plant was maintained after two weeks from emergence for further observations Moisture levels as indicated were maintained throughout the life period of crop All treatments were replicated thrice Nodulation count was taken at half flowering stage Other observations were made at the appropriate time

Results and Discussion

Morphological characters It is evident from the data in Table I and II that the differences in per cent germination and days to first flower were not significant

Table II

Effect of soil compactness and moisture on nodulation and yield components in Groundnut

			Moisture		Density		Moisture × Density	
	SE	CD	SE	CD	SE	CD	SE	CD
% Germination	10 99							
Days to first flower	3 12							
Total flowers produced/plant	2 47	7 25	1 23	3 62	1 42	4 19	2 47	7 26
Pegs/plant	1 39	4 10	0 69	2 05	0 80	2 36	1 39	4 09
Pegs as % of total flowers	4 30							
Pod bearing pegs/plant	0 83	2 43	0 41		0 48	1 41	0 83	2 44
Pod bearing pegs as % of total flowers	15 89	46 61	1 69		1 95	5 85	3 38	9 93
Pegs without pods	2 14	6 41	1 07	3 21	1 23	3 63	2 14	6 28
Pegs without pods as % of total flowers	5 93	17 41	2 96	8 71	3 42	10 05	5 93	
% flowers not producing pegs	4 31							
Days after sowing to produce 50 % of total flowers	0 70	2 05	0 12	0 35	0 16	0 47	0 69	2 05
Days after start of flowering to produce 50 % of flowers	0 66	1 95	0 33	0 97	0 38	1 15	0 66	1 95
Total pods/plant	0 75	2 21	0 37		0 43	1 28	0 75	2 22
Pod yield mg/plant	0 40	1 17	0 20	0 59	0 22		0 39	1 16
No of undeveloped pods/plant	0 55							
No of developed pods/plant	0 81	2 38	0 42		0 46	1 35	0 81	2 38
Dry weight of plant at harvest	0 92	2 70	0 45		0 52	1 55	0 92	2 70
No. of nodules/plant	2 92	8 58	1 46	4 29	1 69	4 95	2 92	8 58

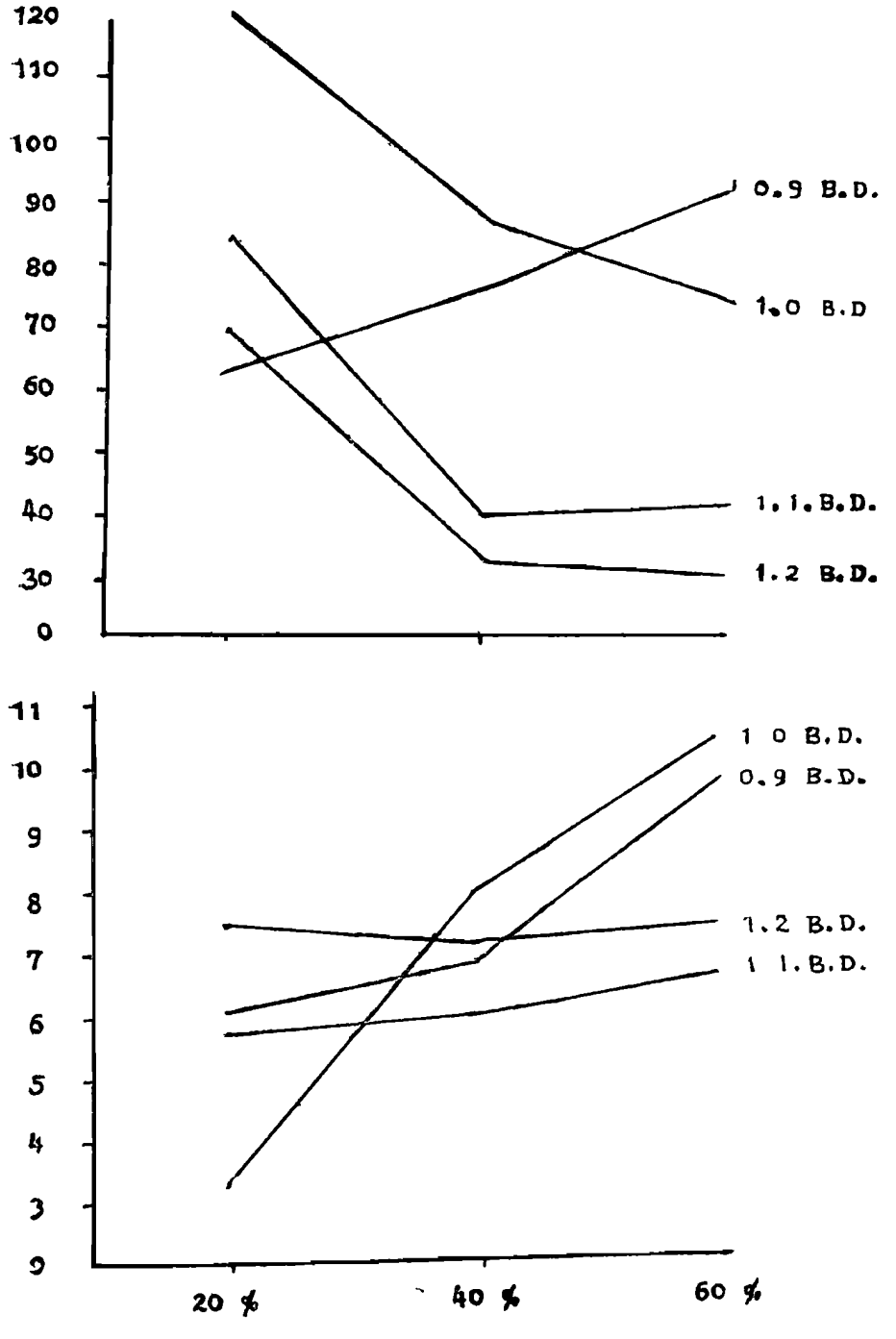


Fig 1 Nodulation and dry matter yield in groundnut as affected by compactness (B D.) and moisture availability in soil

However, differences in total flower production per stand in all treatments were significant There was an increasing trend in flower production in 0 9 to 1.2 bulk density at 20 per cent and 60 per cent available moisture Bulk density of 1 2 at 40 per cent available moisture produced maximum (63) flowers in groundnut in contrast to 15 flowers in a treatment of 0 9 bulk density at 20 per cent available moisture The interaction effects were significant Flocker *et al.* (1959) showed that compacting soil to a certain density increased the per cent germination in tomato plant Compacting the soil delayed the seedling emergence The differences in the total peg production per stand were significant for different moisture levels Interaction effects were also significant 1 2 bulk density at 40 per cent available moisture produced 53 pegs per stand representing 84 74 per cent pegs as per cent of total flowers This treatment was significantly superior over other treatments An increasing trend in peg production per stand was observed from 0.9 to 1 2 B D at 20 per cent and 60 per cent available moisture The number of pod bearing pegs per stand was increased with increasing B D at 40 per cent and 60 per cent available moisture levels The number of pegs without pods was increased with increasing moisture levels in 0 9 and 1 0 B D and with increasing density at 20 per cent available moisture level B D of 1 2 at 40 per cent available moisture produced 79 42 per cent pegs without pods as per cent of total flowers in contrast to 33 22 per cent in 0 9 B D at 60 per cent available moisture The interaction effects were at par In all the treatments the differences in the per cent flowers not producing pegs were not significant B D of 1 0 at 20 per cent available moisture required 54 33 days after sowing to produce 50 per cent of total flowers in contrast to 44 days in 0 9 B D at 60 per cent available moisture level The differences between different moisture and density levels and interactions were significant 1 1 B D at 40 per cent available moisture regained 10 days after flowering to produce 50 per cent of to the flowers in contrast to 19 33 days in 1 0 B D at 20 per cent available moisture Flocker *et al* (1959) reported that compression of soil beyond certain limits resulted in an overall inhibition of some of the physiological processes of plant growth in tomato Bertrand and Kohnke (1957) reported that the influence of soil compaction appeared to be related to many soil-plant-climate interaction

Nodulation, A significant negative response to nodulation was observed with increasing soil B D and mositure levels 1 0 B D at 20 per cent available moisture level produced 120 nodules per stand in contrast to 31 33 nodules in 1 2 B D at 60 per cent available moisture Significant differences were also observed between moisture and density levels 0 9 B D increased nodules per stand with increasing moisture levels significantly, on the other hand 1 0, 1 1 and 1 2 B D decreased nodules with increasing moisture levels It might be due to poor root growth because of lack of oxygen supply, lesser porosity, poor

water utilization and restricted nutrient uptake Veihmeger and Hendrickson (1948) reported that restricted root growth is a function of B D

Yield and yield components A decreasing trend in pod yield per stand was observed with increasing bulk density and moisture levels 0 9 bulk density significantly increased pod yield/plant with increasing moisture levels The differences within bulk density were at par 0 9, 1 0 and 1 1 bulk density increased dry matter with increasing moisture levels However, Wittsell and Hobbs (1965) found reduction in legume yield due to compaction of soil Adams *et al* (1960) reported that increasing soil bulk density from 1 07 to 1 14 g/cm^3 lowered the yield of potato, sugar beet, wheat and corn Yields of sorghum grains, Sudan grass and wheat were also depressed by soil compaction (Wittsell and Hobbs, 1965)

References

Adams, F P , Blake, G R , Martin, W P and Boelter, P H , 1960, Influence of soil compaction on crop growth and development *Trans 7th Intern Congress of Soil Sci* , **1** 609–615

Bertrand, A R and Kohnke, H , 1957, Subsoil conditions and their effects on oxygen supply and the growth of corn roots *Soil Sci Soc Amer Proc* , **21** 135–140

Flocker, W J , Vomocil, J A and Howard, F D , 1959, Some growth responses of tomatoes to soil compaction *Soil Sci Soc Amer Proc* , **23** 188–191

Flocker, W J , Vomocil, J A and Vittum M T , 1958, Response of winter cover crops to soil compaction *Soil Sci Soc Amer Proc* , **22** 181-184

Veihmeger, F J and Hendrickson, A H , 1958, Soil density and root penetration *Soil Sci* , **65** 487–493

Wittsell, L E and Hobbs, J A , 1965, Soil compaction effects on field plant growth *Agron J* , **57** 534–537

Effect of pH, Moisture and Temperature on the Survival of *Rhizobium* sp in Sterile Soil

R Swaminathan and N N Prasad
Division of Microbiology, Faculty of Agriculture, Annamalai University
Annamalainagar-608 181, Tamil Nadu

Abstract

The effect of pH, moisture and temperature on the growth of rhizobium (AUB 2) was studied The optimum pH, moisture and temperature were found to be 9, 60 per cent and 30°C respectively

Introduction

The process of nodule formation by rhizobia has been influenced by pH, moisture and temperature (Yadav and Vyas, 1971, Roughley, 1968 and Roughley and Vincent, 1967) The present study deals with the influence of such factors on the survival of *Rhizobium*

Material and Methods

An isolate of *Rhizobium* sp (AUB 2) from blackgram grown in yeast extract mannitol broth was inoculated into sterilized soil, maintained under different pH (7 0 to 10 0), moisture (20 to 100 per cent) and temperature (30 to 45°C) levels The rhizobial populations in the soil was estimated at 20 days interval by dilution plate technique using yeast extract mannitol agar medium containing congo-red

Results and Discussion

The rhizobial population was more on 60th day at pH 9 and it declined thereafter (Table I)

The inoculated rhizobial cells multiplied well at 60 per cent moisture level Maximum population was observed on 120th day at 60 per cent moisture level (Table II)

The rhizobial population was more at 30°C and at other levels the population was very much affected (Table III)

With most legumes nodule formation takes place in a narrow range of H-ion concentration Yadav and Vyas (1971) reported that the optimum pH for growth of *Rhizobium* spp varied between strains, for berseem strains it was pH 7–8, for cowpea pH 5–7, for daincha pH 6–7, for gram pH 7–9, for

TABLE I

Effect of pH on the survival of inoculated Rhizobium *sp in sterile soil* (10^6/g)

pH	Sampling time in days						Mean
	20	40	60	80	100	120	
7	24 0	19 0	17 5	14 0	10 0	7 5	15 3
8	20 0	29 0	33 0	18 0	12 0	9 0	20 1
9	23 0	34 0	43 5	33 0	24 0	13 0	28 4
10	23 0	30 0	20 0	17 5	12 5	8 0	18 5
Mean	22 5	28 0	28 5	20 6	14 6	9 3	

Initial inoculum 23×10^6/g of soil
Significant at 1 per cent level
Treatment $\overline{T_1\ T_4}\ \underline{T_2\ T_3}$
Period $\overline{P_6\ P_5}\ P_4\ \underline{P_1\ P_2\ P_3}$

Treatment S E D 2 8
C D 6 0
Period S E D 3 5
C D 7 4

TABLE II

Effect of moisture on the survival of inoculated Rhizobium *sp in sterile soil* (10^6/g)

Moisture per cent	Sampling time in days						Mean
	20	40	60	80	100	120	
20	23 0	10 5	5 5	3 5	2 0	1 5	7 6
30	28 0	12 5	6 5	4 0	3 5	2 0	9 4
40	30 5	14 5	7 5	6 0	5 0	2 5	11 0
50	36 0	19 0	9 5	8 0	6 5	5 0	13 9
60	55 0	20 0	14 0	10 5	9 0	8 0	19 4
70	45 0	29 0	12 0	9 0	8 0	7 0	18 3
80	39 0	19 5	10 0	8 0	6 5	6 0	14 8
90	37 0	13 5	9 0	7 0	5 0	4 0	12 5
100	37 0	8 5	8 5	6 0	3 5	2 0	10 9
Mean	36 2	16 2	9 1	6 9	5 5	4 2	

Initial inoculum 23×10^6/g of soil
Significant at 1% level
Treatment $\overline{T_1\ T_2\ T_9\ T_3\ T_8}\ \overline{T_4\ T_7\ T_6\ T_5}$
Period $\overline{P_6\ P_5}\ \underline{P_4\ P_3}\ P_2\ P_1$

Treatment S E D 2 0
C D 4 0
Period S E D 1 3
C D 2 6

groundnut pH 5-7 and for guwar pH 5-6 In the present study the isolate from blackgram was found to survive well at pH 8 0 and 9 0 in the sterile soil up to 60 days after inoculation

TABLE III

Effect of temperature on the survival of inoculated Rhizobium *sp in sterile soil (10⁶/g)*

Temperature	Sampling time in days						Mean
	20	40	60	80	100	120	
30°C	33 0	13 0	4 5	2 0	1 5	0 5	9 1
35°C	17 0	7 0	3 0	1 5	0 5	—	4 8
40°C	11 0	6 0	3 0	0 5	—	—	3 4
45°C	7 0	4 5	2 0	0 5	—	—	2 3
Mean	17 0	7 6	3 1	1 1	0 5	0 12	

Initial inoculum 23×10^6/g of soil Not significant

In the present study, the population level was more at 60 per cent moisture level and this higher population load was maintained up to 40 days This finding is in agreement with the report by Roughley (1968) The introduced rhizobia tolerate a higher moisture level *i e*, 40–60 per cent and the most favourable moisture level depends on the soil type The population level varied with the range of moisture level At extreme levels of moisture conditions *i e*, 20 per cent and 100 per cent the rhizobial population was low This observation supports the report of Vandecaveye (1927) and Heldin and Neuton (1948) that counts of bacteria in soil are closely related to the moisture level

The influence of temperature on the survival of rhizobia was studied by Vandecavaye (1927) and Date (1959) In this study the optimum temperature for the multiplication was found to be 30°C, however this was statistically not significant

REFERENCES

HELDIN, R A AND NEUTON S B, 1948, Some factors influencing the growth and survival of *Rhizobium* in humus and Soil cultures *Can J Res*, 26 174–187

ROUGHLEY, R J 1968, Some factors influencing the growth and survival of the root nodule bacteria in peat culture *J Appl Bacteriol*, **31** 259–265

ROUGHLEY, R J AND VINCENT, J H 1967, Growth and survival of *Rhizobium* spp in peat culture *J Appl Bacteriol*, **30** 259–265

VANDECAVEYE, E G, 1927, Effect of moisture, temperature and other climatic conditions on *Rhizobium leguminosarum* in the soil *Sci*, **23** 355–362

YADAV, V N. K AND VYAS, S R, 1971, Effect of salts and pH in the gravity of *Rhizobium* strains *Indian J Microbiol*, **11** 92–102

Effect of Moisture Stress and Inoculum Placement on Nodulation, Yield and Protein Content of Soybean

C P Ghonsikar, V G Musande and R S Raut
Department of Soil Science and Agricultural Chemistry, Marathwada Agricultural University, Parbhani

Abstract

A greenhouse study with soybean var BC-574 grown in a black clay soil exhibited significant effect of moisture stress and inoculum placement on nodulation, yield and protein Number of nodules, grain yield and seed protein was significantly higher under 80 per cent of field capacity as compared to 60 per cent and 40 per cent of field capacity Similarly the number of nodules, grain yield and seed protein recovery were significantly higher at 50 mm inoculum depth as compared to 100 and 200 mm inoculum depths Maximum dry matter yield and total plant protein recovery were obtained at 80 per cent of field capacity and 50 mm inoculum depth The highest grain yield 14 15 g/plant was obtained with soil moisture at 80 per cent field capacity and inoculum placed at 50 mm below the soil surface

Introduction

Soil moisture is known to have a great influence on symbiotic N fixation. Moisture stress in the root zone causes considerable reduction in N fixation. Water stress can seriously reduce nitrogen fixation by depressing the activity of existing nodules and by reducing nodulation (Engin and Sprent, 1973, Sprent, 1976, Weber, 1966) Increased rates of moisture evaporation at the soil surface induce almost a condition of desiccation in upper portions of soil while a good level of moisture still may be present in lower depths Under normal cultural practices the development of nodules in soybean occurs on roots close to soil surface and this is the layer of soil that is subject to the greatest fluctuation in moisture The establishment of an effective symbiotic N fixing system deeper in the soil could minimize these environmental fluctuations and permit greater N fixation by the nodules

Relatively high soil moisture (75 per cent or more of field capacity) is necessary to ensure intensive multiplication of the bacteria and a high degree of nodulation (Masefield, 1961, Mackee, 1961) Soil water stress affects the growth of young nodules and also dry matter production in soybean Barnett and Naylor (1966) observed inhibition of protein synthesis and decreased levels of protein in bermuda grass growing under water stress

The purpose of the study was to determine whether nodule position could be controlled by inoculum placement. Further the study was also aimed to determine the optimum level of moisture for an effective N fixation, yield and protein content of soybean.

Material and Methods

The experiment was conducted in greenhouse with soybean var. EC–574, grown in 250 mm dia. and 350 mm high plastic pots to hold 12 kg soil. Soil used was black clay soil with pH 8.5, $CaCO_3$ 10.5 per cent clay, 69.14 per cent CEC 54.53 m.e./100 g of soil and water holding capacity 65 per cent. Soil was mixed with ammonium sulphate and super phosphate to supply corresponding amounts of 15 kg N and 60 kg P_2O_5/ha, respectively. Twenty treatment combinations comprising of four moisture levels (field capacity, 80 per cent of field capacity, 60 per cent of field capacity and 40 per cent of field capacity designated as M_1, M_2, M_3 and M_4, respectively) and five levels of inoculum placement (0, 25, 50, 100 and 200 mm below soil surface designated I_1, I_2, I_3 I_4 and I_5, respectively) were tested. The treatments were replicated four times.

Sufficient inoculum (50 ml) was added as a liquid suspension of *Rhizobium japonicum* containing approximately 10^{10} cells/ml. The pot was filled with soil to the desired level, activated carbon was sprinkled at that depth and inoculum was then dispensed into the surface and was covered with the soil. Five seeds

Table I

Effect of moisture regimes and inoculum placement on nodulation, grain yield, dry matter, seed protein and total plant proteins in soybean

Treatments	No. of nodules/ plant	Grain yield (g/plant)	Dry matter yield (g/plant)	Seed protein yleld (g/plant)	Total plant protein (g/plant)
Moisture levels					
Field capacity	339.7	13.15	16.62	4.88	5.80
80 per cent field capacity	348.4	13.11	16.46	4.91	5.83
60 per cent field capacity	117.7	6.73	11.27	2.19	2.72
40 per cent field capacity	27.4	3.90	10.86	1.25	1.75
S.Em. ±	3.23	0.1316	0.140	0.0516	0.0879
CD at 5 per cent	9.56	0.3895	0.414	0.1527	0.2601
Inoculum placement mm below soil surface					
0	219.3	9.48	14.37	3.41	4.17
25	221.2	9.39	14.46	3.40	4.17
50	231.0	9.86	14.91	3.62	4.43
100	190.0	8.73	12.69	3.09	3.73
200	179.8	8.65	12.56	3.01	3.63
S.Em. ±	3.61	0.147	0.157	0.0577	0.0983
CD at 5 per cent	10.68	0.435	0.465	0.1708	0.2909

were placed in each pot at a depth of 15 mm After emergence, three seedlings were maintained per pot Nodule count was taken depthwise at the time of pod formation Nitrogen of seed and dry matter was determined by a micro-kjeldahl procedure (Jackson, 1967)

Results and Discussion

The results (Table 1) exhibited significant effect of moisture and inoculum depth on nodulation, yield and protein content of soybean Twelve fold nodule count was observed at moisture level of 80 per cent field capacity as against nodulation at 40 per cent field capacity moisture level Similarly higher nodule count (221 nodules/plant) was observed at 50 mm inoculum depth whereas 179 nodules/plant were recorded at 200 mm inoculum depth Less oxygen supply to the nodules may be responsible for the observed reduction in nodulation at lower depths (Bergerson and Goodchild, 1973) Inoculum depth and moisture levels governed the nodulation and this is in conformity with the observation made by Wilson (1975) Water stress affected the growth of young nodules and also formation of further nodules (Sprent, 1976)

Grain yield and dry matter yield of soybean were considerably lower when moisture was limiting than at optimum moisture level Moisture levels at field capacity and 80 per cent of field capacity did not affect the grain yield and dry matter production, whereas moisture at 60 per cent of field capacity reduced

Table

Effect of moisture and inoculum depth interaction on nodulation,

Treatment	Field Capacity (M_1)					80% Field Capacity (M_2)				
	No of nodules plant	Grain yield (g/plant)	Dry matter yield (g/plant)	Seed protein yield (g/plant)	Total plant protein (g/plant)	No of nodules plant	Grain yield (g/plant)	Dry matter yield (g/plant)	Seed protein yield (g/plant)	Total plant protein (g/plant)
Inoculum depth (mm)										
0 (I_1)	358 5	13 41	17 13	4 96	5 91	361 0	13 50	16 90	5 06	6 02
25 (I_2)	353 5	13 61	17 20	5 22	6 18	366 5	13 08	17 25	4 19	5 86
50 (I_3)	372 0	13 65	17 85	5 20	6 30	384 0	14 15	18 10	5 47	6 56
100 (I_4)	313 5	12 72	15 45	4 63	5 47	318 5	12 30	14 78	4 47	5 30
200 (I_5)	301 0	12 35	15 45	4 35	5 15	312 0	12 50	15 25	4 61	5 42
			M × I							
S Em	7 22	0 294	0 314*	0 115	0 196*					
CD at 5%	21 37	0 873	0 929	0 340	0.580					

* Non significant

the grain yield to one half of the yield observed at field capacity 16 46 g/plant was the dry matter yield obtained at 80 per cent of field capacity, while it was only 10 86 g/plant when moisture was maintained at 40 per cent of field capacity Satyanarayana and Ghildyal (1970) have also reported beneficial effect of moisture levels on yield of rice crop It is also evident from the data that inoculation depth strongly influenced the grain yield and dry matter production. Highest grain yield (9 86 g/plant) and 14 91 g dry matter were obtained when inoculum was placed at 50 mm below the soil surface (Table I) There was considerable reduction in grain yield and dry matter production when inoculum was placed at 100 and 200 mm below the soil surface

Moisture levels and inoculum depth also influenced significantly the seed protein and total plant protein Restricted soil moisture decreased the seed protein and total plant protein Maximum seed protein (4 91 g/plant) and total plant protein (5 83 g/plant) were observed under 80 per cent of field capacity Considerable low seed protein (1 25 g/plant) was recovered at 40 per cent of field capacity Similarly maximum of (3 62 g/plant) seed protein and total plant protein (4 43 g/plant) were recorded in the treatments where inoculum depth was 50 mm The seed protein and total plant protein were found reduced when inoculum was placed 100 and 200 mm below the soil surface

Effect of moisture–inoculum interaction was significant on nodulation, grain yield and seed protein (Table II) Accordingly, the highest nodulation

II

grain yield, dry matter, seed protein and total plant protein in soybean

60% Field Capacity (M_3)					40% Field Capacity (M_4)				
No of nodules/ plant	Grain yield (g/plant)	Dry matter yield (g/plant)	Seed protein yield (g/plant)	Total plant protein (g/plant)	No of nodules/ plant	Grain yield (g/plant)	Dry matter yield (g/plant)	Seed protein yield (g/plant)	Total plant protein (g/plant)
128 0	6 98	12 10	2 32	2 90	30 5	4 03	11 35	1 30	1 85
128 5	6 88	11 70	2 23	2 80	36 5	3 98	11 70	1 25	1 84
141 5	7 28	11 95	2 35	2 94	26 5	4 35	11 75	1 40	1 94
102 0	6 28	10 35	2 09	2 55	26 0	3 63	10 20	1 18	1 61
88 5	6 23	10 25	1 97	2 42	18 0	3 53	9 28	1 13	1 52

(384 nodules/plant), grain yield (14 15 g/plant) and seed protein (5 47 g/plant) were obtained when inoculum was placed at 50 mm depth and soil moisture maintained at 80 per cent of field capacity Soil moisture at field capacity and inoculum depth of 200 mm greatly affected nodulation in soybean Increasing moisture levels increased nodulation, grain yield and seed protein content irrespective of inoculum depth Further increase in inoculum depth decreased the nodulation, grain yield and seed protein 366 5 nodules/plant in soybean was the nodule count when inoculum depth was adjusted at 50 mm below the surface in a soil maintained at 80 per cent of field capacity The nodule count was found reduced to 128 nodules/plant at moisture level of 60 per cent field capacity The nodule count further dropped to 88 by lowering the inoculum depth to 200 mm

References

Barnett, N M and Naylar, A W , 1966, Amino acid and protein metabolism in bermudagrass during water stress *Plant Physiol* , **41** 122–123

Bergerson, F J and Goodchild, D , 1973, Aeration pathways in soybean root nodules *Aust J Biol Sci* , **26** 729–740

Engin, M and Sprent, J I , 1973, Effect of water on growth and nitrogen fixing activity of *Trifolium repens New Phytologist*, **72** 117–126

Jackson, M L , 1967, *Soil chemical Analysis* , Prentice-Hall Inc N J

Masefield, G B , 1961, The effect of irrigation on nodulation of some leguminous crops *Emp J Expil Agri*, **29** 51–59

Mackee, G W , 1961, Some effects of liming, fertilization and soil moisture on seedling growth and nodulation in birdsfoot trefoil *Agron J* , **53** 237-240

Satyanarayana, T and Ghildyal, B P , 1970, Influence of soil water regimes on the growth and nutrient uptake by rice *(Oryza sativa) J Indian Soc Soil Sci* , **18** 41

Sprent, J I , 1976, Nitrogen fixation by legumes subjected to water and light stress In *Symbiotic Nitrogen Fixation in Plants* (Ed) P S Nutman IBP 7 405-420

Weber, C R , 1966, Nodulating and nonnodulating soybean isolines I Agronomic and Chemical attributes *Agron J* , **58** 43-46

Wilson, D O , 1975, Nitrogen fixation by soybeans as influenced by inoculum placement greenhouse studies *Agron J* , **67** 76-78

Review on *Rhizobium* Inoculation of Principal Legumes in Karnataka

D J Bagyaraj, J V D K Kumar Rao, J H Kulkarni,
H R Raghava Reddy, Gita Mahalingam and R B Patil
Department of Agricultural Microbiology, UAS, Bangalore 560 065

Rhizobial inoculation of grain and forage legume is a well established practice in most pulse growing areas of the world namely, Europe, Australia, New Zealand and U S A In India, Karnataka is one of the important legume growing States Out of a total area of 10 5 million hectares of land under cultivation in Karnataka 1 28 million hectares has been reported to be under pulse cultivation and 0 8 million hectares under groundnut cultivation A project was formulated at the Department of Agricultural Microbiology, UAS, Bangalore during the year 1972 for a duration of five years, to obtain efficient strains of rhizobia for different pulse and to popularise the use of these among the farmers in the State Some of the results obtained during the last five years have been highlighted here

During the initial stages, tour were undertaken to the major legume growing areas *viz* , Dharwad, Raichur, Bailhongal, Annigeri, Doddaballapur, Kolar, Kodigehalli, Gulbarga, Gadag, Nelamangala, Bidar and Belgaum Healthy and pink nodules were collected from standing crops of different legumes and a total of 238 isolates of *Rhizobium* were obtained These were subjected to various laboratory tests and 143 isolates turned out to be true rhizobia on the basis host legume These isolates were tested for their efficiency in pot culture trials Cultures of proven efficiency on the basis of pot culture studies were used in field trials to test their performance in various soils and under different agro-climatic conditions

In all the field trials, the experimental design used was randomized block design with three replications in each treatment Basal dressing consisted of 40 kg P_2O_5 + 20 kg K_2O per ha Fertilizer nitrogen was applied only in N-Control plots at the rate of 25 kg N per ha Seeds were treated with lignite based culture of rhizobia before sowing When the crop was six to eight weeks old, observations on the number and dry weight of nodules per plant, dry weight of plant tops and finally the grain yields were recorded The results of the field trials are furnished below

Groundnut (Arachis hypogaea) All the 22 efficient strains obtained from pot culture trial were tested at Bangalore, Dharwad and Bijapur initially

Isolate G-28 (UAS B-94) proved effective during this trial, and its efficiency was tested for two consecutive years at Bangalore, Dharwad, Raichur, Bijapur and Bidar During the third year trial eight exotic strains were also tested, of which CB-512, CB-530 and CB-661 performed well at one or more test locations (Table I) The field trials showed that rhizobial inoculation increased the yield

TABLE I

Effect of rhizobial inoculation on the yield of groundnut (q/ha)

Treatment	Bangalore	Bidar	Bijapur	Dharwad	Raichur
Control	16 19	21 52	1 78	31 30	2 61
N-Control	15 74	19 44	2 53	30 67	2 29
UASB-94	18 28	20 36	3 36	25 19	2 08
CB-512	17 82	20 36	4 17	36 86	2 18
CB-661	15 74	22 68	4 38	29 81	2 77
Per cent increase due to					
Rhizobium	4 3	3 2 to	18 5 to	4 2 to	6 1
	12 9	7 5	227 5	17 7	
Per cent increase due to N application	—	—	42 1	—	—

Economics of results

Treatment	Profit/ha (Rs + or −)
With *Rhizobium*	+ 484 97
With fertilzer nitrogen	+ 49 50

from 3 2 to 227 5 per cent at five places whereas, nitrogen application increased the yield by 42 1 per cent at one place only The economics of results clearly indicated that profit obtained due to rhizobial inocultion was about ten times higher as compared to nitrogenous fertilizer application

Blackgram (Vigna mungo) Field trials with the above 22 strains were conducted at Bangalore, Dharwad and Bijapur Cultures UASB-120 (B-29) and UASB-125 (B-73) recorded 59 4 per cent and 51 3 per cent increase in yield respectively over uninoculated control Further trials were conducted at Bangalore, Dharwad, Raichur, Bijapur, Annigeri and Bidar to confirm the performance of the two isolate as well as that of some exotic strains over uninoculated control UASB-125 proved better of the two native isolates in a number of places and CB-512 and CB-530 gave highest yields among the exotic strains (Table II) Seed inoculation with *Rhizobium* gave yield increases ranging from 0 4 to 33 0 per cent 15 per cent with fertilizer nitrogen over uninoculated control. Economics of results showed that there was benefit by rhizobial inoculation as compared to fertilizer nitrogen application

TABLE II

Effect of rhizobial inoculation on the yield of blackgram (q/ha)

Treatment	Annigeri	Bidar	Bangalore	Bijapur	Dharwad	Raichur
Control	5 72	6 36	1 89	6 12	15 65	7 05
N-Control	4 89	4 90	5 91	5 86	14 90	8 11
UASB-125	5 46	5 31	6 14	7 89	12 92	7 90
CB-512	5 46	5 56	3 87	5 00	13 78	8 81
CR-530	6 85	5 90	6 84	3 91	16 00	7 47
CB-627	5 48	6 71	4 87	4 87	15 39	7 79
% increase due to *Rhizobium*	0 4-33 0	1 1-5 5	28 9	2 2	7 5-24 9	
% increase due to N application	-	-	-	-	15 0	

Economics of results

Treatment	Profit/ha (Rs ±)
With *Rhizobium*	+ 264 97
With fertilizer nitrogen	+ 111 50

Redgram (Cajanus cajan) Microplot trials revealed that 22 isolates could nodulate redgram efficiently These were tested for their performance under field conditions at Bangalore, Dharwad, Raichur and Bijapur Results of the multilocational field trials showed that no single culture was uniformly effective at all the places However, nine strains *viz* UAS B–111, 120, 104, 106, 101, 118, 96 and 105 appeared promising For confirming the performance of these strains, trials were conducted at Bangalore, Dharwad, Raichur Bijapur and Bailhongal Performance of some exotic strains was also tested Results indicated that four native isolates *viz* , UAS B–111, UAS B–118, UASB–104 and UAS B–101 and three exotic strains *viz* , CB–661, CB–1057 and CB–2832 performed well at most centres (Table III) Increase in yield due to *Rhizobium* inoculation ranged from 1 2 to 162 3 per cent (at six places) whereas, with nitrogen fertilizer application, it was 2 3 to 74 3 per cent (at four places) over uninoculated control The economics of the results indicated that seed inoculation with *Rhizobium* was better than nitrogen fertilizer application

Greengram (Vigna radiata) First year field trials with 22 proven isolates conducted at Bangalore, Dharwad, Raichur and Bijapur showed that strains UAS B–113, 106, 107 95, 96 and 105 were effective Second year field trials were conducted at Bangalore, Dharwar Annigere Bijapur, Bidar and Raichur, to confirm the performance of the above–said strains as well as that of some exotic strains Three out of the seven local isolates *viz* , UAS B–95, UAS B–96 and UAS B–107, and three out of the four exotic strains CB–661, CB–756 and CB–530 were found to perform well at most place (Table IV) The increase in

TABLE III

Effect of rhizobial inoculation on the yield of redgram (q/ha)

Treatment	Bidar	Bijapur	Bailhongal	Dharwad	Raichur
Control	16 48	8 97	13 62	2 73	7 65
N-Control	15 00	10 82	14 20	4 76	5 55
UASB - 101	17 96	12 45	17 82	5 65	12 35
UASB - 111	16 48	14 48	20 83	7 16	3 99
CB - 661	15 74	13 73	20 53	5 42	8 89
CB - 1057	15 37	15 01	22 32	3 76	10 00
CB - 2832	15 00	13 14	20 36	6 45	6 67
% increase due to *Rhizobium*	1 2–8 9	9 4 – 67 3	4 2–68 6	37 7–162 3	6 1–83 3
% increase due to N application	-	20 6	4 2	74 3	-

Economics of results

Treatment	Profit/ha (Rs ±)
With *Rhizobium*	+ 456 57
With fertilizer nitrogen	+ 132 30

TABLE IV

Effect of rhizobial inoculation on the yield of greengram (q/ha)

Treatment	Annigeri	Bangalore	Bijapur	Raichur	Bidar	Dharwad
Control	4 03	5 50	5 13	5 61	7 96	8 61
N - Control	4 06	2 94	4 40	6 83	7 38	8 57
UASB - 95	4 12	6 63	5 16	7 24	6 94	11 29
UASB - 96	4 24	12 03	4 51	6 94	7 91	10 10
UASB - 107	4 83	5 69	3 96	7 95	6 78	8 93
CB - 530	4 28	6 31	4 73	7 47	6 67	10 51
CB - 661	4 56	5 31	4 85	6 35	8 14	6 39
CB - 756	4 35	6 40	3 53	6 73	7 06	9 68
% increase due to *Rhizobium*	1 7–19 8	3 5–118 7	6-1 6	12 2–41 7	2 2–8 1	3 7–31 1
% increase due to N application	1 5	–	–	21 7	–	–

Economic of results

Treatment	Profit/ha (Rs ±)
With *Rhizobium*	+ 355 37
With fertilizer N	— 93 40

yield ranged from 0 6 to 118 7 per cent due to rhizobial inoculation at six places, while with nitrogen fertilizer the increase in yield ranged from 1 5 to 21 7 per cent at two places From the economics of results, it was clear that rhizobial inoculation was profitable as compared to fertilizer nitrogen application.

Bengalgram (Cicer arietinum) Twenty-eight strains which proved promising under pot culture trials were tested for their efficiency under field conditions at Dharwad, Raichur and Bijapur There was increased yields ranging from 0 4 to 23 4 per cent at Dharwad, 0 7 to 32 4 per cent at Raichur and 0 7 to 37 3 per cent at Bijapur as a result of inoculation with different strains of *Rhizobium* This multilocational field trial revealed that isolates UAS B-3, UASB-55 UASB-57, UASB-67 and UASB-71 were efficient Second year field trial at Raichur showed that UASB-59 and UAS B-13 were the best followed by UASB-66, UASB-67, UASB-60 and UASB-18 The economics of the results showed that *rhizobial* inoculation was profitable

Demonstration trials on cultivators' fields These trials were conducted in collaboration with the Karnataka State Department of Agriculture and the Directorate of Extension, UAS, Bangalore, during *kharif* 1975 The object of these trials was (a) to know the performance of selected rhizobia on important pulse crops in different soils and agro-climatic zones of the State, (b) to bring out the importance of *Rhizobium* inoculation for obtaining higher yields of pulses and groundnut to the extension staff and farmers of major pulse growing areas of Karnataka

TABLE V

Effect of rhizobial inoculation on the yield of bengalgram (q/ha)

Treatment	Bijapur	Raichur	Dharwad
UASB—3	9 16	9 81	12 27
UASB—55	10 01	11 42	9 56
UASB—57	10 96	9 97	9 18
UASB—67	10 05	8 95	10 67
UASB—71	8 18	8 33	9 98
	7 98	9 81	9 94
% increase due to *Rhizobium*			

Economics of results

Treatment	Profit Rs/ha (+ or —)
With *Rhizobium*	+ 289 97

Conducted by Karnataka State Department of Agriculture

Avare (Field Bean) (Dilichos lab lab) A demonstration trial was conducted at Mysore There were ten treatments, which included eight cultures, one nitrogen control (25 kg N/ha) and one uninoculated control There was significant increase in bean yield ranging from 52 0 to 74 2 per cent as a result of inoculation with different rhizobial cultures compared to uninoculted control (Table VI).

TABLE VI

Effect of rhizobial inoculation of avare (Mysore)

Treatment	Yield (q/ha)	% increase over uninoculated control
Control	6 25	—
N— Control	9 25	48 3
CB— 512	10 89	74 2
CB— 627	10 63	70 0
CB— 661	9 72	55 5
CB— 756	10 19	63 0
CB— 1057	10 89	74 2
CB— 2832	9 50	52 0
Composite	10 41	66 6
UAS Composite	9 72	55 5

Economics of the results

Treatment	Profit/ha (Rs ±)
With *Rhizobium*	+ 677 47
With fertilizer nitrogen	+ 342 50

However, nitrogen fertilizer application increased the bean yield by 48 3 per cent The results indicated that a substantial increase in bean yield could be obtained by seed treatment with an efficient culture of *Rhizobium* as compared to fertilizer nitrogen application Economics of results are presented in the table

Redgram (Cajanus cajan) , The trial was conducted at four places, *viz* , Bidar, Mysore, Gulbarga and Bijapur Here again, the trial included 10 treatments which included eight cultures, one nitrogen control (25 kg N/ha) and one uninoculated control There were significant differences in nodule number and plant weight in *Rhizobium* inoculated treatments as compared to uninoculated control InMysore and Gulbarga, the increase the grain yield was 24 9and 7 5 per cent respectively The increase due to the rhizobial inoculation ranged from 71,8 to 142 2 per cent at Mysore and from 7 5 to 34 9 per cent at Gulbarga, compared to uninoculated control The data clearly showed that rhizobial inoculation gave profitable yield as compared to nitrogen fertilizer application (Table VII)

Groundnut (Arachis hypogaea) The trial was conducted at six places *viz* , Raichur, Dharwad, Jamkhandi, Bagalkot, Jewargi and Chincholi. The treatments included eight cultures, one nitrogen fertilizer application (25 kg N/ha) and one uninoculated control There was an increase in yield ranging from 4 0 to 20 0 per cent at Jamkhandi 1 1 to 14 3 per cent at Bagalkot, 11 4 to 50.0 per cent in Jewargi, 38 7 to 69 9 per cent at Dharwad and 16 6 to 58 3 per cent at Raichur due to *Rhizobium* inoculation as compared to uninoculated control Though application of fertilizer nitrogen gave more yield as compared

to *Rhizobium* inoculation, it may be noted that this increase was noted only at two out of five places (Table VIII) Conducted by U A S , Directorate of Extension.

TABLE VII

Effect of rhizobial inoculation of the yield of redgram

Treatment	Mysore	Chincholi
Control	4 50	11 12
N-Control	5 62	11 95
UASB-96	8 13	13 90
UASB-101	10 90	15 00
UASB-104	8 47	13 34
UASB-105	7 73	11 95
UASB-106	9 91	10 00
UASB-111	10 11	15 00
UASB-118	8 34	14 45
Composite	8 50	10 00
% increase due to *Rhizobium*	24 88—142 2	7 4—34 9
% increase due to N application	24 88	7 4

Economics of results

Treatment	Profit/ha (Rs ±)
With *Rhizobium*	+517 77
With nitrogen fertilizer	+ 17 10

*Groundnut (*Arachis hypogaea*)* The trial was conducted on five farmers' fields using composite cultures only The results revealed that there was an increase in yield ranging from 11 03 to 20 50 per cent over uninoculated control An increase in net profit ranged from Rs 38 00 to Rs 157 00 per acre compared to uninoculated control and nitrogen fertilizer application

Bengalgram (Cicer arietinum) These demonstration trials were conducted at 10 taluks using composite cultures supplied by the University of Agricultural Sciences Yield of Bengalgram increased from 1 55 to 76 66 per cent because of rhizobial inoculation

Blackgram (Vigna mungo) These trials were conducted at five taluks of Belgaum district *viz* , Athani, Chikkodi, Raibag, Hukkeri and Bailhongal Increase in yields due to rhizobial inoculation ranged from 6 51 to 83 97 per cent over uninoculated control

*Horsegram (*Phaseolus vulgaris*)* The trial was laid out at seven taluks of Belgaum district The results showed that rhizobial inoculation increased the yields from 3 31 to 49 65 per cent over uninoculated control

In conclusion it can be said that majority of the farmer in Karnataka have become aware of the usefulness of rhizobial inoculation on crop yields This can be noticed from the increase in demand for cultures at the time of termination of the scheme as compared to the demand during the initial stages Table VIII shows the increase supplies of inoculants over the years It may be noted that only a small portion of the total demand could be met by the Department

TABLE VIII

Effect of Rhizobial inoculation on the yield of groundnut

Treatment	Bagalkot	Dharwad
Control	19 67	10 27
N-Control	18 75	17 50
UASB-94	19 89	17 45
CB-512	21 79	16 86
CB-530	21 53	15 46
CB-627	21 53	14 25
CB-756	22 44	16 33
CB-1057	20 36	16 66
Composite	20 13	17 03
UAS Composite	20 36	15 55
% increase due to *Rhizobium*	1 1 to 13 8	38 6 to 70 4
% increase due to N application	—	70 4

Economics of results

Treatment	Profit/ha (Rs +)
With *Rhizobium*	+432 97
With nitrogen fertilizer	+677 50

Efficient and proven strains for most of the pulses *viz* , groundnut, blackgram, geeengram, redgram and bengalgram have been isolated From the reports obtained from farmers, it has been noticed that the multistrain inoculant containing six proven isolates, UASB-94, UASB-101, UASB-120, UASB-125, CB-530 and CB-1024 performed well at most places on many pulse crops In case of Bengalgram an UAS composite of 3 proven isolates, UASB-67, B D and Revadin is being used

NOTE Strains CB are from C S I R O Division of Tropical Agronomy, Brisbane
Strains B D (Beldagan) and Revadin are from Israel

A Review of Research on Soybean Microbiology in Karnataka

M V Shantaram and R B Patil*
University of Agricultural Sciences, G K V K Campus, Bangalore-560 065

There has been an increasing emphasis in our country towards the problems of solving protein-calorie malnutrition in human diets The traditional milk and meat proteins and other pulse proteins being expensive and out of reach of the poor and middle class strata of Indian Society, soybean may serve well as a valuable source of protein in Indian diets Nutritive value of soybean protein conforms very well with FAO standards for dietary proteins In addition soybean being rich in oil content, edible oil of high nutritive value is being extracted and used for human consumption In the state of Karnataka, cultivation of improved varieties of soybean was first introduced about decade ago (Krishnamurthy and Shivashankar 1975) During the early period of introduction of the crop it was found that the yield of grain was suboptimal and this was presumed to be due to the absence of soybean-specific rhizobia in several soil types of the State To overcome this difficulty studies on isolation, testing and selection of efficient strains of rhizobia for use as inoculants to soybean were undertaken The results of these studies (Katti *et al* , 1970) indicated that the nodulation and yield of soybean can be significantly improved by seed inoculation with soybean-specific rhizobia The results of a two-year field trial indicate that among the three rhizobial cultures used namely, E-67 and E-88 obtained from JNKVV, Jubbulpore and UAS composite from this laboratory, the latter was more effective in nodulating and increasing the yield of soybean Another noteworthy finding from these studies was that phosphate application to soil with seed inoculation of rhizobia proved better in increasing the yield of soybean Katti *et al* (1970) further conducted crop rotation trials to ascertain whether wheat or ragi (finger millet) could be grown after soybean without additional fertilizers These results indicated that the grain yield of both ragi and wheat increased considerably in treatments where soybean inoculated with *Rhizobium* and raised with phosphate application was the previous crop in the rotation

Further studies on the efficacy of some commercial and institutional inoculants on nodulation and yield of soybean by Patil *et al* (1972) indicated that during *kharif* 1971, out of the seven rhizobial inoculants used, seed

* Microbiologist, AICRP on Soybean and Professor and Head, Department of Agricultural Microbiology, respectively

inoculation with IARI and 'NITRAGIN' cultures significantly increased the nodulation in plants Even though bean yields increased by 24 5 per cent and 26 0 per cent with the two cultures respectively the results were not statistically significant In the succeeding *rabi* season the residual effect of soybean cultivation on the succeeding crop of wheat (variety Kalyan 227) was tested With wheat grown after soybean the grain yield increased in plots wherein soybean inoculated with IARI culture was grown as the preceding crop in the rotation. The extent of yield increase was 27 0 per cent as compared to control where no crop was grown previously These studies were conducted in a red sandy loam soil (Rhodustalf) of pH 6 8 with an application of 60 kg P_2O_5/ha and 40 kg K_2O/ha to soybean crop only

Under the All India Coordinated Research Project on Soybean, single strains as well as multi strain cultures of rhizobia were used as inoculants for soybean to test their efficiency In these studies the preliminary work on isolation and field testing of rhizobia were undertaken by Patil *et al* (1972) They isolated 14 strains of rhizobia from soybean plants grown in some soil types of Karnataka and screened them for their purity Only six strains were identified with genes *Rhizobium* A mixed culture of the six strains was used as seed inoculum for studying nodulation in soybean cultivar-Davis First, second and third plant passage isolates were obtained from nodulated plants In all seven strains of *Rhizobium* representing all the three plant passages were isolated All these appeared to belong to the 'Cowpea' group rather than *Rhizobium japonicum* whereas the other two strains used, for comparison, namely E-67 and E-88 obtained from J N K V V , Jubbalpure, conformed to the characteristics of *R japonicum* In a multilocation trial conducted at six different locations the nodulating efficiency was tested by recording nodule number and plant weight The results presented on Table I indicate that the third plant passage isolates proved to be better in nodulating the soybean plants which suggested the possibility of increasing nodulating efficiency of rhizoba by repeated plant passage The data also indicate that five out of six soils lacked native rhizobia which could nodulate soybean and in only one location Sirsi, nodulation in uninoculated control was recorded

The perfoimance of various composite cultures of *Rhizobium* specific for soybean was field tested during the past four years Kumar Rao (1974) reported that among the seven composite cultures tested IARI–2, G B P U A T (Pantnagar) and U A S Bangalore cultures increased the bean yield of soybean cultivar Davis Kumar Rao (1975) further testing the efficacy of five composite cultures in a red sandy loam soil type reported that IARI, G B P U A T Pantnagar and U A S Bangalore cultures were more efficient than uninoculated control or the inoculants supplied by J N K V V Jubbalpure or C S Azad University of Agriculture and Technology, Kanpur

TABLE I

Nodulation and dry matter yield of soybean inoculated with Rhizobium *in different soil types*

Observations		Rhizobial strain number									
		E-67	E-88	S-5	S-6	S-7	S-8	S-11	S-12	S-13	Control
Nodule Number Per Plant*	Sirsi	13	6	3	4	6	3	8	14	14	3
	Mudigere	—	13	—	20	—	—	—	65	10	—
	Mandya	—	—	—	—	—	—	—	19	30	—
	Hebbal	—	—	—	—	—	—	—	32	—	—
	Raichur	—	—	3	—	—	—	—	14	4	—
	Dharwad	—	—	—	—	—	—	—	26	24	—
Nodule Size and Wt (g) per plant*	Sirsi	L3 0	M1 5	S0 8	MS1 3	M1 4	S1 4	S1 0	LM4 0	LM4 6	S1 2
	Mudigere	—	M0 11	—	S0 03	—	—	—	L0 24	S2 04	—
	Mandya	—	—	L0 16	—	—	—	—	L0 36	LS0 63	—
	Hebbal	—	—	—	—	—	—	—	—	L1 07	—
	Raichur	—	—	—	—	—	—	—	L0 13	M0 06	—
	Dharwad	—	—	—	—	—	—	—	L1 80	L1 55	—

* Average of 6 plants

L=Large (Larger than sorghum grains),

M=Medium (Size of sorghum grains)

S=Small (Smaller than sorghum grains),

MS=Medium and Small Patil *et al* (1972)

S-5, S-6 First plant passage isolates

S-7, S-8 Second plant passage isolates

S-11 S-12 S-13 Third plant passage isolates

E-67, E-88 Obtained from JNKVV, Jabbalpure

The compitability of different strains of *Rhizobia* with varieties of soybean was investigated in field trials in a red sandy loam soil of Bangalore by Kumar Rao (1975) and the experiment was repeated by Shantharam and Patil (1976) Both the investigations revealed the absence of any interaction between the five different strains of *Rhizobia* used *viz* UASB Nos 126, 127, 128, 137 and 143 and the four cultivars of soybean *viz*, Davis, Hardee, 33-2 (locally evolved at U A S, Bangalore) and UPSS-38 (U P Agril University, Pantnagar)

Relative efficiency of different strains of Rhizobia on nodulation and yield of soybean Kumar Rao and Patil (1974) observed that the soils of Karnataka are usually deficient in soybean-specific *Rhizobium* and therefore recommended inoculation with efficient strains of Rhizobia for obtaining better soybean yields During their field trials with inoculants from different sources they observed that a few plants without any rhizobial seed inoculation were healthy and comparable to stand to the plant raised from inoculated seeds This led them to isolate a few strains of *Rhizobium* from such nodulated plants

An initial screening of the 31 isolates yielded ten promising strains with better nodulating ability These ten isolates were further field–tested with eight other exotic (obtained from U S A and Australia) strains Dry matter yield data indicate that compared to the exotic strain UASB 136 the relative efficiency of native strains ranged from 101 66 to 70 54 as against 104 53 to 164 05 by exotic strains The relative efficiency of native strains as compared to exotic strain UASB 140 ranged from 77 0 to 129 17 as against the efficiency of exotic strains which ranged from 76 0 to 124 25 (Table II) Bean yield also increased

TABLE II

Nodulation and growth soybean inoculated with native and inoculant strains of Rhizobium japonicum *under field conditions*

Culture No	No of nodules plant		Dry Wt of nodules/plant (mg m)		Dry Wt of plant top (mg)		Dry Wt of plant top in comparison to	Dry Wt of plant top in comparison to
	1	2	1	2	1	2	UASB–136	UASB–140
UASB								
126	17 5	76 2	95	784	0 63	9 45	142 75	108 12
127	15 5	51 0	100	825	0 70	10 87	164 20	124 37
128	12 7	77 0	85	828	0 63	8 40	126 89	96 00
129	27 0	50 0	68	892	0 59	7 79	117 68	89 00
130	16 2	80 8	95	822	0 72	11 29	170 54	129 17
131	12 7	81 0	93	838	0 78	8 01	120 99	91 00
132	15 2	71 2	88	828	0 66	6 98	105 43	79 00
133	15 5	40 5	102	608	0 71	8 24	124 47	94 00
134	13 0	41 7	83	787	0 58	7 00	105 74	80 00
135	19 7	42 8	70	610	0 75	6 73	101 66	77 00
136	11 8	50 0	85	640	0 57	6 62	100 00	76 00
137	12 2	60 7	85	1007	0 77	8 54	129 00	98 00
138	9 5	108 7	62	698	0 66	7 74	116 91	88 00
139	9 7	71 0	50	563	0 48	8 63	130 35	99 00
140	14 5	70 0	63	1053	0 71	8 74	132 02	100 00
141	5 2	27 3	38	157	0 56	7 12	107 55	81 00
142	18 0	96 2	87	703	0 50	6 92	104 00	79 00
144	16 2	65 5	92	818	0 82	10 86	164 05	124 25

1 30–day period of plant growth
2 60–day period of plant growth

Kumar Rao and Patil (1974)

significantly as a result of inoculation with native and exotic strains of *Rhizobium*. The relative efficiency of native strains as compared to exotic strain No UASB 136 ranged from 89 to 145 while that of the exotic strains was 93 to 143 Compared to exotic strain No UASB 140 the relative efficiency of native and exotic strains ranged from 76 to 123 and 79 to 121 respectively These results are presented in Table III In view of this it may be concluded that some of the native strains of *Rhizobium* were as good as exotic strains in nodulating and increasing bean yield in Soybean The authors suggested that such proven native

strains should be recommended to replace the exotic strains for inoculation for such native strains may be endowed with better adaptability to local soil and other environmental conditions

TABLE III

Bean yield of soybean inoculated with native and inoculant strains of soybean Rhizobium

Culture Number	Yield/ha in* Quintals	% increase over control	Relative efficiency in comparison to	
			Strain No UASB 134	UASB 140
UASB				
126	39 29	320 94	145 14	123 35
127	29 24	214 07	108 01	92 00
128	30 29	225 34	111 89	95 00
129	28 20	202 90	104 17	88 00
130	30 39	226 42	112 26	95 00
131	32 42	248 23	119 76	101 79
132	32 59	250 05	120 39	102 32
133	30 51	227 71	112 70	96 00
134	24 07	158 54	89 00	76 00
135	34 63	271 97	127 92	108 72
136	27 07	190 76	100 00	85 00
137	35 07	276 69	129 55	110 10
138	36 86	295 92	136 16	115 73
139	28 98	211 27	107 05	91 00
140	31 85	242 11	117 65	100 00
141	26 61	185 82	98 00	83 00
142	25 15	170 14	93 00	79 00
144	38 61	314 71	142 63	121 22
Control	9 31	—	—	—

* Results significant at 1% level C D , at 1% level is 9 315 q/ha

Kumar Rao and Patil (1974)

TABLE IV

Effect of lime pelleting the Rhizobium *inoculated seed on nodulation and growth of soybean*

Treatment	No of nodules per plant	Dry wt of nodules per plant in mgm	Dry Wt of whole plant in g	Average yield per plant g	% increase over control
T_1 Uninoculated unpellted	0	0	2 0	1 18	—
T_2 Uninoculated pelleted with $CaCO_3$	0	0	2 9	1 35	14 41
T_3 Inoculated unpelleted	20	214	2 9	2 66	125 42
T_4 Inoculated pelleted	31	401	5 4	2 98	152 54
C D at 5%	1 99	5 51	1 20	0 833	—

Kumar Rao (1973)

Inoculant establishment in acidic soil conditions The efficiency of lime pelleting in establishment of inoculated *Rhizobium* was tested by Kumar Rao (1973) In a pot culture trial using variety 'Davis' and UAS-Composite-1 culture of *Rhizobium* in an acidic soil of pH 4 0, it was observed that seed pelleting with lime during inoculation increased nodulation, dry matter and grain yields The data presented in Table IV indicate that increase in grain yield as a result of inoculation was 125–42 per cent as compared to uninoculated control but it was enhanced to 152 54 per cent when inoculated seeds were lime pellted

Interaction between inoculated Rhizobium *and* Azotobacter *on nodulation and yield of soybean* Kumar Rao and Patil (1976) evaluated the effect of inoculation of *Rhizobium* and *Azotobacter* on soybean The data presented in Table V indicated that the nodulation pattern was not affected to any significant

TABLE V

Effect of Rhizobium *and* Azotobacter *inoculation on Soybean*

Treatment	No of nodules/ plant a	b	Dry Wt of nodules/ plant (mg) a	b	Dry Wt of plant top (mg) a	b	Bean yield/ha quintals	Per cent increase in bean yield over control
Control (Uninoculated)	2 3 (1 26)	2 6 (1 55)	4 0 (1 47)	52 0	1 19	2 31	6 37	
Rhizobium only	20 1 (4 49)	47 5 (6 81)	135 0 (11 54)	801 0	1 25	7 32	20 22	217 4
Azotobacter only	8 0 (2 69)	13 4 (3 63)	7 5 (2 56)	325 0	1.27	2 71	12 14	90 6
Rhizobium + *Azotobacter*	20 6 (4 55)	44 1 (6 53)	148 0 (11 78)	838 0	1 36	6 16	21 11	231 2
CD at 1 per cent	1 48	2 59	2 792	281 97	NS	2 11	6 82	
CD at 5 per cent							4 86	
SE							3 53	
CV							23 4%	

Plant growth A 30 days B 60 days

Values in parenthesis are transformed using $\sqrt{X + 0.5}$ treatments

Kumar Rao & Patil (1976)

extent There was also no significant difference in dry matter production at 60 day period between *Rhizobium* treatment alone and *Rhizobium* with *Azotobacter* Bean yield data showed that *Azatobacter* seed treatment alone increased the yield to an extent of 90 6 per cent as compared to uninoculated control The differences in grain yield with *Rhizobium* inoculation alone or *Rhizobium* with *Azotobacter* was not significant. It was therefore concluded that seed inoculation of soybean with an efficient strain of Rhizobium was as good as a mixed

inoculation with *Rhizobium* and *Azotobacter* and *Azotobacter* treatment did not contribute either to nodulation or nitrogen fixation efficiency by *Rhizobium* However treatment with *Azotobacter* alone seemed to be beneficial which was reported to be possibly to the production of biologically active compounds stimulatory to plant growth

Effect of Agricultural Chemicals on Symbiotic Nitrogen Fixation by Rhizobium *in Soybean* This aspect of research has not been dealt with in detail with respect to soybean specific rhizobia The one report by Reddy and Kumar Rao (1974) on the effect of certain non-volatile nematicides on nodulation and yield of soybean indicated no apparent deleterious effect on either nodulation or yield of soybean

References

Katti, C P, Krishnamurthy, K, Patil, R B and Rangaswamy, G S 1970, Studies on *Rhizobium* treatment and phosphate application to soybean and their residual effect on cereals *Mysore J agric Sci* 4 (1) 88–96

Krishnamurthy, K and Shivashankar, K, 1975, *Soybean Production in Karnataka,* UAS Tech. Series No 12 University of Agricultural Sciences, Bangalore

Kumar Rao, J V D K, 1973, Report on Microbiological experiments on soybean conducted at University of Agricultural Sciences, Bangalore, during 1972

Kumar Rao, J V D K and Patil, R B 1974, Response of soybean to inoculation with different strains of *Rhizobium japonicum Andhra Agric J 21 133–141*

Kumar Rao, J V D K, 1974, Report on Microbiological Experiments on soybean conducted at University of Agricultural Sciences, Bangalore, during 1973 and 1974

Kumar Rao, J V D K and Patil, R B, 1975, Report on Microbiological Experiments on Soybean conducted at University of Agricultural Sciences, Bangalore, during 1975

Kumar Rao, J V D K and Patil, R B, 1976, Effect of Inoculation with *Rhizobium* and *Azotobacter* on nodulation, growth and yield of soybean *Curr Sci* 45 (14) 523-523

Patil, R B, Gantotti, B V and Bagyaraj, 1972, Comparative field trials on the performance of different soybean specific *Rhizobium* inoculants *Proc Symposium on legume inoculants Science and Technology INSA New Delhi, Bull* No 51, 1976 pp 952-654

Patil, R B, Pugashetty, B K and Bagyaraj, D J, 1972, Isolation of efficient strains of *Rhizobium* for soybean root nodulation *Proc Symposium on legume inoculant science and Technology INSA, New Delhi, Bull No 51,* 1976, pp 641-743

Reddy, D D R and Kumar Rao, J V D K 1974 In Report of Microbiological Experiments on Soybean conducted at University of Agricultural Sciences, Bangalore during 1973 and 1974

Shantaram, M V and Patil, R B Report on Microbiological Experiments on soybean conducted at University of Agricultural Sciences, Bangalore during, 1976

SESSION II

Crop Response to Inoculation—Legumes

Effect of Bio-fertilizers on Rice Crop

G Oblisami*, P Santhanakrishnan* and J Chandramohan**
Tamil Nadu Agricultural University, Coimbatore-3

Abstract

A field trial on the effect of bio-fertilizers employing efficient strains of *Azotobacter chroococcum, Bacillus polymyxa* and *B megaterium* was conducted on the long duration group rice variety, *viz*, CO 40-Rajarajan, under the conditions prevailed in Coimbatore, along with four levels of fertilizer nitrogen The bio-fertilizers were compatible with each other and all the three organisms put together performed better giving the maximum grain yield of 7 19 tonnes/ha, effecting a saving of fertilizer nitrogen to the tune of 30 to 40 per cent

Rice cultivation is being carried out traditionally in major portion of the country and in Tamil Nadu it is grown in an area of 27 lakh hactares, having an annual production of 2 10 tonnes/ha The non-symbiotic nitrogen fixation associated with cereal crops is potentially most valuable for tropical agriculture Certain strains of *Azotobacter* adopted to rice soil ecosystem as well as facultatively anaerobic organisms like *Bacillus polymyxa* were believed to play an important role in nitrogen fixation under submerged soil conditions (Mishustin, 1970, Nutman, 1971, Brown, 1974, Dobereiner, 1974, Rangaswami, 1975) Inoculation of *Azotobacter* to the paddy seeds seedlings and soil is well known to establish under rice soil conditions and bring about various types of beneficial effects to the rice crop (Balandreau *et al*, 1974, Broughton *et al*, 1976,, Oblisami *et al*, 1976) Various varieties of rice crop respond varyingly to the treatment of *Azotobacter* and other nitrogen fixing organisms (Oblisami *et al*, 1976, Broughton *et al*, 1976) and short duration and medium duration varieties are performing well in association with *Azotobacter* whereas not much information is available on the performance of long duration group of rice varieties The present paper reports on the performance of the latest rice variety from Tamil Nadu Agricultural University, CO 40–Rajarajan, to the treatment of *Azotobacter* and other bacterial inoculants along with graded levels of fertilizer nirrogen

Material and Methods

The field trial on the effect of bio-fertilizers employing efficient strains of *Azotobacter chroococcum* and *Bacillus polymyxa* and *Bacillus megaterium* on CO

*Department of Biology, Tamil Nadu Agricultural University, Coimbatore-3
**Department of Agricultural Botany, Presently Regional Research Station, Kaveripattinam, Dharmapuri Dt T N

40 (Rajarajan) a high yielding long duration rice variety was carried out in the Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, during the season July, 1976-January, 1977 Peat soil based inoculants were prepared and applied to the crop following the methods reported earlier (Oblisami *et al*, 1976) The bio-fertilizer treatments were given along with different combinations of fertilizer nitrogen in the form of urea The crop was supplied with P and K, respectively in the form of superphosphate and muriate of potash as the basal application Data on various plant parameters meant for yield attributes as well as grain yield were collected The yield data were put to statistical scrutiny

Results and Discussion

The results on the effect of bio-fertilizers on the rice variety (Rajarajan) are presented in Tables I to IV

Table I

Effect of bacterial inoculants on rice (CO 40–Rajarajan) in combination with 100 per cent of the recommended level of fertilizer nitrogen

(Fertilizer nitrogen applied as 80+80 kg N/ha)

Treatment	Height (cm/plant)			No of tillers per hill	Panicle length (cm)	Grain yield kg/ha
	60th day	120th day	160th day			
A chroococcum	54 3	95 9	152 7	8 1	22 7	6845
A chroococcum+B polymyxa	54 8	99 6	119 7	8 3	22 6	6895
A chroococcum+B megaterium	52 6	99 5	115 5	8 8	22 2	6565
A chroococcum+B polymyxa+ B megaterium	55 2	99 6	110 8	8 5	22 5	6660
No bacterial inoculant (control)	51 1	93 0	114 2	8 2	22 1	6595

Table II

Effect of bacterial inoculants on rice (CO 40–Rajarajan) in combination with 75 per cent of the recommended level of fertilizer nitrogen

(Fertilizer nitrogen applied as 40+80 kg N/ha)

Treatment	Height (cm/plant)			No of tillers per hill	Panicle length (cm)	Grain yield kg/ha
	60th day	120th day	160th day			
A chroococcum	48 1	93 9	119 2	7 6	22 2	6880
A chroococcum+B polymyxa	45 2	90 4	113 7	8 3	22 3	6460
A chroococcum+B megaterium	47 4	91 3	113 7	7 5	23 6	6890
A chroococcum+B polymyxa+ B megaterium	46 6	94 6	123 3	7 6	22 3	6920
No bacterial inoculant (control)	46 0	94 0	115 8	8.2	22 5	6710

TABLE III

Effect of bacterial inoculants on rice (CO 40–Rajarajan) in combination with 62 5 per cent of the recommended level of fertilizer nitrogen

(Fertilizer Nitrogen applied as 20+80 kg N/ha)

Treatment	Height (cm/plant)			No of tillers per hill	Panicle length (cm)	Grain yield kg/ha
	60th day	120th day	160th day			
A chroococcum	49 2	96 8	119 2	8 6	22 7	6930
A chroococcum+Polymyxa	44 9	90 4	113 7	7 6	22 8	6795
A chroococcum+megaterium	47 0	91 3	113 7	7 5	22 9	6610
A chroococcum+B polymyxa+ megaterium	48 3	94.6	123 3	8 3	22 2	7190
No bacterial inculant (control)	48 8	94 0	115 8	7 6	22 1	6440

TABLE IV

Effect of bacterial inoculants on rice (CO 40–Rajarajan) in combination with 50 per cent of the recommended level of fertilizer nitrogen

(Fertilizer nitrogen applied as 0+80 kg N/ha)

Treatment	Height (cm/plant)			No of tillers per plant	Panicle length (cm)	Grain yield kg/ha
	60th day	120th day	160th day			
A chroococcum	46 9	93 3	145 2	8 5	22 5	6960
A chroococcum+B polymyxa	49 8	92 4	111 0	7 5	22 0	6895
A chroococcum+B magaterium	48 3	91 2	114 2	8 0	22 7	7150
A chroococcum+B polymyxa+ B megaterium	48 6	93 8	115 9	7 6	22 9	6820
No bacterial inoculant (control)	45 2	89 8	110 0	7 7	22 8	6545

The plant height and number of tillers per hill at different age of the crop exhibited slight variations due to the bio-fertilizer treatments The panicle length did not show any variation between the treatments The *Azotobacter* inoculant alone was found to give better grain yield than the fertilizer alone *Azotobacter* and *B polymyxa* performed better with the reduced level of nitrogenous fertilizer application *A chroococcum* and *B megaterium* performed better with 50 0 per cent fertilizer nitrogen alone When all the three organisms were inoculated together, maximum grain yield of 7190 kg/ha was recorded with the application of 62 5 per cent of fertilizer nitrogen *Azotobacter* inoculant could bring out the benefit to the farmers with the application of reduced level of fertilizer nitrogen, which is in conformity with the earlier reports (Oblisami, *et al*, 1976, Patil *et al*, 1976, Shivappa Shetty *et al*, 1976) The performance of *B polymyxa* in association with *Azotobacter* was encouraging and probably

due to its capability to adopt to the rice soil conditions (Doberiner, 1974) Similarly the beneficial performance of *B megaterium* in effecting higher grain yield of the crop might be due to its activity in releasing the phosphorus for the benefit of the rice crop However, detailed studies are needed to establish the exact role of *B megaterium* in combination with *Azotobacter* The compatibility between all the three organisms put together is established in the present study and this might be responsible for getting the maximum grain yield These results are of great significance in the present context of high cost of fertilizer nitrogen Thus 25 0 to 40 0 per cent of fertilizer nitrogen could be saved by employing the bio-fertilizers in rice crop production Also, the beneficial performance of the combined inoculants in the case of long duration rice variety is of great significance because of the probability of any one of these organisms or all of them might get adjusted to the varying environmental factors and bring about the beneficial effects to the rice crop under the field conditions

References

Balandreau, J , Rinaudo, G , Fares-Hamad, I and Dommergues, Y , 1974, Nitrogen fixation in the rhizosphere of rice plants In "*Nitrogen fixation by free-living microorganisms*" (E D) W D P Stewart, Cambridge Univ Press, London, pp 57–69

Broughton, W J , Hong, T K , Rajoo, M J and Ratnasabapathy, M , 1976, Nitrogen fixation in some Malaysian paddy fields Paper presented in Somiplan Symp , Univ Kuala Lumpur, pp 39

Brown, M E , 1974, Seed and root bacterization *Ann Rev Phytopathol* , **12** 181–197

Dobreiner, J , 1974, Nitrogen fixing bacteria in the rhizosphere In *The Biology of Nitrogen Fixation* (E D) A Quispel, North Holland Publishing Co , Amsterdam pp 86–120

Mishustin, E N , 1970, The importance of non-symbiotic nitrogen fixing microorganisms in agriculture *Pl Soil*, 32 545–554

Nutman, P S , 1971, Perspective in biological nitrogen fixation *Sci Prog* , **59** 55–74

Oblisami, G , Natarajan, T and Balaraman, K , 1976, Effect of *Azotobacter* on rice crop. *Madras agric J* , **63** 590–594

Patil, S P , Patil, P L and More, B B , 1976, Effect of association of *Azotobacter* with nitrogenous fertilizer on yield of paddy *Madras agric J* , **63** 600–602

Rangaswami, G , 1975, Bacterial fertilizers *Pesticides*, 9 (9) 58-60

Shivappa Shetty, K S , Khan, A N A , Subramanyam, M B and Patil R B , 1976, Studies on the *Azotobacter* inoculation of crop plants *Madras agric J* , **63** 606–610

Response of IR 20 Rice Variety to *Azotobacter* Inoculant in Farmer's Holding

T S Theetharappan, N Kamalam, P Krishnamoorthy and G Oblisami
Tamil Nadu Agricultural University, Coimbatore

Abstract

A field trial under the conditions of farmer's holding was undertaken to assess the performance of *Azotobacter* inoculant to the IR 20 rice crop A saving of fertilizer nitrogen to the tune of 18 75 to 75 0 kg/ha was observed without affecting the grain yield The water holding capacity and percentage of pore space of the soils from *Azotobacter* treated plots were better over the control Nitrogen balance studies indicated that with reduced fertilizer nitrogen, the performance of *Azotobacter* inoculant was better and maximum quantity of available nitrogen was recorded in such plots

Azotobacter inoculants are well known to perform better under gardenland soil conditions and number of crops derived the benefit of better growth and even increased yield of grains, vegetables, kapas, etc occasionally (Mishustin, 1970, Mehrotra and Lehri, 1971, Rao *et al*, 1974, Brown, 1974, Oblisami *et al*, 1976, Shivappa Shetty *et al*, 1976, Badigire and Bindu, 1976) The response of rice crop to *Azotobacter* inoculation varied with the variety, soil types, pH and agro-climatic conditions (Oblisami *et al*, 1976, Patil *et al*, 1976, Rangarajan and Muthukrishnan, 1976) The performance of *Azotobacter* inoculant in combination with varying levels of fertilizer nitrogen application to a popular variety of rice (IR 20) under the conditions prevailed in the farmer's holding is presented in the present paper

Material and Methods

The field trial was taken up during October 1975 – February 1976, season, in a farmer's holding at Keelanchikulam, Madurai district, Tamil Nadu The *Azotobacter* inoculant treatment was given to IR 20 variety of rice as seed treatment, seedling root dipping and soil application as reported by Oblisami *et al* (1976) The following treatments were taken up in the present study

T_1 – No Azotobacter + 75 kg N basal + 75 kg N top dressing (100 0 per cent N alone)–control

T_2 – Azotobacter + 75 kg N basal + 75 kg N top dressing (100 0 per cent N + Azotobacter)

T_3 – Azotobacter + 56 25 kg N basal + 75 kg N top dressing (87 5 per cent N + Azotobacter)

T_1 – Azotobacter + 37 50 kg N basal + 75 kg N top dressing (75 0 per cent N + Azotobacter)

T_5 – Azotobacter + 18 75 kg N basal + 75 kg N top dressing (62 5 per cent N + Azotobacter)

T_6 – Azotobacter + 0 kg N basal + 75 kg N top dressing (50 0 per cent N + Azotobacter)

The phosphorus and potassium were applied at 75 kg/ha each as superphosphate and muriate of potash, respectively as the basal dose The plot size was five cents The plant parameters like plant height, number of productive tillers per hill and grain yield were recorded The soil samples were collected before and after the field trial and analysed for the physical characteristics like maximum water holding capacity and pore space using the Kee Raczkowski brass cup (Piper, 1950) The available and total nitrogen content of the soils and the total nitrogen content of the plant samples were estimated following the standard procedures (Piper, 1950, Subbiah and Asija, 1956)

Results and Discussion

The results on the effect of *Azotobacter* inoculant along with varied levels of nitrogenous fertilizers under the field trial as existed in the farmer's holding are presented in Table I The treatments receiving *Azotobacter* along with

Table I

Effect of Azotobacter inoculant on IR-20 rice under the conditions of farmer's holding

Treatment	Plant height (cm)	Productive tillers (No /plant)	Grain yield (kg/ha)	% increase
No Azotobacter+100 per cent fertilizer —(control)	71 3	6 9	5090	—
Azotobacter+100 0 per cent fertilizer N	74 4	8 1	5250	3 1
Azotobacter+87 5 per cent fertilizer N	77 3	8 0	5500	8 1
Azotobacter+75 0 per cent fertilizer N	73 5	7 1	5380	6 0
Azotobacter+62 5 per cent fertilizer N	74 4	7 8	5400	6 1
Azotobacter+50 0 per cent fertilizer N	75 1	9 0	5615	10 3

reduced levels of fertilizer nitrogen recorded better plant height and number of tillers when compared to the treatment receiving 100 per cent fertilizer nitrogen alone The grain yield from the treatments receiving *Azotobacter* inoculant gave more or less similar yield as that of the control indicating the saving of fertilizer nitrogen to the tune of 18 75 to 75 0 kg N/ha without affecting the grain yield These results are in agreement with the earlier reports (Oblisami *et al*, 1976, Rangarajan and Muthukrishnan, 1976) A marginal increase in the grain yield (160 to 525 kg/ha) was recorded in plots receiving *Azotobacter*

treatment in combination with different levels of fertilizer nitrogen which is similar to the earlier reports (Oblisami *et al* 1976, Patil *et al*, 1976, Rangarajan and Muthukrishnan, 1976) The performance of *Azotobacter* inoculant was better with the minimum fertilizer nitrogen application However, the beneficial effect of *Azotobacter* inoculant along with the 100 per cent fertilizer N application was poor which might be probably, because of the deleterious effect of readily available nitrogen in inhibiting the activities of *Azotobacter* (Rangaswami, 1975)

The maximum water holding capacity of the field samples from *Azotobacter* treated plots got increased by 1 6 to 7 0 per cent Also, the percentage of pore

TABLE II

Effect of Azotobacter *inoculant on certain physical properties of soil grown to IR-20 rice under the conditions of farmer's holding*

Treatment	Maximum water holding capacity %	Pore space %
No Azotobacter+100 0 per cent fertilizer N —(control)	37 89	54 72
Azotobacter+100 0 per cent fertilizer N	41 61	60 89
Azotobacter+87 5 per cent fertilizer N	44 94	65 10
Azotobacter+75 0 per cent fertilizer N	39 50	55 50
Azotobacter+62 0 per cent fertilizer N	40 88	60 46
Azotobacter+50 0 per cent fertilizer N	40 33	60 49

TABLE III

Nitrogen balance in the field due to treatment with Azotobacter *inoculant on IR-20 rice under the conditions of farmer's holding*

Treatment	Initial available N in soil (kg/ha)	Fertilizer N added to soil (kg/ha)	Total available N (kg/ha)	Total available N removed by crop (kg/ha)	Balance N (probable) (kg/ha)	Final available N in soil (kg/ha)	Difference (kg/ha)
No Azotobacter+ 100 0 per cent fertilizer N —(control)	158 6	150 0	308 6	129 7	178 9	193 7	+14 8
Azotobacter +100 0 per cent fertilizer N	158 6	150 0	308 6	126 6	182 0	169 4	—12 6
Azotobacter+87 5 per cent fertilizer N	158 6	131 2	289 2	140 1	149 7	161 9	+12 2
Azotobacter+75 0 per cent fertilizer N	158 6	112 2	270 8	128 0	142 8	186 7	+43 9
Azotobacter+62 5 per cent fertilizer N	158 6	93 7	252 3	139 1	113 2	180 2	+67 0
Azotobacter+50 0 per cent fertilizer N	158 6	75 0	233 7	135 2	98 4	200 5	+102 1

space was found to increase by 0 7 to 10 4 in the samples from *Azotobacter* treated plots Maximum increase in these two characteristics were recorded in the *Azotobacter* treatment along with 87 5 per cent of the fertilizer nitrogen (Table II) Such an observation on the better physical characteristics of the soil due to *Azotobacter* treatment might have been due to the proliferation of the bacterial cells and production of polysaccharides by the *Azotobacter* cells (Mishustin, 1970, Brown, 1974), which in turn might have been responsible for improving the aggregation of the soil particles

The nitrogen balance in the soil after the trial (Table III) indicated that the treatments receiving *Azotobacter* along with reduced fertilizer nitrogen doses showed greater value of available nitrogen in soil This would mean that the *Azotobacter* inoculant might have played a greater beneficial role in the nitrogen fixation and release to the soil by different means such as excretion of amino acids into soil and mixing of cell constituents after lysis The increase in the available nitrogen in the control plot might be due to the activities of native microflora involved in the nitrogen fixation This study needs further testing under varied agro-climatic conditions for assessing nitrogen economy due to the application of *Azotobacter* inoculant

The authors are thankful to the farmer, Thiru S Shanmugavelu, Keelanchikulam, Madurai District, Tamil Nadu, for providing all the facilities to carry out this trial

References

Badigire, D R and Bindu, K J, 1976, Effect of *Azotobacter* seed inoculation on wheat (*Triticum vulgare*) *Madras agric J*, 63 603-605

Brown, M E, 1974, Seed and root bacterization *Ann Rev Phytopathol*, 12 181-197

Mehrotra, C L and Lehri, K, 1971, Effect of *Azotobacter* inoculation on crop yields *Indian Soc Soil Sci*, 19 243-248

Mishustin, E N, 1970, The importance of non-symbiotic nitrogen fixing microorganisms in agriculture *Pl Soil*, 32 545-554

Oblisami, G, Natarajan, T and Balaraman, K, 1976, Effect of *Azotobaeter* on rice crop *Madras agric J*, 63 590-594

Patil, S P, Patil, P I and More, B B, 1976, Effect of Association of *Azotobacter* with nitrogenous fertilizer on yield of paddy *Madras agric* J, 63 600-602

Piper, C S, 1950, *Soil and Plant Analysis* Inter Sci Pub. Inc, New York, pp 1-368

Rangarajan, M and Muthukrishnan, P, 1976, Effect of various organic manures and *Azotobacter chroococcum* on the growth and yield of paddy. *Madras agric J*, 63 611-615

Rangaswami, G, 1975 Bacterial fertilizers – Prospects and limitations Proc FAI–FAI Seminar Optimising Agril Production, New Delhi, pp 453-458

Rao, S B P, Ramachandran, K and Kanitkar, U K, 1974, *Azotobaeter* an effective tool to meet fertilizer shortage *Agri & Agro-Indust Jour*, 2 1-3

Shivappa Shetty, K S., Khan, A N.A, Subramanyam, M B and Patil, R B, 1976, Studies on the *Azotobacter* inoculation of crop plants *Madras agric J*, 63 606-610

Subhiah, B V and Asija, G L, 1956, A rapid procedure for estimation of available nitrogen in soils *Curr Sci*, 25 259-260

Effect of Graded Levels of Nitrogen Fertilization and *Azotobacter* Inoculation on Yield of Paddy

R Prakash and N N Prasad
Microbiology Division, Faculty of Agriculture
Annamalai University, Annamalainagar-608 101

Abstract

The effect of graded levels of nitrogenous fertilization *viz*, 25 00, 31 25, 37 50 and 43 75 kg/ac together with *Azotobacter* inoculation was compared with 50 00 kg/ac level of nitrogen fertilization with two varieties of paddy *viz*, AU 1 and Madhu *Azotobacter* inoculation with 43 75 kg/ac level of N fertilization gave grain and straw yields similar to 50 00 kg N/ac without *Azotobacter* inoculation

Introduction

The improved crop yields and soil fertility due to *Azotobacter* inoculation have been reported by several workers (Greaves and Jones, 1942, Garbosky 1956, Gopalakrishnamurthy *et al*, 1967) That the nitrogenous fertilization level could be reduced, if supplemented with *Azotobacter* inoculation has been reported by Oblisami *et al* (1976) and Patil *et al* (1976) The results on the effect of graded levels of nitrogen fertilization combination with *Azotobacter* inoculation when compared with 50 kg N/ac level without *Azotobacter* inoculation are presented in this paper

Material and Methods

A field trial was conducted with a plot size of 4m × 3m, in split plot design The varieties AU 1 and Madhu were used in this study A common basal dressing of K_2O, as muriate of potash, and P_2O_5 as super phosphate at the rate of 25 kg/ac was given to all the treatments Graded levels of nitrogen were applied as urea at 25 00, 31 25, 37 50 and 43 75 kg/ac The full dose of nitrogen at 50 kg/ac with no *Azotobacter* inoculation was included to serve as control *Azotobacter* inoculum (AU 3 isolate) obtained from Microbiology Division, Faculty of Agriculture, Annamalai University was used The soil treatment was given at the rate of 6 packets (each weighing 150 g) per acre just before transplantation

Results and Discussion

In AU 1 decrease in height to the extent of 10 72 4 47, 7 78 & 1 18 per cent was obtained with *Azotobacter* inoculation in combination with 25 00, 31 25,

37 50 and 43 75 kg N/ac levels, respectively over 50 00 kg N/ac application without *Azotobacter* In Madhu increase in height of 1 22 per cent was obtained with treatment of *Azotobacter* in combination with 43 75 kg N/ac over 50 00 kg N/ac without *Azotobacter* (Table I) The *Azotobacter* treatment with 43 75 kg N/ac gave equal yield to 50 kg N/ac without *Azotobacter* in AU 1 variety (Table II) In Madhu *Azotobacter* treatment with 43 75 kg N/ac gave 5 61 per

TABLE I

Effect of graded levels of nitrogen application and Azotobacter *inoculation on the height + at of rice*

Treatments	AU 1					Madhu				
	Sampling time after transplantation in days				Mean	Sampling time after transplantation in days				Mean
	15	35	55	75		15	35	55	75	
Azotobacter + 25 kg N/ac (No nitrogen*+25 00 kg**)	32 7	49 3	68 9	84 4	58 82	29 6	48 4	70 9	86 8	58 92
Azotobacter + 31 25 kg N/ac (6 25 kg*+25 00 kg**)	33 6	51 1	72 3	86 2	60 80	30 6	50 2	71 8	88 1	60 17
Azotobacter + 37 5 kg N/ac (12 50 kg* +25 00 kg**)	35 6	53 1	74 9	88 2	62 9	32 3	52 1	75 0	91 3	62 67
Azotobacter + 43 75 kg N/ac (18 75 kg* + 25 00 kg**)	37 0	54 7	77 7	91 1	65 12	33 8	56 3	77 7	93 8	66 40
50 00 kg N/ac (25 00 kg* + 25 00 kg**)	36 1	56 8	78 7	92 0	65 90	33 8	52 4	78 1	94 6	64 60

+ at Height in cm * Basal dressing ** Top dressing

TABLE II

Effect of graded levels of nitrogen application and Azotobacter *inoculation on the grain yield of rice*

Treatment	AU 1		Madhu	
	Per plot in kg	Per acre in kg	Per plot in kg	Per acre in kg
Azotobacter + 25 00 kg N/ac (No nitrogen* + 25 00 kg**)	4 50	1518	4 80	1619
Azotobacter + 31 25 kg N/ac (6 25 kg* + 25 00 kg**)	4 80	1619	5 00	1687
Azotobacter + 37 5 kg N/ac (12 50 kg* + 25 00 kg**)	5 20	1746	5 33	1798
Azotobacter + 43 75 kg N/ac (18 75 kg* + 25 00 kg**)	5 30	1822	5 63	1899
50 00 kg N/ac (25 00 kg* + 25 00 kg**)	5 30	1822	5 33	1798

* Basal dressing ** Top dressing C D (P=0 05) = 0 572

TABLE III

Effect of graded levels of nitrogen application and Azotobacter *inoculation on the straw yield of rice*

Treatment	AU 1		Madhu	
	per plot in kg	per acre in kg	per plot in kg	per acre in kg
Azotobacter + 25 00 kg N/ac (No nitrogen* + 25 00 kg**)	7 60	2564	7 80	2631
Azotobacter + 31 25 kg N/ac (6 25 kg* + 25 00 kg**)	8 10	2732	8 30	2800
Azotobacter + 37 5 kg N/ac (12 50 kg* + 25 00 kg**)	8 50	2867	8 60	2900
Azotobacter + 43 75 kg N/ac (18 75 kg* + 25 00 kg**)	9 50	3204	9 60	3238
50 00 kg N/ac (25 00 kg* + 25 00 kg**)	9 50	3204	9 36	3157

* Basal dressing ** Top dressing C D (P=0 01) = 0 909

cent increased yield over 50 00 kg N/ac Oblisami *et al* (1976) reported that *Azotobacter* treatment with 45 kg N/ha gave higher yields of grain ranging from 1 7 to 8 0 per cent over the nitrogen (60 kg N/ha) Patil *et al* (1976) also reported an increase of 28 per cent in the yield of paddy when nitrogen was applied with *Azotobacter* *Azotobacter* inoculation with 43 75 kg N/ac and 50 00 kg N/ac without *Azotobacter* inoculation gave similar straw yield in AU 1 On the other hand, in Madhu variety an increase of 2 1 per cent in the former treatment was found compared to the latter (Table III)

REFERENCES

GARBOSKY, A J , 1956, Distribution of *Azotobacter* in depth in soils of Fucuman *Rev Fac* Laplata *Agron* 33 65-72

GOPALAKRISHNAMOORTHY, A , MAHADEVAN, A AND RANGASWAMI, G , 1967, Effect of bacterization of rice seeds on the rhizosphere microflora and plant growth *Indian J Microbiol* , 7 21-28

GREAVES, J E AND JONES, L W , 1942, Inoculation of soil with *Azotobacter chroococcum* *Soil Sci* , 53 229-232

OBLISAMI, G , NATARAJAN, T AND BALARAMAN, K , 1976, Effect of *Azotobacter* on rice crop *Madras J Agric* , 63 590-594

PATIL, S P , PATIL, P L AND MORE, B B , 1976, Effect of association of *Azotobacter* with nitrogenous fertilizers on yield of paddy *Madras J agric* , 63 600-602

Effect of Certain Organic Amendments on the Efficiency of Inoculation with *Azotobacter*

R Prakash and N N Prasad
Microbiology Division, Faculty of Agriculture, Annamalai University
Annamalainagar-608 101

Abstract

Application of certain organic amendments *viz*, Sesbania, glyricidia, sunnhemp and paddy straw gave an increased grain yield over control Application of these amendments with *Azotobacter* inoculation gave higher yields compared to their application without *Azotobacter* Sesbania + *Azotobacter* and glyricidia + *Azotobacter* combinations were found to be very effective Application of organic amendments in combination with *Azotobacter* increased the nitrogen content of shoot and nitrogen and organic matter contents of the soil compared to the application of organic matter alone

Introduction

Several workers have suggested the use of bacterial fertilizers to improve the rice (*Oryza sativa* L) yields (Manna *et al*, 1962, Shivappa Shetty *et al*, 1976, Patil, 1976) The beneficial effect of organic amendments added to soil on its *Azotobacter* population was reported by Ekbote (1973) and Singh *et al* (1975) The effect of application of certain organic amendments in combination with *Azotobacter* inoculation to soil on yield of rice and nitrogen content of plant and soil are reported in this paper

Material and Methods

A field trial was conducted with IR 20 rice variety The plots were laid out in split plot design with three replications The plot size was 4m × 3m Green manures Sesbania, glyricidia and sunnhemp and paddy straw were incorporated at the rate of 2 5 tonnes per acre and left for decomposition for 30 days The plots without *Azotobacter* and organic amendments served as control Basal dressing of K_2O and P_2O_5 was given at the rate of 25 kg/ac to all the plots Nitrogen was applied, as Urea at 25 kg/ac level in two equal split doses, one at the time of transplantation and another at 25th day after transplantation Soil treatment with *Azotobacter* (isolate AU 3 of *Azotobacter chroococum*) inoculum was done with 6 packets each weighing 150 g, of lignite-based culture per acre, obtained from Microbiology Division, Faculty of Agriculture, Annamalai University The nitrogen content of shoot and soil samples collected

from rhizosphere was estimated following the method of Biemner (1960) The organic matter content of the rhizosphere soil was estimated following the method of Walkley and Black (1947)

Results and Discussion

Increased growth of the plant was observed in all the treatments generally over the unamended control The height of plants with glyricidia + *Azotobacter* was maximum over the unamended control (Table I) Among the treatments

Table I

Effect of application of certain organic amendments and Azotobacter *inoculation on the height of rice plants*

Treatment	Height of the plants in cm				Mean	Percentage increase or decrease over control
	Sampling time after transplantation in days					
	15	35	55	75		
Control	36 4	55 9	66 9	89 7	66 22	—
Azotobacter alone	37 5	58 7	70 5	91 9	64 65	+3 37
Sesbania	38 4	56 3	73 7	90 7	64 77	+4 09
Sesbania+*Azotobacter*	39 0	59 3	75 8	92 1	66 55	+6 95
Glyricidia	37 4	56 4	73 1	92 1	64 77	+4 09
Glyricidia+*Azotobacter*	38 0	59 1	76 8	95 9	67 45	+8 80
Sunnhemp	38 6	58 9	69 8	92 8	65 02	+4 50
Sunnhemp+*Azotobacter*	39 0	60 9	73 6	94 3	66 95	+7 60
Paddy straw	35 6	59 9	64 6	86 4	60 62	—2 57
Paddy straw+*Azotobacter*	37 5	57 4	68 3	92 1	63 82	+2 57

Table II

Effect of application of certain organic amendments and Azotobacter *inoculation on grain yield of rice*

Treatments	Grain yield in kg		Percentage of increase over control
	per plot	per acre	
Control	3 76	1269	—
Azotobacter alone	4 23	1427	12 5
Sesbania	4 60	1552	22 3
Sesbania + *Azotobacter*	4 95	1669	31 7
Glyricidia	4 23	1427	12 5
Glyricidia + *Azotobaeter*	4 93	1663	31 1
Sunnhemp	4 16	1403	7 9
Sunnhemp + *Azotobacter*	4 63	1562	23 1
Paddy straw	4 03	1359	7 1
Paddy straw + *Azotobacter*	4 33	1461	15 1

C D (P=0 05) = 0 481

sesbania + *Azotobacter* gave the maximum increase (31 7 per cent) in the grain yield over the control followed by glyricidia + *Azotobacter* and sunnhemp + *Azotobacter* Application of different organic manures in combination with *Azotobacter* gave higher yields compared to their application without *Azotobacter* (Table II) Ekbote (1973) reported that incorporation of compost, sunnhemp, paddy straw and jowar straw increased soil microbial activity considerably, with highest increase in *Azotobacter* population with jowar straw Singh] *et al* (1975) reported that Mahua cake increased *Azotobacter* population in soil at concentration of 0 25 per cent and above In the present study the straw yield was maximum with glyricidia and *Azotobacter* inoculation compared to control (Table III) The shoot samples from the sesbania + *Azotobacter,*

TABLE III

Effect of the application of certain organic amendments and Azotobacter *inoculation on straw yield of rice*

Treatment	Straw yield in kg		Percentage increase over control
	per plot	per acre	
Control	12 00	4048	—
Azotobacter	13 93	4699	16 08
Sesbania	15 23	5136	26 91
Sesbania + *Azotobacter*	16 83	5676	40 25
Glyricidia	15 31	5164	27 58
Glyricidia + *Azotobacter*	17 60	5936	48 75
Sunnhemp	13 90	4688	15 83
Sunnhemp + *Azotobacter*	15 06	5079	25 55
Paddy straw	13 13	4429	9 41
Paddy straw + *Azotobacter*	14 00	4722	16 66

C D (P=0 01) = 1 428

TABLE IV

Effect of the application of certain organic amendments and Azotobacter *inoculation on nitrogen content of shoots (expressed in percentage)*

Treatment	Sampling time after transplantation in days			
	15	35	55	75
Control	0 504	0 812	1 421	1 708
Azotobacter alone	0 588	0 952	1 528	1 792
Sesbania	0 644	1 120	1 680	1 904
Sesbania + *Azotobacter*	0 812	1 260	1 764	2 492
Glyricidia	0 784	1 204	1 540	1 944
Glyricidia + *Azotobacter*	0 896	1 344	1 792	2 200
Sunnhemp	0 644	1 120	1 652	1 904
Sunnhemp + *Azotobacter*	0 700	1 260	1 792	2 380
Paddy straw	0 784	0 896	1 540	1 848
Paddy straw + *Azotobacter*	0 952	0 980	1 568	1 988

glyricidia + *Azotobacter* and sunnhemp + *Azotobacter* contained 2 492, 2 200 and 2 380 per cent nitrogen compared to 1 708 per cent in the shoots of plants raised from *Azotobacter* alone inoculated plots (Table IV) Similar results were found with respect to nitrogen content of the rhizosphere soil in the above mentioned treatments (Table V) Beishova (1954) claimed that application of

TABLE V

Effect of the application of certain organic amendments and Azotobacter *inoculation on total nitrogen content of rhizosphere soil (expressed in percentage)*

Treatment	Sampling time after transplantation in days			
	15	35	55	75
Control	0 031	0 037	0 044	0 056
Azotobacter alone	0 039	0 046	0 056	0 067
Sesbania	0 038	0 047	0 058	0 070
Sesbania + *Azotobacter*	0 048	0 058	0 068	0 084
Glyricidia	0 038	0 048	0 056	0 075
Glyricidia + *Azotobacter*	0 052	0 064	0 069	0 092
Sunnhemp	0 036	0 048	0 060	0 073
Sunnhemp + *Azotobacter*	0 050	0 068	0 075	0 087
Paddy straw	0 036	0 056	0 061	0 071
Paddy straw + *Azotobacter*	0 048	0 067	0 073	0 082

TABLE VI

Effect of application of certain organic amendments and Azotobacter *inoculation on the organic matter content of soil (expressed in percentage)*

Treatments	Sampling time after transplantation in days			
	15	35	55	75
Control	0 86	0 99	1 19	1 27
Azotobacter alone	0 93	1 26	1 02	1 48
Sesbania	0 99	1 12	1 28	1 57
Sesbania + *Azotobacter*	1 19	1 31	1 49	1 72
Glyricidia	1 07	1 19	1 32	1 59
Glyricidia + *Azotobacter*	1 19	1 31	1 43	1 80
Sunnhemp	1 12	1 19	1 38	1 48
Sunnhemp + *Azotobacter*	1 31	1 41	1 51	1 63
Paddy straw	0 89	1 08	1 24	1 80
Paddy straw + *Azotobacter*	1 04	1 19	1 50	1 94

Azotobacter increased the nitrogen status in the rhizosphere soil Purushothaman *et al* (1976) reported more nitrogen fixation in the rice rhizosphere soil than in the uncropped soil The rhizosphere soil samples from plots in which the organic amendments were added in combination with *Azotobacter* inoculation recorded a relatively higher organic matter content compared to samples collected from plots in which either organic amendments are added without *Azotobacter* or *Azotobacter* inoculated without organic amendments (Table VI) This may be due to the favourable effect of the treatments on the root growth with consequent increase in the sloughed off root materials and microbial activity in this metabolically active ecological region

References

Bershova, O A , 1954, *Azotobacter* in applications to the soils of the Polesya *Ukrain Mierobiol Zh Akad Nauk* Ukrain, *U S S R* , **16** 3-13

Bremner, J M , 1960, Determination of nitrogen by Kjeldhal method *J Agri Sci* , **55** 11-33

Ekbote, N V , 1973, Decomposition of organic matter in medium black soil and its effects on nutrient availability and microbial population *Fourteenth Ann Conf Assoc Microbiologists of India, Poona*

Manna, B B , Mahapatra, I C and Vachani, M V , 1962, Azotobacter in a bacterial fertilizer for rice crop *Rice News Letter*, **10** 83-84

Patil, S P , Patil, P L and More, B B , 1976, Effect of Association of *Azotobacter* with nitrogenous fertilizers on yield of paddy *Madras agric J* **63** 600-602

Purushothaman, D , Oblisami, G and Balasundaram, C S , 1976, Nitrogen fixation by *Azotobacter* in rice rhizosphere *Madras agric J* , **63** 595-599

Shivappa Shetty, K , Khan, N A , Subramanian, M B and Patil, R B , 1976, Studies on *Azotobacter* inoculation to crop plants *Madras agric J* , **63** 606-610

Singh, D P , Batra, M L and Mishra, M M , 1973, Effect of mahua cake on *Azotobacter* and nitrogen fixation *Fourteenth Ann Conf Assoon Microbiologist of India* Poona, Abstr

Walkley, A and Black, C A , 1947, Chromic acid titration method for determination of soil organic matter *Soil Sci* , **63** 251-257

The Response of Maize and Sorghum to *Azotobacter* Inoculants

P SANTHANAKRISHNAN AND G OBLISAMI
Department of Biology, Tamil Nadu Agricultural University, Coimbatore-3

ABSTRACT

Field trials to assess the response of two cereal crops like sorghum and maize to *Azotobacter* inoculant in combination with three levels of fertilizer nitrogen indicated that maize was more responsive to *Azotobacter* inoculant than the sorghum crop Maximum benefit due to *Azotobacter* inoculants was obtained when the fertilizer nitrogen level was reduced to 75 per cent of the recommended level, in both the crops

BACTERIZATION of crops with *Azotobacter* is being extensively practiced in various countries as well as India (Mishustin, 1970, Brown 1974, Oblisami *et al*, 1976) The response of cereal crops to *Azotobacter* treatment has been reported by number of earlier workers (Patel, 1969, Brown, 1974, Mishustin, 1970, Oblisami *et al* 1976) The field performance of *Azotobacter chroococcum* inoculant on two important cereal crops *viz*, sorghum and maize under field conditions, is presented in the present paper

MATERIAL AND METHODS

Randomized and replicated trials were taken up at the Agricultural Research Station, Bhavanisagar Sorghum seeds (Co 21 variety) and maize seeds (Ganga 5 variety) were treated with peat soil based *Azotobacter chroococcum* inoculant The soil application of the inoculant was also done following the procedure reported earlier (Oblisami *et al*, 1976) The fertilizer nitrogen was applied at three different levels *viz*, 100, 75 and 50 per cent of the recommended levels for both the crops The phosphatic and potassic fertilizers were applied at the recommended levels as basal application The height of the plant at different intervals and the grain yield were recorded

RESULTS AND DISCUSSION

The performance of the *Azotobacter* inoculant on the cereal crops are presented in Tables I and II The sorghum plants from *Azotobacter* treated seeds were comparatively taller than the uninoculated plants Whereas the height of the maize plant receiving *Azotobacter* treatment was some what smaller than the uninoculated treatment The grain yield of *Azotobacter* treated sorghum plants was more or less similar to that of the uninoculated plants with the treatments receiving 100 and 50 per cent of the recommended fertilizer

nitrogen, however, there was a marginal increase in *Azotobacter* treated plants with 75 per cent of the fertilizer nitrogen (Table I) *Azotobacter* inoculated maize plots gave increased yield of grain over that of uninoculated treatment with all the three levels of fertilizer nitrogen, the increase varied from 8 8 to 19 4 per cent (Table II) The present results are similar to the earlier reports

TABLE I

Effect of Azotobacter *inoculant in combination with three levels of fertilizer nitrogen on Co 21 variety of sorghum*

Treatment	Plant height (cm/plant)			Grain yield (kg/ha)	% increase
	40th day	70th day	100th day		
No Azotobacter treatment					
45 0+45 0 kg N/ha (100 per cent N)	108 3	220 0	223 3	3765	—
22 5+45 0 kg N/ha (75 per cent N)	103 2	210 9	213 9	3470	—
0+45 0 kg N/ha (50 per cent N)	101 6	202 4	210 6	3465	—
Azotobacter treatment					
45 0+45 0 kg N/ha (100 per cent N)	116 2	220 2	221 8	3735	—0 8
22 5+45 0 kg N/ha (75 per cent N)	111 7	220 5	225 4	3820	+10 0
0+45 0 kg N/ha (50 per cent N)	108 5	210 4	215 5	3460	— 0 1

TABLE II

Effect of Azotobacter *inoculant in combination with the three levels of fertilizer nitrogen on Ganga 5 variety of maize*

Treatment	Plant height (cm/plant)		Grain yield (kg/ha)	per cent increase
	40th day	70th day		
No Azotobacter treatment				
67 0+67 0 kg N/ha—(100 per cent N)	183 4	249 1	4411	—
33 5+67 0 kg N/ha—(75 per cent N)	193 5	255 6	4116	—
0+67 0 kg N/ha—(50 per cent N)	165 2	221 8	4033	—
Azotobacter treatment				
67 0+67 0 kg N/ha (100 per cent N)	159 0	229 5	4566	3 8
33 5+67 0 kg N/ha (75 per cent N)	180 3	242 3	4915	19 4
0+67 0 kg N/ha (50 per cent N)	169 4	224 4	4443	10 2

by various workers with other cereal crops as well as others (Brown *et al*, 1963, Patel, 1969, Badgire and Bindu, 1976 , Oblisami *et al*, 1976 , Patel *et al*, 1976 , Shivappa Shetty *et al*, 1976) It is well known that C_4 plants are responsive to *Azotobacter* treatments (Purushothaman *et al* , 1976) In the

present study though both the plants are C_4 plants, maize exhibited better response to *Azotobacter* than the sorghum crop Dobereiner (1974) has also reported that maize could be better responsive to *Azotobacter* and bacterial treatments and the present results confirms the above view The response of sorghum with reduced level of fertilizer nitrogen (*i e* 75 per cent N) was however, better and the grain yield was more or less similar to that of the treatment receiving 100 per cent fertilizer nitrogen alone These results indicated the possibility of saving of fertilizer nitrogen to the tune of 25 per cent of the recommended level of nitrogen without effecting the grain yield by the application of *Azotobacter* inoculants

References

Badgire, D R and Bindu, K J , 1976, Effect of *Azotobacter* seed inoculation on wheat (*Triticum vulgare*) *Madras agric J* , **63** 603–605

Brown, M E , Buralingham, S K and Jackson, R M , 1964, Studies on *Azotobacter* species in soil III Effects of artificial inoculation on crop yields *Pl Soil* **20** 194–214

Brown, M E , 1974, Seed and root bacterization *Ann Rev phytopathol* , **12** 181–197

Dobereiner 1974, Nitrogen fixing bacteria in the rhizosphere In *The Biology of Nitrogen Fixation*, (Ed) A Quispel, North Holland Publishing Co , Amsterdam, pp 86–120

Oblisami G , Natarajan, T and Balaraman, K , 1976, Effect of *Azotobacter* on rice crop *Madras agric J* , **63** 590–594

Patil, S P Patil, P L and More, B B , 1976, Effect of association of *Azotobacter* with nitrogenous fertilizer on yield of paddy *Madras agric J* , **63** 600–602

Patel, J J , 1969, Microorganisms in the rhizosphere of plants inoculated with *Azotobacter chroococcum Pl Soil* **31** 209–223

Purushothaman, D , Kasirajan, C and Rangaswami, G , 1976 Nitrogen fixation by *Azotobabter* occurring in the rhizosphere of C_4 plant species *Curr Sci* , **45** 807–809

Shivappa Shetty, K S , Khan, A N A , Subramanyam M B and Patil, R B , 1976, Studies on the *Azotobacter* inoculation of crop plants *Madras agric J* , **63** 606–610

Effect of Combined Inoculants on Potato

P SANTHANAKRISNAN, K BALARAMAN, N RAMADOSS* AND G OBLISAMI
Department of Biology, Tamil Nadu Agril University, Coimbatore-3 Tamil Nadu

ABSTRACT

Treatment of potato seed tubers and soil application with *Azotobacter*, *Beijerinckia* and *Bacillus megaterium* either alone or in combinations gave higher yields of potato tubers over the uninoculated control The combined inoculant with all the three bacterial cultures gave the maximum tuber yield, IAA, nitrogen and available phosphorus content were also maximum with all the three inoculants put together Similarly, maximum phosphatase activity in soil was recorded with all the three inoculants Considering individual inoculants, *Beijerinckia* performed better over the *Azotobacter* or *Bacillus megaterium*

SEED bacterization with bacterial inoculants is well known to improve the plant growth and vigour of the plant in the early stages of the crop because of their role in nitrogen fixation and mineralization as well as release of phosphorus in soil by the introduced organisms (Mishutin, 1970, Doberemer, 1974, Rangaswami, 1975) Bacterial fertilizers improved the yields of a wide range of crop plants, especially vegetables including tubers (Brown *et al*, 1964, Brown, 1974; Shivappa shetty *et al* 1976, Oblisami *et al*, 1976 and 1977) The Western workers have reported that in case of potato the yield increase due to *Azotobacter* inoculation ranged 18–25 per cent (Rakhno and Ryys, 1963, Brown *et al*, 1964, Volodin, 1969) However, no information is available on potato crop under South Indian conditions The present paper deals with the performance of biofertilizers on potato crop grown under Nilgiris conditions

MATERIAL AND METHODS

A randomized, replicated field trial was conducted at the State Seed Farm Thummanatty, near Ootacamund, Nilgris, Tamil Nadu, using Kufri Jothi variety of potato The seed tubers were treated with peat soil based *Azotobacter* inoculant following the methods suggested (Oblisami by *et al*, 1977) and planted adopting a spacing of 40 × 40 cm The recommeded levels of fertilizers were applied (N P K = 75 150 75 kg/ha) The other bacterial inoculants *Beijerinckia*, *Bacillus megaterium* and combinations of either of the two or all the three bacterial inoculants were also applied similarly. The plot which received only the fertilizers and not bacterial inoculants served as control

*Indo-German Nilgiris Development project, Ooty, Nilgiris, Tamil Nadu

The indole acetic acid production potential in the soil was estimated following the procedures detailed in the earlier report (Purushothaman *et al*, 1973) The phosphatase activity in the soils of the experimental plots was assessed as per the standard procedure The nitrogen and phosphorus contents of the soil samples from the experimental plot were estimated following the standard procedures (Bremner, 1960 , Jackson, 1962)

Results and Discussion

The results on the effect of bacterial inoculants on the yield of potato tuber are presented in Table I Maximum yield of potato tubers (20 34 tonnes/ha)

Table I

Effect of bacterial inoculants on Kufri Jothi variety of potato

Treatment	Potato tuber yield (Tonnes/ha)	Percentage increase
No bacterial inoculant (control)	16 42	—
Azotobacter	18 67	13 7
Beijerinckia	19 42	18 3
Bacillus megaterium	18 88	15 0
Azotobacter+Beijerinckia	17 04	3 8
Azotobacter+B megaterium	16 79	2 3
Beijerinckia+B megaterium	15 30	—
Azotobacter+Beijerinckia+B megaterium	20 34	23 9

was recorded in the treatment receiving all the three inoculants, *viz* , *Azotobacter*, *Beijernickia* and *Bacillus megaterium* giving 23 9 per cent increased yield over the uninoculated control Among the single inoculants, *Beijeinckia* gave an yield of 19 42 tonnes/ha followed by *Bacillus megaterium* and *Azotobacter* However, when two of the inoculants were employed there was not much increased in yield *Bacillus megaterium* seems to be not compatible with the other two inoculants When all the three inoculants are introduced simultaneously *Beijerinckia* and *Azotobacter* might have suppressed the effect of *B megaterium* and this needs further critical study The present results of the increased tuber yield of the potato due to bacterial inoculants is in conformity with the earlier reports of Western workers (Rakhno and Ryys, 1963 , Brown *et al* , 1964 , Volodin, 1969) The beneficial effect of the combined inoculants involving the three bacteria as observed in the present study is interesting and needs further critical assessment under varied soil and agro-climatic conditions.

The results on the total nitrogen, available phosphorus, phosphatase activity and IAA production potential in soil as influenced by the bacterial inoculants are presented in Tables II and III The maximum nitrogen content of

TABLE II

Effect of bacterial inoculants on total nitrogen and available phosphorus content of the potato field soil

Treatment	Total 'N'		Available 'P'	
	Per cent	% increase over control	kg/ha	% increase over control
No bacterial inoculant (control)	0 101	—	15 75	—
Azotobacter	0 101	—	15 75	—
Beijerinckia	0 103	2 0	16 45	4 4
Bacillus megaterium	0 108	6 9	16 80	6 7
Azotobacter + Beijerinckia	0 130	28 7	17 15	8 9
Azotobacter + B megaterium	0 120	18 8	17 85	13 3
Beijerinckia + B megaterium	0 120	18 8	18 20	15 6
Azotobacter + Beijerinckia + B megaterium	0 133	31 7	17 85	13 3

TABLE III

Effect of bacterial inoculants on phosphatase activity and IAA production potential of the potato field soil

Treatment	Phosphatase activity (Pyrogallol equivalents)		IAA content	
	mg/g soil	% increase over control	mg/g soil	% increase over control
No bacterial inoculant (control)	0 32	—	0 038	—
Azotobacter	0 35	9 3	0 044	15 8
Beijerinckia	0 33	3 1	0 041	7 9
Bacillus megaterium	0 38	18 8	0 044	15 8
Azotobacter + Beijerinckia	0 32	—	0 044	15 8
Azotobacter + B megaterium	0 44	37 5	0 047	23 7
Beijerinckia + B megaterium	0 46	43 8	0 041	7 9
Azotobacter + Beijerinckia + B megaterium	0 54	68 8	0 085	123 7

the soil was recorded in the soil receiving all the three inoculants followed by two inoculants series and individual series So also IAA content of the soil treated with all three inoculants was maximum The available phosphorus content of the soils receiving either two or three inoculants were better over single inoculant treatment Also, the maximum phosphatase activity was exhibited in the soils receiving all the three inoculants

The increased phosphatase activity in the soils receiving the inoculants is responsible for the increased available phosphorus content in the soil Similarly the inoculants might have played a major role in the availability of increased nitrogen content which in turn might be responsible for the increased tuber yield The present results corroborates the earlier reports (Mishustin 1970, Brown, 1974, Doberiener, 1974)

References

Bremner, J M, 1960, Determination of nitrogen in soil by Kjeldhal method *J agric Sci*, **55** 11-33

Brown, M E, 1974, Seed and root bacterization *Ann Rev Phytopathol*, **12** 181-197

Brown, M E, Burlingham, S K and Jackson, R M, 1964, Studies on *Azotobacter* species in soil III Effect of artificial inoculation on crop yields *Pl Soil* **20** 194-214

Doberreiner, J, 1974, Nitrogen fixing bacteria in the rhizosphere In *The Biology of Nitrogen Fixation*, (Ed) A Quispel, North Holland Pub Co, Amsterdam, pp 86 120

Jackson, M L, 1962, *Soil Chemical Analysis* Prentice Hall Inc, Englewood Cliffs, New Jersey

Mishustin, E N, 1970, The importance of non-symbiotic nitrogen fixing microorganisms *Pl Soil*, **32** 545-554

Oblisami, G, Natarajan, T, Thamburaj, S, Palanisamy, R and Muthukrishnan, C R, 1976, Effect of *Azotobacter* treatment on sweet potato *Madras agric J*, 63 658-660

Oblisami, G, Natarajan, T, Arumugam R and Muthukrishanan, C R, 1977, Effect of *Azotobacter* treatment on Okra *South Indian Hort*, **25** 83-84

Purushothaman, D, Balaraman, K and Oblisami, G, 1973, Indole acetic acid metabolism in soil as influenced by pesticide application *Curr Sci*, **42** 365-366

Rakhno, P Kh and Ryys, G G, 1963, The use of *Azotobacter* preparations *Microbiologiza*, **32** 558-561

Rangaswami, G, 1975, Bacterial fertilizers *Pesticides*, **9(9)** 58-60

Shivappa Shetty, K S, Khan, A N A, Subramanayam, M B and Patil, R B, 1976, Studies on the *Azotobacter* inoculation of crop plants *Madras agric J*, **63** 606-610.

Volodin, V, 1969, Effect of treating seed potatoes with MO and Azotobacterin *Kartofel Groshchi*, 6 46

Effect of Bacterization with Combined Inoculants on Carrot

G Oblisami, P Santhanakrishanan, K. Balaraman and N Ramadoss*
Department of Biology, Tamil Nadu Agril University, Coimbatore-3, Tamil Nadu

Abstract

Bacterization of carrot seeds and soil application of *Azotobacter*, *Beijerinckia* and *Bacillus megaterium* either alone or in combinations resulted in significantly higher yields of tubers than the uninoculated control *Beijerinckia* inoculant performed better in giving the maximum carrot yield *Beijerinckia* and *B megaterium* were compatible and better phosphatase activity and available phosphorus content were resulted The total nitrogen and available phosphorus contents as well as phosphatase activity in the soil receiving all the three inoculants were better indicating their compatibility to bring out the benefits to the crop

Bacterization of vegetable seeds with *Azotobacter* inoculants is well known to result in a number of beneficial effects on the various vegetable crops like tomato, brinjal, cabbage, carrot etc (Mishustin *et al*, 1963, Brown *et al*, 1964, Patel, 1969, Mehrotra and Lehri, 1971, Oblisami *et al*, 1977) However, there seems to be no information available on the performance of *Azotobacter* inoculants as well as other bacterial inoculants on carrot under field conditions as existed in the Nilgiris The effects of bacterization of carrot seeds and soil application with *Azotobacter* inoculant along with other bacterial inoculants like *Beijerinckia* sp, *Bacillus megaterium* on carrot and certain field soil characters are presented in this paper

Material and Methods

A randomized replicated field trial was conducted at the State Seed Farm, Thummanatty, near Ootacamund, Nilgiris, Tamil Nad u, using Danvers Half Long variety of carrot The seeds were treated with peat soil based *Azotobacter* inoculant having approximately 3×10^3 viable *Azotobacter* cells per seed Ten packets of *Azotobacter* inoculants (200g/pkt) were mixed with 10 kg of FYM and then mixed with 10 kg of soil to treat one hectare of land The *Azotobacter*-FYM soil mixture was placed on the holes where the seeds were dibbled. The recommended levels of fertilizer were applied (N P K = 135 135 135 kg/ha) and adopting a spacing of 20×5 cm The other bacterial inoculants like *Beijerinckia* and *B megaterium* and the combinations of two or three bacterial inoculants were also applied similar to that of *Azotobacter* inoculants The plot

*Indo-German, Nilgiris Development Project, Ootacamund, Tamil Nadu

which did not receive any of the bacterial inoculants and only the recommended levels of fertilizers served a control.

The Indole acetic acid production potential in the soil was estimated following the procedure detailed by the earlier workers (Purushothaman *et al.*, 1973). The phosphatase activity in the soils from experimental plots was assessed as per the standard procedure. The soil samples were analysed for total nitrogen as well as available P contents following the standard procedures (Bremner, 1960 ; Jackson, 1962).

Results and Discussion

The effect of bacterial inoculants on the yield of carrot are presented in the Table I. Maximum yield of the carrots was recorded due to the treatment of

Table I

Effect of bacterial inoculants on Danvers Half Long variety of carrot

Treatment	Carrot yield (Tonnes/ha)	Per cent increase
No bacterial inoculant (control)	17.22	—
Azotobacter	21.00	22.0
Beijerinckia	24.43	41.8
Bacillus megaterium	22.60	31.3
Azotobacter+Beijerinckia	21.00	22.0
Azotobacter+B. megaterium	19.14	11.1
Beijerinckia+B. megaterium	20.67	20.0
Azotobacter+Beijerinckia+B. megaterium	21.88	21.1

S.E : 3.50 C.D. at 5 per cent 7.40

Beijerinckia (24.4 tonnes/ha) while the minimum yield was recorded in the control plot being 17.2 tonnes/ha. The treatment receiving *B. megaterium* (phosphobacteria) gave the next best yield, followed by *Azotobacter* alone and the combinations of two bacterial cultures. When all the three bacterial inoculants were applied together, the performance was better than *Azotobacter* alone. The carrot yield increase varied from 11 to 42 per cent due to the activities of different bacterial inoculants employed.

The total nitrogen and available phosphorus content of the field soil after the harvest of the crop (Table II) exhibited considerable variation. The nitrogen content increased from 1.3 to 17.5 per cent, the maximum was recorded in the treatment containing all the three inocultants put together. Similarly the available phosphorus content showed an increase over the that of the control ; the increase varied from 16.3 to 73.5 per cent. Here again the maximum

TABLE II

Effect of bacterial inoculants on total nitrogen and available phosphorus content of the carrot field soil

Treatment	Total 'N'		Available 'P'	
	Per cent	% increase over control	kg/ha	% increase over control
No bacterial inoculant (control)	0 080	—	17 15	—
Azotobacter	0 080	—	17 85	—
Beijerinckia	0 085	6 3	21 70	26 5
Bacillus megaterium	0 093	16 3	31 50	83 7
Azotobacter+Beijerinckia	0 092	15 0	31 50	83 7
Azotobacter+B megaterium	0 086	7 5	19 95	16 3
Beijerinckia+B megaterium	0 081	1 3	23 10	34,7
Azotobacter+Beijerinckia+B megaterium	0 094	17 5	29 75	73 5

increase was recorded in the treatment receiving combination of all the three inoculants

The phosphatase activity in the soil samples indicated an increase ranging from 8 5 to 140 4 per cent Maximum phosphatase activity was recorded in the treatment receiving all the three inoculants resulting in the content of available phosphorus at higher level The IAA content of the soil samples indicated that the treatment receiving all the three bacterial inoculants contained the maximum IAA content (Table III)

TABLE III

Effect of bacterial inoculants on phosphatase activity and IAA production potential of the carrot field soil

Treatment	Phosphatase activity (py rogallol equivalents)		IAA Content	
	mg/g soil	% increase over control	mg/g soil	% increase over control
No bacterial inoculant (control)	0 470	—	0 041	—
Azotobacter	0 570	21 3	0 050	22 0
Beijerinckia	0 510	8 5	0 050	22 0
Bacillus megaterium	0 720	53 2	0 050	22 0
Azotobacter+Beijerinckia	0 570	21 3	0 041	—
Azotobacter+B megaterium	0 570	21 3	0 077	87 8
Beijerinckia+B megaterium	0 670	42 6	0 044	7 3
Azotobacter+Beijerinckia+B megaterium	1 130	140 4	0 066	61 0

The combination of all the three inoculants exhibited the maximum nitrogen content in the soil which might be due to the beneficial activities of all the three organisms either by way of exretion of fixed nitrogen into the environment or lysis of the cells (Mishustin, 1970, Brown, 1974, Dobereiner,

1974) The IAA content of the soils collected from treatments receiving combination of *Azotobacter* and *B megaterium* (phosphobacteria) was better which might be due to the synergistic activity of these two organisms *Beijerinckia* in the presence of other organisms seemed to inhibit the IAA production by the other two organisms, indicating the possibility of the non-compatible nature of *Beijerinckia* with the other two organisms Similar studies were reported earlier on the role of IAA production potential of *Azotobacter* and other organisms in soils, phyllosphere and other ecosystems (Purushothaman *et al*, 1973, Bhaskaran *et al*, 1974, Oblisami *et al*, 1974) However, detailed studies are needed to establish the role of the synthesis of IAA in the rhizosphere and the growth characteristics of the plant systems resulting in a better tuber yield

References

Bhaskaran, R, Kandasamy, D and Oblisami, G, 1974 Effect of granular insecticides on indole acetic acid metabolism in soil *Madras agric J*, **61** 228–229

Bremner J M, 1960, Determination of nitrogen in soil by the Kjeldhal method *J agric Sci* **55** 11-33

Brown, M E, 1974, Seed and root bacterization *Ann Rev Phytopathol*, **12** 181-197

Brown, M E, Burlingham, S K and Jackson, R M, 1964, Studies on *Azotobacter* species in soil III Effect of artificial inoculation on crop yields *Pl soil*, **20** 194-214

Dobereiner, J 1974, Nitrogen fixing bacteria in the rhizosphere In *The Biology of Nitrogen Fixation*, (Ed) A Quispel, North Holland Pub Co, Amsterdam, pp 86-120

Jackson, M L, 1962, *Soil Chemical Analysis* Prentice Hall Inc Englewood Cliffs, New Jersey

Mishustin, E N, 1970, The importance of non-symbiotic nitrogen fixing microorganisms in agriculture *Pl Soil* **32**, 545-554

Mishustin, E N, Naumova, A N and Marienko, V G, 1963, *Azotobacter* and its effectiveness *Izy timiryazev Selkhoz Akad*, **4** 42 54

Mehorotra, C L and Lehri L K, 1971, Effect of *Azotobacter* inoculation on crop yields *J Indian Soc Soil Sci* **19** 243-248

Oblisami, G, Kandasamy, D, Marimuthu, T and Subramaniam, T R, 1974, IAA synthesis in relation to phyllosphere microflora of *Solanum melongena* L sprayed with pesticides *Curr Sci*, **43** 589-590

Oblisami, G, Natarajan, T, Arumugam, R and Muthukrishnan, C R, 1977, Effect of *Azotobacter* treatment on Okra *South Indian Hort*, **25** 83–84

Patel, J J, 1969, Microorganisms in the rhizosphere of plants inoculated with *Azotobacter chroococcum Pl Soil*, **31** 209-223

Purushothaman D, Balaraman, K and Oblisami, G, 1973, Indole acetic acid metabolism in soil as influenced by pesticide application *Curr Sci* **42** 365 366

Effect of Moisture and Temperature on the Survival of *Azotobacter* in Soil

P Prakash and N N Prasad
Microbiology Division, Faculty of Agriculture, Annamalai University
Annamalainagar-608 101

Abstract

The effect of different levels of soil moisture and temperature on the survival of *Azotobacter* was studied up to a period of 120 days The inoculated cells multiplied well at 60 per cent moisture level compared to at other levels The population was more at 30°C than at 35 and 40°C

Introduction

Several workers have reported on the effect of moisture (Dommergues, 1962 , Lazarev, 1964 and Mahmoud and Ibrahim, 1971) and temperature (Green, 1932) on the survival of *Azotobacter* in soil The results on the effect of different levels of moisture and temperature on the survival of *Azotobacter* inoculated into soil are reported in this paper

Material and Methods

An efficient isolate (AU 3) of *Azotobacter* was inoculated into the sterile broth and grown on a rotary shaker The broth was adjusted to 50 per cent transmittance by addition of distilled water, using Spectronic 20 at 495 mμ wave length Well dried, powdered and sieved paddy field soil (100 g) was taken in bottles and sterilized for one hour at 20 lb pressure To find out the effect of moisture on the survival of inoculated *Azotobacter* isolate, different levels of soil moisture *viz* , 20, 40, 60, 80 and 100 per cent were maintained in the bottles Ten ml of the yeast extract mannitol broth culture of *Azotobacter*, multiplied in shake culture was aseptically added to the sterilized soil in each bottle The bottles were incubated at room temperature for 120 days Moisture levels were maintained throughout the period of study by periodically weighing the bottles and adding the required quantity of sterile distilled water

To find out the effect of temperature on the survival of inoculated *Azotobacter* in soil , 30, 35 and 40°C temperature levels were chosen. The bottles were inoculated as detailed earlier and incubated in incubators at the above mentioned temperature levels up to 120 days The soil was maintained at flooded condition during the period of study

Results and Discussion

In the present study the population of the inoculated *Azotobacter* cells increased up to 20th day at all the soil moisture levels, excepting at 20 per cent level and declined thereafter However, from the initial inoculum of 24.0×10^3 cells/g of soil, a population of 2.0×10^3 cells/g of soil survived up to 120th day (Table I) Dommergues (1964) noted that cultures of *Azotobacter* from tropical

Table I

Effect of moisture on the survival of inoculated Azotobacter† *in soil*
(Population expressed in 10^3/g of soil)

Moisture per cent	Sampling time in days					
	20	40	60	80	100	120
20	22 5	12 0	6 5	4 0	2 5	2 0
40	30 0	20 0	10 5	6 5	4 5	2 5
60	53 0	24 0	16 5	11 5	10 0	9 0
80	37 0	20 5	10 0	7 0	5 5	5 5
100	34 5	11 0	8 5	5 5	3 5	3 0

† Initial inoculum 24.0×10^3/g of soil

regions tolerate drying best Among the different moisture levels tested, the inoculated *Azotobacter* survived better at 60 per cent level The population was maximum in 120th day sampling at the above moisture level Mahmoud and Ibrahim (1971) reported that the optimum level of moisture for the growth and proliferation of *Azotobacter* was at 52 to 70 per cent of water holding capacity in clay loam soil

At 30°C the initial inoculum level was found to maintain up to 20th day, and declined thereafter and at 120th day sampling 0.5×10^3 cells/g of soil survived At 35 and 40°C the population declined at all the sampling periods and the survival was up to 100 and 80 days only, respectively (Table II) The

Table II

Effect of temperature on the survival of inoculated Azotobacter† *in soil*
(Population expresses 10^3/g of soil)

Temperature	Sampling time in days					
	20	40	60	80	100	120
30 C	25 0	18 0	6 0	2 5	2 0	0 5
35 C	17 0	9 0	4 0	2 0	0 5	—
40 C	13 0	8 0	4 0	1 0	—	—

† Initial inoculum 24.0×10^3/g of soil

results in the present study clearly revealed that the population of inoculated *Azotobacter* of paddy soil can tolerate relatively high soil temperatures for a considerable period Green (1932) established that the *Azotobacter* from soils of Arizona desert fixes no less nitrogen at 40°C than at 30°C

REFERENCES

DOMMERGUES, J , 1962, Contribution a le'tude de la dynamique microbienne des sols en zone semiaride at et zone tropical seche *Ann agron* , **12** 262-324

DOMMERGUES, K , 1964, Etude de quelques facteures influant sur le comportment de la microflora du sol au cours de la dessiccation *Sci Soil*, 2 141-155

GREEN, R, A , 1932, The effect temperature upon fixation of nitrogen by *Azotobacter Soil Sci* , 33 153-161

LAZAREV, S F , 1964, Microbiological processes in the soils of the natural zones of central Asia *Agrobiologia*, 1 29-36

MAHMOUD, S A Z AND IBRAHIM, A N , 1971, Non-symbiotic nitiogen fixation as influenced by soil moisture *Acta Agron Acad Sci Hung* , **20** 157-163

Effect of Decomposed Wheat Straw Incorporation on Asymbiotic Nitrogen Fixation by *Azotobacter*

SUHAS P WANI* AND P A SHINDE

Department of Plant Pathology and Agricultural Microbiology

Mahatma Phule Krishi Vidyapeeth, Rahuri-413722 Maharashtra

ABSTRACT

The incorporation of partially decomposed wheat straw into broth medium showed varied effects on nitrogen fixation by *Azotobacter* In some cases nitrogen fixation was favoured, whereas in others it was adversely affected In general, incorporation of two per cent wheat straw favoured nitrogen fixation

INTRODUCTION

A maximum quantity of asymbiotically fixed nitrogen is contributed by Azotobacter depending on the availability of carbon source In tropical countries like India, where the maintenance of soil organic matter is a problem, the survival and multiplication of Azotobacter becomes difficult Gaur *et al* (1968, 71) observed that addition of wheat straw alone in soil increased Azotobacter population appreciably The effect of incorporation of wheat straw

(decomposed by different microorganisms) in broth culture on nitrogen fixation by Azotobacter is presented in this paper

Material and Methods

Wheat straw decomposed by different microorganisms (Wani and Shinde, 1977, 1979) was used in the present investigation The wheat straw was analysed for its organic carbon, total nitrogen, phosphorus and potash contents by following standard procedures (Jackson, 1973) Fifty millilitres of nitrogen-free Jensen's broth medium was distributed in each of 250 ml capacity conical flasks Wheat straw from different treatments was incorporated in broth medium at one per cent and two per cent levels The sterilized flasks were inoculated with Azotobacter and then incubated at room temperature (30±2°C) for 21 days under stationary conditions At the end of incubation period, total nitrogen content of broth culture was determined by macrokjeldhal method The nitrogen fixed by Azotobacter per 50 ml of broth culture was calculated by substracting the amount of nitrogen present in decomposed straw from total nitrogen content of broth culture

Results and Discussion

The analysis of straw decomposed by different microorganisms (Table I) indicated that treatments vary in their organic carbon, total nitrogen, available

Table I

Analysis of the decomposed wheat straw

Treatment	Organic carbon	Total Nitrogen	Mineral N (mg/100g)	Phosphorus (mg/100g)	Potash (mg/100g)
Serratia kiliensis	17 25	895	26 47	4 69	92 40
Cytophaga rubra–I	20 25	915	24 21	5 37	118 07
Cytophaga rubra–II	14 25	825	30 50	6 15	82 28
Bacillus macerans–I	18 00	925	33 11	3 90	117 52
Cytophaga rubra–III	18 75	1030	38 54	3 32	119 96
Bacillus macerans II	18 00	875	20 36	3 99	125 18
Cytophaga rubra IV	21 00	1050	42 26	3 25	141 67
Cellulomonas uda	18 00	950	15 72	3 05	68 14
Pencillium sp	17 25	760	17 48	7 32	214 45
Aspergillus sp I	21 75	1010	24 79	2 71	69 97
Aspergillus sp II	15 00	880	53 73	4 99	128 00
Aspergillus sp III	18 50	885	29 76	4 28	102 14
Composite culture	15 75	875	14 62	2 10	201 30
Undecomopsed straw	36 00	450	—	—	—

phosphorus and potash contents The organic carbon content of straw used varied from 14 62 to 36 00 per cent and mineral nitrogen content varied from 14 62 to 57 73 mg per 100 g of straw

The response of Azotobacter to fix atmospheric nitrogen varied in different treatments A maximum increase of 134 02 per cent nitrogen over control was recorded in Azotobacter inoculated broth containing straw decomposed by *Penicillium* sp at two per cent level The treatments where one per cent straw was incorporated, six out of 14 treatments showed positive increase in nitrogen fixation by Azotobacter, whereas remaining eight treatments showed negative response over control About 85 56 per cent increase in the amount of nitrogen fixed over control was recorded wherein, the carbon content of straw was less than half of the undecomposed straw On the other hand, 75 25 per cent reduction in the amount of nitrogen fixed was recorded in *Cytophaga rubra* IV at one per cent level, where organic carbon content was higher than in treatments showing positive response There was an inverse relationship existing between mineral nitrogen and nitrogen fixation The treatment showing

TABLE II

Effect of decomposed wheat straw incorporation on nitrogen fixation by Azotobacter in vitro
(Data represent the average of two replications)

Treatment	1 per cent straw		2 per cent straw	
	Amount of N_2 fixed (mg/100ml broth)	% increase over control	Amount of N_2 fixed (mg/100 ml broth)	% increase over control
Serratia kiliensis	23 80	22 68	26 50	38 65
Cytophaga rubra I	20 60	11 34	29 50	52 06
Cytophaga rubra II	17 00	—12 37	26 60	37 11
Bacillus macerans I	12 30	—36 59	14 60	—24 74
Cytophaga rubra III	27 30	40 72	8 40	—56 70
Bacillus macerans II	16 60	—14 43	17 60	—11 34
Cytophaga rubra IV	4 80	—75 25	12 80	—38 14
Cellulomonas uda	10 10	—55 15	25 60	31 95
Penicillium sp	31 80	63 91	45 40	134 02
Aspergillus sp I	15 30	—21 13	14 40	—25 77
Aspergillus sp II	13 80	—28 86	38 40	100 00
Aspergillus sp III	11 90	—38 65	26 00	34 02
Composite culture	36 00	85 56	20 40	5 15
Undecomposed straw*	19 40	—	23 00	18 53
Control	18 00	—	—	—
Cal 'F' value		—	826 25**	
C D at 1 per cent		—	1 27	

Note *=Considered as control for calculating percentage increase

maximum amount of nitrogen fixed, had lowest content of mineral nitrogen This correlation holds good in all treatments where one per cent straw was incorporated except *C rubra* III *Bacillus macerans*, and *Cellulomonas uda* treatments However, this did not hold good when two per cent straw was incorporated The fact that biologically decomposed wheat straw favoured or inhibited the nitrogen fixation by Azotobacter indicated that probably straw decomposing microorganisms synthesized a variety of substances during the process of degradation and these substances had varied effects on the nitrogen fixing ability of Azotobacter The presence of toxic substances in most crop plant residues have been reported by various workers (Borner, 1960, Guenzi and McCalla, 1964, Guenzi *et al*, 1967 and Patrick and Koch, 1963) In general, incorporation of two per cent straw gave better results than one per cent straw

References

Borner, H, 1960, Liberation of organic substances from higher plants and their role in soil sickness problem *Bot Rev*, **26** 393-425

Gaur A C, Mathur, R S and Kavimandan, S K, 1968, *In vitro* nitrogen fixation by *Azatobacter* as influenced by plant materials and their extracts *Agrochemica*, **13** 81–84

Gaur, A C, Sadasivam, K V, Vimal, O P and Mathur, R S, 1971, A study on the decomposition of the organic matter in an alluvial soil, CO_2 evolution, microbiological and chemical transformations *Pl Soil*, **35** 17–28

Guenzi, W D and McCalla, T M, 1964, Residue substances affected crop growth *Crops and Soils* **16** 25

Guenzi, W D, McCalla, T M and Norstadt, F A, 1967, Presence and persistence of phytotoxic substances in wheat, oat, corn, and sorghum residues *Agron J*,, **59** 163–165

Jackson, M L, 1973, *Soil Chemical analysis* Prentice Hall of India, Pvt Ltd, New Delhi

Patrick, Z A and Koch, L W, 1963, the adverse effect of phytotoxic substances from decomposing plant residues on resistance of tobacco to black root rot *Can J Bot*, **41** 447–458

Wani, S P and Shinde, P A, 1977, Studies on biological decomposition of wheat straw I Screening of straw decomposing microorganisms *in vitro* *Pl Soil*, (In Press)

Wani, S, P and Shinde, P A, 1979 Studies on biological decomposition of wheat straw II Screening of wheat straw decomposing microorganisms under field conditions *Mysore J Agril Sci*, (In Press)

Nitrogen Fixation by *Azotobacter* Occurring in the Rhizosphere of Certain Tropical Plant Species

D Purushothaman and N Dhanapal
Department of Biology, Tamil Nadu Agricultural University, Coimbatore-641 003

Abstract

The distribution of *Azotobacter* in the rhizosphere of sixteen grass species and eight weed plant species (C_4 plants) has been examined and compared with that of some crop plants (C_3 plants) There was not much difference in the rhizosphere effect for *Azotobacter* between the two groups of plants However, the *Azotobacter* isolate from C_4 plants recorded greater N-fixing ability that the C_3 isolates Seed inoculation of rice seeds with *Azotobacter* of C_4 plant species resulted in better growth of radicle and plumule than that observed with C_3 isolate When the isolates were tested in a field trial with the rice variety, Vaigai (Co 37) C_4 isolate gave more grain yield than the C_3 isolate

The luxuriat growth of certain tropical grasses and weeds like *Cynodon dactylon*, *Cyperus rotundus* and *Digitaria* sp even under extremely poor soil conditions is known to be at least in part, due to the association of efficient nitrogen fixing microorganisms in their root system (Dobereiner and Day, 1974, Day *et al*, 1975, Purushothaman *et al*, 1976) Several species of free-living nitrogen fixing microorganisms like *Azotobacter*, *Klebsiella*, *Derxia* and *Beijerinckia* have been recognised in the root environments of the plants (Dart, 1976, Tjepkema and Burris, 1976) Recently, Barber and Evans (1976) observed that as much as 3–10 g of N fixation/ha/day in the root environment of the grass, *Digitaria sanguinallis* Though they were unable to fix up the identity of the organisms implicated, suggesed that they closely resembled the members of the family, *Azotobacteriaceae*

Strangely enough these grass species and weeds exhibit a mechanism of CO_2 fixation quite distinct from the conventional Calvin photosynthetic (C_3) pathway Plant physiologists admit that the C_4 pathway of CO_2 fixation is an ecological adaptation in plants helping them to grow efficiently even in regions of higher temperature and light intensity with the limited availability of water (Hatch and Slack, 1970). In as much as the very physiology of C_4 plant species is distinctly different from that of C_3 plants in respect of CO_2 fixation it is reasonable to expect subtle qualitative and quantitative differences in the root surface microflora and nitrogen fixing microorganisms in particular A few investigators (Dobreiner *et al*, 1972), Paul *et al* (1971) and Raju *et al* (1972)

have already come out with valuable information on the fixation of nitrogen in the root surface of grasses The distribution of *Azotobacter* and their activities in the rhizosphere of certain C_3 and C_4 plant species are presented in this paper

Material and Methods

Sixteen species of grasses and eight species of dicotyledonous weeds (C_4 plants) growing in wilderness were identified at the Tamil Nadu Agricultural University Campus Eight species of crop plants like cotton, tomato, chillies representing the C_3 group plants were also selected The rhizosphere soil samples of these plant species were carefully collected from their natural habitats following standard microbiological techniques (Pramer and Schmidt, 1966) For each plant species, the non-rhizosphere soils were also collected to arrive at the "rhizosphere effect"

The distribution of *Azotobacter* in the rhizosphere as well as the non-rhizosphere soil samples was determined following the standard dilution plate technique using Waksman No 77 agar medium *Azotobacter* colonies from the rhizosphere samples of the plants were carefully transferred to Waksman 77 agar slants and maintained Only those colonies exhibiting distinctly different morphological characteristics alone were picked up

The isolates of *Azotobacter* were tested for their N-fixing capacity under laboratory conditions following the procedure detailed earlier (Purushothaman *et al*, 1976) For preparing the inoculum, the isolates were grown in Burks liquid medium (Aaronson, 1970) for three days, under shake culture conditions A standardised inoculum of 0 1 ml cell suspension (Ca 3×10^8/ml) was added to 100 ml of Waksman 77 broth (devoid of calcium carbonate) taken in 250 Ehrlenmeyer flasks The flasks were incubated over a rotary shaker at room temperature (28 ± 1°C) for 7 days From representative aliquots withdrawn from the flasks, the total nitrogen content was determined in a micro Kjeldhal apparatus (Bremner, 1960)

Surface sterilised seeds of the rice varieties Bhavani and Vaigai (Co 37) were inoculated with a heavy suspension of *Azotobacter* cells (Ca 10^9 cells/seed) and the seeds were air dried The seeds were allowed to germinate between folds of moistened filters paper rolled in the form of cylinders (Anon 1966). The cylinders were placed in an upright position in darkness for five days after which the growth of plumule and radicle was measured Seeds soaked in water served as control

At the wet lands of the Tamil Nadu Agricultural University a field trial was laid out during October-December, 1976 with the rice variety, Vaigai The efficient *Azotobacter* isolate from C_3 (AB 3) and C_4 (Cyp 3) plants were used.

These cultures were separately multiplied and processed into peat soil based inoculants It was applied as seed, seedling and soil inoculant It was a randomized block design with plot size being 6×2 m At five levels of nitrogen viz , 0, 25, 50, 75 and 100 per cent of the recommended dose and with a standard level of P_2O_5 30 kg/ha and K_2O 30 kg/ha the trial was conducted

Results and Discussion

The occurence of *Azotobacter* was noted in all the crop species examined Between the C_3 and C_4 plants the rhizophere effect for *Azotobacter* is not very much distinctly pronounced (Tables I and II)

Table I

Rhizosphere effect for Azotobacter *in* C_4 *plant species*

Plant species	Rhizosphere effect
Cvanddon dactylon	2 60
Cyperus rotundus	1 80
Zea mays	3 50
Sorghum vulgare	2 60
Brachiaria mutica	2 84
Panicum maximum	2 86
Pennisetum purpureum	2 80
Chloris barbata	3 05
Panicum repens	2 80
Panicum antidotale	2 70
Cenchrus ciliaris	4 20
Paspalum weetsonia	3 20
Setaria italica	3 80
Eichnochloa sp	3 60
Digitaria marginata	4 80
Saccharum officinarum	3 10
Amaranthus viridis	4 50
Amaranthus spinosa	3 20
Amaranthus quadrifoliata	4 00
Amaranthus pentaphylla	2 10
Portulaca oleracea	2 60
Portulaca tuberosa	2 60
Mollugo oppositefolia	1 20
Alternanthera sessilis	3 6

There existed wide differences between the C_3 isolates and C_4 isolates in respect of N-fixation In general the C_4 isolates exhibited greater N-fixing ability than the C_3 isolates While most isolates of C_3 plant species recorded on an average 18 mg of N/g of carbon source, the C_3 isolates could fix only 8–10 mg of N/g of carbon source.

TABLE II

Rhizosphere effect for Azotobacter *in certain* C_3 *plants*

Plant species	Rhizosphere effect
Oryza sativa var Bhavani	4 6
Gossypium hirsutum	4 8
Capsicum annum	2 6
Lycopersicon esculentum	3 2
Solanum melongena	3 8
Phaseolus aureus	2 6
Phaseolus mungo	1 8
Vigna catjang	3 6

TABLE III

Nitrogen fixation by different isolates of Azotobacter *from* C^4 *plant species*

Plant species	Isolate No	N-fixation (mg of N/g of mannite)
1	2	3
Panicum maximum Jacq	pm 1	11 50
	pm 2	12 60
	pm 3	18 60
Panicum repens L	Pr 1	22 40
	Pr 2	18 20
Panicum antidotale Retz	Pa 1	12 60
	Pa 2	18 20
Paspalum weetsonia	Pw 1	18 26
	Pw 2	22 00
	Pw 3	16 40
Pennisetum purpureum Sch	Pp 1	11 40
	Pp 2	24 80
	Pp 3	20 00
Chloris barbata Sw	Cb 1	22 10
	Cb 2	16 20
Cenchrus ciliaris L	Cc 1	8 60
	Cc 2	18 20
	Cc 3	19 60
Echinochloa sp Beaur	Ech 1	17 00
	Ech 2	21 40
	Ech 3	18 20
Digitaria marginata Link	Dig 1	22 60
	Dig 2	24 00
	Dig 3	18 20
Setaria italica Beaur	Si 1	16 80
	Si 2	20 00

1	2	3
Amaranthus viridis L	Am v 1	19 60
	Am v 2	22 40
	Am v 3	19 80
	Am v 4	22 40
Amaranthus spinosus	Am sp 1	18 60
	Am sp 2	21 60
	Am sp 3	22 00
Amaranthus pentaphylla	Am p 1	18 50
	Am p 2	16 50
	Am p 3	31 50
Amaranthus quadrifoliata	Am q 1	12 60
	Am q 2	16 20
	Am q 3	18 50
	Am q 4	21 60
	Am q 5	18 20
Portulaca tuberosa Roxb	P tub 1	19 50
	P tub 2	20 10
	P tub 3	23 60
Portulaca oleracea L	P ole 1	17 10
	P ole 2	12 00
	P ole 3	14 20
	P ole 4	32 00
Mollugo oppositifolia L	Mol 1	8 20
	Mol 2	14 60
Alternanthera sessilia		
	Alt 1	18 20
	Alt 2	17 18
	Alt 3	20 00
Zea mays L	Zm 1	16 80
	Zm 2	12 80
	Zm 3	17 20
Sorghum vulgare Pers	Sorg 1	21 00
	Sorg 2	12 00

The result of the studies made on the influence of seed inoculation with *Azotobacter* on seedling growth are presented in Table V Both the rice varieties responded well to *Azotobacter* inoculation The growth of plumule and radicle in both Bhavani and Vaigai varieties was significantly more in C_4 isolate treated seeds when compared to the C_3 isolates treated seeds

The grain yields obtained from the field study of *Azotobacter* isolates are set out in Table VI Though the yield increase in grains due to C_4 plant isolate (Cyp 3) was not statistically significant, increase in yield was found in all the

TABLE IV

Nitrogen fixation by Azotobacter *isolates from C_3 plant species*

Plant species	Isolate No	N-fixation (mg of N/g mannite)
Oryza sativa L	Ab 1	16 80
	Ab 3	17 60
	Ab 6	8 60
	Ab 9	15 60
Gossypium hirsutum	Gh 1	7 60
	Gh 2	8 20
	Gh 3	8 60
Capsicum annuum	Ca 1	9 50
	Ca 2	9 00
Lycopersicon esculentum	Le 1	6 40
	Le 2	9 20
	Le 3	11 00
Solanum melongena	Sm 1	6 80
	Sm 2	12 40
	Sm 3	16 20
Phaseolus aureus	Ph a 1	12 40
	Ph a 2	8 40
Phaseolus mungo	Ph m 1	12 00
	Ph m 2	6 40
Vigna catjang	Vig 1	6 40
	Vig 2	12 60

TABLE V

Effect of seed Inoculation with Azotobacter *on the seedling growth of rice varieties*

Source of Azotobacter	Bhavani		Vaigai	
	Plumule	radicle	Plumule	radicle
C_3 isolates*	12 3	30 5	16 2	42 3
C_4 isolates*	15 8	38 6	26 1	51 8
Water-control	8 6	26 5	15 6	31 60

Data represent average of 25 seedlings measured five days after germination
*Average values of 10-isolates in each group

treatments where C_4 isolate was used The yield difference was more pronounced at 75 per cent and 100 per cent N levels than at lower levels of nitrogen

Dobreiner and Campelo (1971) have indicated that Azotobacter *paspali* occurring in the rhizosphere of tropical grass were more efficient in N-fixation Subsequently, Dobreiner *et al* (1972) at Rhothamsted computed that as much as 100 kg of N/ha/year has been fixed by *A paspali* in the rhizosphere of *Paspalum notatum*

TABLE VI

Influence of Azotobacter *Inoculation (C_3 and C_4 Plant Isolates) on the grain yield of the rice variety, Vaigai (Co 37) (kg/ha)*

Nitrogen level	Without Azotobacter inoculation	Azotobacter isolate (AB 3) from C_3 plant inoculation	Azotobacter isolate (Cyp 3) from C_4 plant inoculation
0 per cent	2133	2767	2233
25 per cent	3066	3267	3260
50 per cent	3175	3517	3670
75 per cent	3283	3678	3833
100 per cent	3383	3717	3975

In the present study, all the plant species harboured Azotobacter in their rhizosphere, the rhizosphere effect (R S ratio) for Azotobacter between the C_3 and C_4 plants indicated not much quantitative variation in their respective habitats rather than under indentical environmental condition

Compared to the isolates of C_3 plants all the C_4 isolates exhibited, a greater N-fixing ability 18-20 mg of N/g of carbon source excepting the isolate Cc 1 from *Cenchrus ciliaris* It is perhaps these efficient N-fixing organisms in the rhizosphere that sustain plant growth even in adverse environments and is amply supported by earlier workers (Dobreiner, 1974, Dart, 1976, Tjepkema and Burris, 1976)

Plants using the C_4 type of CO_2 assimilation obviously possess a rapid rate of photosynthesis, more dry matter production, and highest growth rate in the world (Black, 1973) The C_4 plants species are thought to translocate more of their carbohydrates to the roots and to exude more in their rhizosphere (Dart, 1976) These exuded sugars serve as better energy source for *Azotobacter* activity Seed inoculation of paddy with Azotobacter from the C_4 plants promoted better growth of radicle and plumule than the C_3 plant isolates The growth promoting effect may be due to (i) better N-fixing capacity of the isolates or (ii) the synthesis of growth promoting substances like IAA, GA etc), or (iii) a cumulative effect of both The results of the field trial did not bring out any appreciable difference between these isolates Whenever C_4 isolates was employed, there was increase in grain yield but it was not statistically significant The establishment, activity and competitive survival in the rhizosphere of rice needs to be understood

References

Aaronson, A , 1970, *Experimental Microbial Ecology*, Academic Press, NewYork, p 236

Anonymous, 1966, *Proceedings of the International seed Testing Assoc* International Rules for Seed Testing *Int Seed Testing Assoc* Wageningen, The Netherlands, **31** 152.

Barber, L E and Evans, H J , 1976, Characterisation of nitrogen fixing bacterial strain from the roots of *Digitaria sanguinalis Can J Microbiol* , **22** 254–260

Black, C C , Jr , 1973, Photosynthesis carbon fixation in relation to net O_2 uptake *Ann Rev Plant Physiol* , **24** 283-286

Bremner, J M , 1960, Determination of nitrogen in soil by the Kjeldhal method *J agr Sci* , **55** 11-33

Dart, P J , 1976, Non symbiotic nitrogen fixation In soil pp 225-252 in *Soil Microbiology*, Ed N Walker, Butterworths, London

Day, J M , Neves, M C P and Dobereiner, J , 1975, Nitrogenase activity on the roots of tropical forage grasses *Soil Biol Biochem* , 7 107-112

Dobereiner, J and Campelo, A B , 1971, Non-symbiotic nitrogen fixing bacteria in tropical soils *Pl Soil* (Special Volume), 457-470

Dobereiner, J , Day, J M and Dart, P J , 1973, Nitrogenase activity and oxygen sensitivity of the *Paspalum notatum - Azotobacter paspali* association *J Gen Microbiol* , **71** 103-116

Dobereiner, J , 1974, Nitrogen fixing bacteria in the rhizosphere pp 86-120 In *The biology of nitrogen fixation* A Quispel Ed North Holland Publishing Co , Amsterdam

Dobereiner, J and Day, J M , 1974, Associative symbiosis in tropical grasses Characterisation of microorganism and dinitrogen fixation sites *International symp on Nitrogen fixation*, Pullman, Washington, U S A

Hatch, M D and Slack, 1970, C R *Ann Rev Plant Physiol* , **21** 141-162

Paul, E A Myers, R J K and Rice, W A , 1971, Nitrogen fixation in grassland and associated cultivated ecosystems *Pl Soil* (special volume), 495-507

Pramer, D and Schmidt, E L , 1966, *Experimental Soil Microbiology* Burgess Publ Co , Minneapolis, Minn p 146

Purushothaman, D , Oblisami, G and Balasundaram, C S , 1976, Nitrogen fixation by *Azotobacter* in rice rhizosphere *Madras agric J* , 63 595-599

Purushothaman, D , Kasirajan, C and Rangaswami, G , 1976, Nitrogen fixation by *Azotobacter* occurring in the rhizosphere of C_4 plant species *Curr Sci* , **45** 807-809

Raju, P N , Evans, H J and Seildler, R J , 1972, An asymbiotic nitrogen fixing bacterium from the root environment of corn *Proc Natl Acad Sci , USA*, **69** 3474-3478

Tjepkema, J D and Burris, R H , 1976, Nitrogenase activity associated with some Wisconsin Prarie grasses *Pl- Soil* , **45** 81-94

Biochemical Comparison of *Azotobacter* Isolates from C_3 and C_4 Plant species

S Sadasivam, S, Pandian and D Purushothaman
Tamil Nadu Agricultural University, Coimbatore

Abstract

A comparative study has been made between the *Azotobacter* isolates of C_3 and C_4 plant species The respiratory activity of the C_4 isolates are far more greater than that of C_3 isolates A C_4 isolate from *Cyperus rotundus* preferred xylose, malate and succinate for maximum growth, while the C_3 isolate from *Oryza sativa* preferred mannitol and glucose As regards the ammonia assimilating enzyme, GDH, there is not much difference among the two groups of isolates, however, the C_4 isolates recorded greater activity of GOI than that of C_3 isolates

It has been amply illustrated that the luxurient growth of certain weeds and tropical grasses even in nitrogen poor soils might be due to the occurrence of active species of nitrogen fixing organisms (Paul *et al*, 1971) Though quite a large number of microorganisms have been implicated in the process of nitrogen fixation at the root environments, the work on *Azotobacter*, *Spirillum*, *Derxia* and *Beijerinckia* seems significant (Raju *et al*, 1972, Dobereiner, 1974, Dart and Day, 1976) A spate of papers has been published in recent years on the nitrogen fixing activity of *Azotobacter* associated with the tropical grasses (Dobereiner and Day, 1974, Tjepkema and Burris, 1976) Recently, Purushothaman *et al* (1976) observed subtle differences in the nitrogen fixing capacities of *Azotobacter* isolated from C_3 and C_4 plant species In the present communication, a few biochemical comparisons have been made between *Azotobacter* isolates of C_3 and C_4 plants

Material and Methods

Sources of Azotobacter isolates The *Azotobacter* isolates used in the present study have been obtained from the culture collections of the Microbiology Laboratory, Tamil Nadu Agricultural University, Coimbatore All the cultures were maintained on Waksman 77 agar slants

Utilization of carbon compounds by Azotobacter To find out the differences, if any, among the *Azotobacter* isolates in respect of carbon compounds utilized, Waksman 77 liquid culture was prepared and several carbon sources were used to replace mannitol Only two isolates, one from C_3 plant (AB 3) and another from C_4 plant (Cyp 3) were chosen for the study The flasks containing the sterilized media devoid of $CaCO_3$ were inoculated with the cell suspension of

the isolate and incubated for 7 days at room temperature (28 ± 1°C) over a rotary shaker The biomass (cell growth) was determined by transferring an aliquot of the broth and drying it at 105°C for 8 hours

Respiratory activity of the Azotobacter isolates Relatively young cultures (3 days old) of the organisms grown in Waksman 77 liquid medium was taken for this purpose The respiratory activity was followed in a Warburg respirometer (Umbreit *et al* , 1958) The reaction mixture contained 2 0 ml of the cell suspension, 1 0 ml of 0 1 M phosphate buffer (pH 7 0) in the reaction chamber and 0 2 ml of 40 per cent KOH in the centre well with a filter paper strip Oxygen consumption was determined for a period of 60 min

Determination of Glutamate Dehydrogenase, GDH (E C 1 4 1 4) *and Glutamate oxaloacetate transaminase, GOT* (E C 2 6 1 1)

Three days old cultures of *Azotobacter* isolates, nine from each C_3 and C_4 group of plants were used for enzyme assay After harvesting and washing the cells with 0 25 M sucrose, they were subjected to sonication at 100 mA for 6 minutes in the Vibronics Sonicator During sonication the cell suspension was kept in an ice bath The homogenate was centrifuged at 8000 rpm in an IEC refrigerated centrifuge and the supernatant was used as the enzyme source for determining GDH and GOT activity GDH was estimated by the colorimetric method of King as described by Bergmeyer (1974) substituting NADPH instead of NADH , GOT activity as per the 2,4, dinitrophenyl hydrazine colorimetric estimation of Reitman and Frankel described by Bergmeyer (1974) and protein was by the method of Lowry *et al* (1951)

Results and Discussion

Azotobacter species occurring in the root environments of tropical grasses are recognised to be efficient in nitrogen fixation (Tjekema and Burris, 1976) Surprisingly enough the tropical plant species so far investigated fall under the group of C_4 type plants except rice (Dart and Day, 1976) exhibiting altogether a different pathway of CO_2 fixation (Hatch and Slack 1970) It has been shown that a number of *Azotobacter* isolates from C_4 plant species fixed more nitrogen than that of the isolates from C_3 plants (Purushothaman and Dhanapal, 1977) It has been admitted by plant physiologists that C_4 plants are characterized by a rapid rate of CO_2 assimilation, more dry matter production and highest growth rate As the CO_2 assimilation pathway is different, the root exudates of C_4 plants will also be different

Utilization of different carbon sources by *Azotobacter* isolates are depicted in Table I The isolate Cyp 3 isolated from *Cyperus rotandus,* prefered for its proliferation and N-fixation carbon sources like xylose, malate and succinate,

TABLE I

Utilization of carbon compounds by Azotobacter *from C_3 and C_4 Plants*
(Data represent the mean of two estimations)

Carbon source	Biomass (mg/g of Carbon Source)	
	Isolate AB 3 (C_3 isolate)	Isolate Cyp 3 (C_4 isolate)
Acetate	0 480	0 750
Pyruvate	0 460	0 820
Malate	0 230	1 480
Succinate	0 560	1 230
Glucose	0 860	0 962
Xylose	0 530	1 530
Mannitol	1 860	1 170
Lactose	0 230	0 670
Raffinose	0 430	1 030
Rhamnose	0 260	0 350

TABLE II

Respiratory activity of Azotobacter *from C_3 and C_4 plant species*
(Data represent the mean of two determinations)

Isolates from	(μl of oxygen consumed/g of cell)
C_3 plants	
AB 1	180 0
AB 2	196 0
AB 3	165 0
Gh 1	260 0
GH 2	203 0
Ca 1	265 0
Ca 2	420 0
Le 1	430 0
Le 2	290 0
C_4 plants	
Am v 4	800 0
Bm 3	760 0
Cyp 1	912 0
Cyp 3	915 0
P tub 3	860 0
Zm 3	1100 0
Sorg 1	620 0
P ole 4	560 0
Am P 3	720 0

whereas the isolate AB_3 isolated from *Oryza sativa* accumulated maximum biomass in mannitol and glucose to a large extent Xylose has been reported to be present in the root exudates (Balasubramanian and Rangaswami, 1973) and

malate is the key compound in the C_4 pathway from which succinate could easily be synthesized in the plants The isolates from C_4 plants may be distinctly different in their physiology and this may be the reason for their selective colonization and proliferation in the rhizosphere of C_4 plant species

The respiratory activity of the two groups of *Azotobacter* isolates is presented in Table II While the isolates of C_3 plants registered a low rate of oxygen consumption, that of the C_4 plants have exceedingly higher rate of oxygen consumption The higher rate of respiration may be one of the reasons for high nitrogenase activity by way of scavenging excess oxygen which might otherwise be inhibiting the oxygen labile nitrogenase (Postgate, 1974)

The comparative activity of GDH and GOT in *Azotobacter* isolates are illustrated in Table III Though there is no marked difference in GDH activity between the isolates from C_3 and C_4 plants, isolates from C_4 plants registered more activity of GOT than the isolates from C_3 plants As there is difference in GOT activity, the enzyme responsible for the utilization of assimilated nitrogen, the difference in the capacity of assimilation of fixed nitrogen between

TABLE III

Activity of Glutamate Dehydrogenase (GDH) and Glutamic Oxaloacetic Transaminase (GOT) in Azotobacter Isolates from C_3 and C_4 plant species

(Data represent the mean of three determinations)

Isolates from	GDH activity (X 10^{-1})	GOT activity (X 10^{-3})
C_3 plants		
AB 1	4 1	20
AB 2	1 4	61
AB 3	1 7	83
Gh 1	2 2	37
GH 2	14 8	20
Ca 1	1 8	25
Ca 2	2 1	39
Le 1	33 0	50
Le 2	13 0	15
C_4 plants		
Am v 4	5 6	524
Bm 3	4 1	261
Cyp 3	10 0	250
Cyd 3	17 0	436
P tub 3	6 6	132
Zm 3	32 0	150
Sorg 1	6 1	95
P ole 4	4 2	120
Am P 3	7 2	135

these two groups may lie in the coupled reactions catalysed by glutamine synthetase (GS) and glutamate synthase (GOGAT) as this pathway seems to be predominantly involved in the assimilation of N_2 in most of the N_2 fixing organisms than that of the GDH pathway (Nayatani *et al*, 1971)

References

Balasubramanian, A and Rangaswami, G, 1973, Influence of foliar application of chemicals on the root exudation and rhizosphere microflora of *Sorghum vulgare* and *Crotalaria juncea* *Folia Microbiol*, **18** 492–498

Bergmeyer, H U, 1974, Methods of Enzymatic Analysis **2** 656–658, 735–737, Academic Press, London

Dart, P J and Day, J M, 1976, Non-symbiotic nitrogen fixation in soil, pp 225–252 In *Soil Microbiology*, (Ed) N Walker; Butterworth London

Doberiner, J, 1974, Nitrogen fixing bacteria in the rhizosphere pp 86–120 In *The Biology of Nitrogen fixation* (Ed) A Quispel North Holland Publishers, Amsterdam

Dobereiner, J and Day, J M, 1974, Associative Symbioses in tropical grasses Characterisation of microorganisms and dinitrogen fixation in soils *International Symposium on nitrogen fixation* Pullman Washington, USA

Hatch, M D and Slack, C R, 1970, Photosynthetic CO_2 fixation pathways *Ann Rev Plants Physiol*, **21** 141–162

Lowry, O H, Rose Brough, N J, Farr, A L and Randall, R J, 1951, Protein measurement with Folinciocalteau phenol reagent, *J Biology Chemistry* 193-265

Nagatani, H, Shimizu, M and Valentine, R C, 1971, The mechanism of ammonia assimilation in Nitrogen fixing bacteria *Arch Mikrobiol*, **79** 164–175

Paul, E A, Myers, R J K and Rice, L R, 1971, Nitrogen fixation in grassland and associated cultivated ecosystems *Plant and Soil* (special volume) 495–507

Postgate, J R, 1974, New advances and future potential in biological nitrogen fixation *J appl Bact*, **37** 185

Purushothaman, D, Kasirajan, C and Rangaswami, G, 1976, Nitrogen fixation by *Azotobacter* occurring in the rhizosphere of C_4 plant species *Curr Sci*, **45** 807-809

Purushothaman, D and Dhanapal, N, 1977, Nitrogen fixation by *Azotobacter* occurring in the rhizosphere of C_4 Plant species Paper Presented in Third Southern Regional-Conference on Bacterial Inoculants' at Dharwad

Raju, P N, Evans H J and Seidler, R J, 1972, An asymbiotic nitrogen fixing bacterinum from the root environment of corn *Proc Natl Acad Sci* **62** 3474–3478

Tjepkema, J D and Burris, R H, 1976, Nitrogenase activity associated with some Wisconsin Prairie grasses *Plant* and *Soil* **45** 81–94

Umbreit, W W, Burris, R H and Stanffer, J F, 1964, *Manometric Techniques* 4th Ed Burges Publ Co Minneapolis Minn

Influence of Foliar Application of Chemicals on the *Azotobacter* Population in the Rhizospheres of *Sorghum vulgare* and *Crotalaria juncea*

A Balasubramanian
Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore

Abstract

Azotobacter population in the rhizospheres of sorghum (*Sorghum vulgare* Pers) and sunnhemp (*Crotalaria juncea* Linn) was maximum when the plants were 75 days old, nearing maturity and minimum at 15 days growth The foliar spray treatments with 2, 4-D and sodium nitrate reduced the *Azotobacter* population significantly while disodium hydrogen phosphate and dithane Z-78 treatments increased the population in the rhizospheres of both the crop plants The changes in the *Azotobacter* populations are attributed to the altered root exudations of amino acids due to the foliar chemical treatments

Azotobacter spp are free-living soil microorganisms that are known to fix atmospheric nitrogen They have also been reported to colonize the rhizosphere of cultivated plants in appreciable numbers (Vancura and Macura, 1961 , Bagyaraj and Rangaswami, 1972) However, the ecology and the role of *Azotobacter* in the plant rhizosphere is not clearly understood Patel and Brown (1969) observed in wheat rhizosphere, that the numbers of antagonists to *Azotobacter* increased considerably with increasing plant age with parallel decrease in *Azotobacter* numbers in the rhizosphere While Barea and Brown (1974) observed no nitrogen fixation by *A paspali* in the rhizosphere of *Paspalum notatum*, but found several growth promoting substances Day *et al* (1975) reported considerable fixation of nitrogen by *A paspali* in association with *P notatum* roots In this paper, changes in the population of *Azotobacter* in the rhizosphere of two cultivated plant species, viz , *Sorghum vulgare* Pers and *Crotalaria juncea* Linn as influenced by foliar application of chemicals are reported

Material and Methods

Plant culture Surface sterilized, healthy seeds of sorghum (Co 4 variety) and sunnhemp (local) were sown in cement troughs (50 cm × 35 cm × 25 cm) filled with Hebbal red sandy loam soil (pH 6 6 and 0 48 per cent organic carbon) Twenty seeds were sown in each trough leaving equal space between the seeds At least 12 plants were maintained in each trough with four troughs under each treatment The soil moisture was adjusted every alternate day to 60 per cent of

the maximum water holding capacity and the troughs were maintained under identical conditions in green house

Foliar spray treatment The following foliar spray treatments were given to the plants (i) sodium nitrate, at 0 1 per cent, (ii) disodium hydrogen phosphate, at 0 1 per cent, (iii) 2, 4-D (2, 4 dichlorophenoxy acetic acid), at 25 ppm, (iv) Dithane Z-78 (Zinc ethylene bis dithio carbamate), at 250 ppm, and (v) distilled water (control)

Sufficient volume of the chemical solutions were sprayed on the foliage following the same procedure as described by Balasubramanian and Rangaswami (1973) The foliar spray treatments were started when the plants were 15 days old and were repeated at 20, 25, 30, 45 and 75 days age However, enumeration of the rhizosphere samples was carried out after 36 to 48 hrs after the foliar treatments on 30, 45 and 75 days only

Enumeration of Azotobacter *in the rhizosphere soil* *Azotobacter* population in the rhizosphere and non-rhizosphere soils was enumerated by the dilution plate method on Waksman's medium No 77 The populations were calculated on the basis of one g of moisture free sample and the rhizosphere. soil (R/S) ratio was also computed

Results and Discussion

The populations of *Azotobacter* in the rhizospheres of sorghum and sunnhemp plants at different stages of growth are presented in Table I Sorghum rhizosphere harboured more number of *Azotobacter* than sunnhemp at all the stages of plant growth, however, the maximum population was observed on the 75th day and the minimum on the 15th day in both the plant species The

Table I

Changes in Azotobacter *population in the rhizospheres of sorghum and sunnhemp plants at different stages of growth*

Age of plant (days)	Sorghum Population* (10^2/g)	Sorghum R S ratio	Sunnhemp Population* (10^2/g)	Sunnhemp R S ratio	Soil (10^2/g)
15	1 75	1 73	0 60	—	0 72
30	2 10	1 29	1 42	—	1 62
45	1 50	2 77	0,72	1 33	0 54
75	4 75	2 50	3 16	1 66	1 90

* Data significant at 1% level

Critical difference Plant species at 1% level — 0 100
Plant age — 0 116
Interaction — 0 201

variations in the *Azotobacter* population at different stages of plant growth are in accordance with the observations of Bagyaraj and Rangaswami (1973) in ragi (*Eleusine coracana*) plants The R/S ratio was also more in sorghum plants than in sunnhemp No rhizosphere effect was observed upto 30 days of plant growth in sunnhemp while an increase in R/S ratio was observed later with the growth of the plant

The rhizosphere effect on this organism has been reported to vary with the soil type, plant species and stage of plant growth (Brown *et al*, 1962, Katznelson and Strzelczyk, 1961) Maximum number of *Azotobacter* cells were encountered when the plants were 75 days old, nearing maturity However, Patel and Brown (1969) observed in wheat that the number of antagonists to *Azotobacter* increased with the decrease in *Azotobacter* numbers in the rhizosphere The present results are not in agreement with such a conclusion

The foliar spray treatments with the different chemicals altered the *Azotobacter* population in the rhizospheres of the two plant species significantly (Table II) The spray treatments with 2, 4-D and sodium nitrate drastically

TABLE II

Influence of foliar application of chemicals on the Azotobacter *population in the rhizosphere of sorghum and sunnhemp plants*

Treatment	Population (10^2/g)+			R S ratio		
	*30	45	75	30	45	75
Sorghum						
Sodium nitrate	0 40	0	1 33	0 24	0	0 60
Disodium hydogen phosphate	3 18	4 33	8 66	2 35	9 62	3 93
2,4-D	0 20	0	1 33	0 12	0	0 60
Dithane Z-78	7 41	6,22	9 33	4 57	13 82	4 24
Distilled water (control)	2 20	1 66	5 50	1 35	3 68	2 50
Sunnhemp						
Sodium nitrate	0	0 23	0 46	0	0 51	0,20
Disodium hydrogen phosphate	2 20	1 60	5 14	1 35	3 55	2 33
2,4-D	0	0	0 72	0	0	3 27
Dithane Z-78	3 20	2 40	7 16	1.97	5 33	3 25
Distilled water (control)	1 20	0 75	2 80	0 74	1 66	1 27
Soil	1 62	0 45	2 20			

\+ Data significant at 1% level
* Age of the plant in days

C D at 1% level

Spray	0 1379
Plant age	0.0975
Plant species	0.0796

reduced the *Azotobacter* population in sorghum rhizosphere while disodium hydrogen phosphate and dithane Z–78 sprays increased the population, as compared to the control Similar effect was observed in sunnhemp rhizophere also Negative rhizosphere effect (R/S ratio) was observed at certain stages of plant growth due to 2, 4–D and sodium nitrate treatments in both the plant species

The association of soil microorganisms with plant roots in great numbers in the rhizosphere region is often attributed to the presence of a variety of chemicals exuded by the plant roots (Rovira, 1962) Balasubramanian and Rangaswami (1973) have demonstrated the relationship between the quality and quantity of root exuded chemicals and the rhizosphere microflora of sorghum and sunnhemp The exudation of more of qualitatively and quantitatively rich amino acids by the plants treated on the foliage with 2,4–D and sodium nitrate, more of sugars by 2,4–D and disodium hydrogen phosphate treatments and notable reduction in the exuded chemicals by dithane Z–78 treatment, in general, have been reported by them (*loc cit*) It is, therefore, evident that an increase in the exudation of amino acids suppressed *Azotobacter* population, while no significant effect of the exuded sugars on these bacteria was evident Vancura and Macura (1961) observed that the amino acid fraction of the root exudates inhibited the growth of *Azotobacter* in stationary cultures This probably explains the suppression of *Azotobacter* in the rhizosphere of plants treated with sodium nitrate and 2,4-D which, generally, increased the amino acids in the root exudates Such suppression of *Azotobacter* in the rhizosphere of ragi treated with ammonium sulphate and 2,4–D on the foliage has been reported (Bagyaraj and Rangaswami, 1973) Hence, the results of the present studies suggest that the root exudations, particularly the organic nitrogenous fractions such as amino acids, play an important role in the colonization of the rhizosphere region of plant roots by *Azotobacter* The observations of Balandreau (1975) that, the nitrogenase activity of rhizosphere soil of several grasses were affected by the root exudations, supports such a possibility

The author expresses his gratitude to the Head of the Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore, where a major part of the studies was carried out

References

Bagyaraj, D J and Rangaswami, G , 1972, Studies on the rhizosphere microflora of *Eleusine coracana* under the influence of foliar chemical sprays I Quantitative incidence of microorganisms *Madras agric J*, **59** 517–523

Balandreau, J , 1975 Mesure de l, activite nitrogenasique des microorganismes fixateurs libres d' azote de la rhizosphere de quelques graminees *Rev Ecol Biol Sol* **12** 273–290

Balasubramanian, A and Rangaswami, G , 1973, Influence of foliar application of chemicals on the root exudations and rhizosphere microfolora of *Sorgnum vulgare* and *Crotalaria juncea Folia Microbiol*, **18** 492–498

BAREA, J. M. AND BROWN, M.K., 1974, Effects on plant growth produced by *Azotobacter paspali* related to synthesis of plant growth regulating substances. *J. Appl. Bacteriol.*, **37** : 582–593.

BROWN, M. E., BURLINGHAM. S. K. AND JACKSON, R.M., 1962, Studies on *Azotobacter* species in soil. II. Population of *Azotobacter* in the rhizosphere and the effects of artificial inoculation. *Pl. soil*, **17** : 320–332.

DAY, J. M., NEVES, M. C. P. AND DOBEREINER, J., 1975, Nitrogenase activity on the roots of tropical forage grasses. *Soil Biol. Biochem.*, 7 : 107–112.

KATZNELSON, H., AND STRZELCZYK, E. 1961, Studies on the interactions of plants and free-living nitrogen fixing microorganisms I. Occurrence of *Azotobacter* in the rhizosphere of crop plants. *Can. J. Microbiol.*, 7 : 431–446.

PATEL, J. J. AND BROWN, M.E., 1969, Interactions of *Azotobacter* with rhizosphere and root surface microflora. *Pl. Soil.* **31** : 273–281.

ROVIRA, A.D., 1962, Plant root exudates in relation to the rhizosphere microflora. *Soils* and *Fert.*, **25** : 167–172.

VANCURA, V. AND MAGURA, J., 1961, The effect of root excretions on *Azotobacter Folia Microbiol.*, **6** : 250–259.

SESSION III

Ecology of Nitrogen Fixing Organisms in Soil

Synergistic Effect of Four Granular Insecticides and *Rhizobium* Treatment on the Nodulation and Yield of Blackgram

P Muthukrishnan and M Rangarajan
Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai

Abstract

Seed treatment with *Rhizobium* sp , along with the soil application of granular insecticides such as disyston, phorate aldicarb, and furadon stimulated the growth, nodulation and yield in Co 2 variety of blackgram indicating the synergistic effect of *Rhizobium* sp , and disyston, phorate, aldicarb and furadon

Introduction

The granular insecticides applied to the soil for the control of insect pests of crops are reported to influence rhizobia, which increase the growth, nodulation and yield of legumes D D T applied at the recommended field rates did not have adverse effect on nodulation and yield of greengram (Pareek and Gaur, 1970)

Selim *et al* (1970) reported that dieldrin and lindane applied at the field dose did not have inhibitory effect on growth and nodulation of cowpea Lindane applied at field rate did not show toxic effect on growth and nodulation of bengalgram (Magu *et al* , 1972) Application of certain granular insecticidies *viz* , phorate, endrin, chlorfenvinphos and disulfoton is reported to stimulate the growth, nodulation and yield of leguminous crops (Oblisami *et al* , 1974 , Swamiappan and Chandy, 1975 , Chendrayan and Prasad, 1976) The synergistic effects of the granular insecticides *viz* , disyston, phorate, aldicarb, and furadon applied at 0 5 kg a i/ha and *Rhizobium* sp , with respect to the legume-*Rhizobium* symbiosis in blackgram are presented in this paper

Material and Methods

The field trial was conducted in *kharif* season, 1976–77 under irrigated conditions in a randomised block design with three replications having a plot size of 4 5 × 3m There were ten treatments including control in which no insecticide and no *Rhizobium* were applied application of *Rhizobium* alone formed one treatment The four granular insecticides *viz* , disyston, phorate, aldicarb and furadon were applied alone and also in combination with *Rhizobium* The insecticides were applied at the rate of 0 5 kg a i/ha in long

furrows in between lines to a depth of 4 cm one week after sowing Seeds of Co 2 variety of blackgram were thoroughly coated with the peat soil based *Rhizobium* culture and sown in lines with the spacing of 30 × 15 cm The crop was cultivated with all recommended package of practices Plant samples were collected on 65th and 45th day after sowing and observations were recorded for growth and nodulation Dry weights of shoot and root were considered as parameters of growth Total nodules per plant and dry weight of the nodular tissue were considered as indications of nodulation The grains extracted periodically from three harvests were pooled and final grain yield was assessed

Results and Discussion

The observations recorded on 45 days old crop are given in Table I The combined applications of *Rhizobium* sp , and granular insecticides were significantly superior to individual application of *Rhizobium* or insecticides with regard to growth of plants and nodulation

Table I

Effect of granular insecticides and Rhizobium *on the growth and nodulation of blackgram (Observations recorded on 45th day after sowing)*

Treatment	Dry weight of shoot (g)	Dry weight of root (g)	Total number of nodules per plant	Dry weight of nodular tissue (g)
Control	10 41	0 96	13 0	0 146
Rhizobium sp	12 37	1 20	25 0	0 239
Rhizobium+Disyston	15 52	1 49	34 7	0 391
Rhizobium+Phorate	14 59	1.29	31 7	0 372
Rhizobium+Aldicarb	14 57	1 26	31 3	0 366
Rhizobium+Furadon	13 99	1 16	28 7	0 297
Disyston	12 80	1 14	15 3	0 154
Phorate	11 83	0 99	14 3	4 155
Aldicarb	11.13	1 00	14 0	0 153
Furadon	11 12	1 00	13 0	0 149
SE	0 198	0.103	0 96	0 013
CD at 5%	0 584	0 306	2 86	0 037

The observations recorded on 65 days old crop are given in Table II The soil application of disyston and phorate at 0 5 kg a i/ha along with the seed treatment of *Rhizobium*, gave seed yield of 14 70 and 14 20 q/ha and 45 3 and 45.0 nodules per plant respectively while the *Rhizobium* treatment alone gave seed yield of 9 82 q/ha and 31 0 nodules per plant and disyston and phorate alone gave seed yields of 8 62 and 8 04 q/ha and 17 3 and 16.7 nodules per plant respectively

TABLE II

Effect of granular insecticides and Rhizobium *on the growth, nodulation and yield of blackgram (Observations recorded on 65th day after sowing)*

Treatments	Dry weight of shoot (g)	Dry weight of root (g)	Total number of nodules	Dry weight of nodular tissue (g)	Seed yield (q/ha)
Control	12 330	1 170	16 7	0 1650	7 71
Rhizobium sp	13 967	1 627	31 0	0 3427	9 83
Rhizobium+Disyston	17 657	1 907	45 3	0 4393	14 71
Rhizobium+Phorate	16 853	1 793	45 0	0 6000	14 21
Rhizobium+Aldicarb	16 903	1 553	41 3	0 5426	11 31
Rhizobium+Furadon	16 180	1 520	36 0	0 3930	10 91
Disyston	14 580	1 370	17 3	0 1740	8 63
Phorate	13 633	1 310	16 7	0 1680	8 04
Aldicarb	13 417	1 273	15 7	0 1652	8 14
Furadon	13 039	1 227	15 0	0 1573	7 79
SE	0 061	0 029	1 50	0 0191	0 19
CD at 5%	0 181	0 087	4 46	0 0567	0 57

Soil application of aldicarb and furadon at 0 5 kg a i/ha along with the seed treatment of *Rhizobium* also gave similar increased yields over the application of insecticides or bacterial culture alone, although they recorded lower yields and nodules than disyston and phorate All the four granular insecticides along with *Rhizobium*, were stimulatory to nodulation Similar results were recorded by Magu *et al* (1972) in bengalgram due to lindane

REFERENCES

CHENDRAYAN, K AND PRASAD, N N , 1976, Effect of soil application of phorate and disulfoton on *Rhizobium*-groundnut symbiosis *Madras Agric J* , 63 528–530

MAGU, S P , GAUR, A C , AND SADASIVAM, K V , 1972, Effect of lindane on nodulation, grain yield and nitrogen uptake of *Cicer arietinum* crop *Symp Legume Inoculants-Science and Technology*, p 26–27

OBLISAMI, G , BALARAMAN, K , NATARAJAN, T AND KULANDAIVELU, R , 1974, Effect of granular insecticides on growth and nodulation of groundnut Abst XV Ann Conf AMI, p 86

PAREEK, R P AND GAUR, A C , 1970, Effect of dichlorodiphenyl trichloroethane (D D T) on symbiosis of *Rhizobium* sp with *Phaseolus aureus* (greengram) *Pl Soil* , 33 297–304

SELIM, K G , MAHAMOUD, S A AND MOKADEM, M T , 1970, Effect of dieldrin and lindane on the growth and nodulation of *Vicia faba Pl Soil* 33 325–329

SWAMIAPPAN, M AND CHANDY, K C , 1975, Effects of certain insecticides on the nodulation by nitrogen fixing bacteria in cowpea (*Vigna sinensis* L) *Curr Sci* , 44 558–559.

Effect of Different Insecticides on the Antibiotic Resistant Mutants of *Rhizobium* spp

S JAYACHANDRAN AND G JEGANATHAN
Department of Biology, Agricultural College and Research Institute
Madurai–625 104 Tamil Nadu

ABSTRACT

The influence of different technical grade insecticides *viz*, Lindane and B H C (Organochlorine), Carbofuran and Sevin (Carbamates) and thimet and phosphomidon (Organophosphorus) at 1,5 and 10 μg/ml concentrations on the growth and polysaccharide production of CAM^r and St^r mutants of *Rhizobium* spp was investigated and compared with the wild type All the insecticides at 1 μg/ml concentration promoted the growth of St^r mutant and wild type while Lindane alone inhibited the growth of CAM^r mutant All the insecticides at all the three concentrations inhibited the production of alkali-stable polysaccharides of both the mutants and wild type All the insecticides at 1 μg/ml stimulated the production of water soluble polysaccharides in both the mutants whereas sevin and carbofuran alone at 1 mg/ml stimulated the production of water-soluble polysaccharides in wild type At higher concentrations (5 and 10 μg/ml) all the insecticides inhibited the production of water soluble polysaccharides in both the mutants and wild type The qualitative analysis of the polysaccharides produced by the mutants and wild type in the presence of the insecticides at the lowest concentration (1μg/ml) revealed that the monomeric sugar units of the polysaccharides consisted of both hexoses and pentoses However, in the absence of any of the insecticides the monomeric sugar units of the polysaccharides produced by wild type consisted only of hexoses indicating that the incorporation of insecticides even at the lowest concentration (1 μg/ml) altered the pathway of synthesis of the polysaccharides

INTRODUCTION

ANTIBIOTIC resistant marker strains of rhizobia were employed to study the efficacy of *Rhizobium* legume symbiosis in natural environment (Law and Strijdom 1974) Although different antibiotic resistant mutants of rhizobia tend to lose their infectivity or nodule forming ability, such effects are believed to be governed by the concentration of the antibiotics used to screen specific antibiotic resistant mutants (Schwinghammer, 1967) Notwithstanding the information on the influence of a variety of potent synthetic compounds such as insecticides, fungicides detergents etc On different soil microorganisms (Sreenivasulu and Rangaswami, 1973 Van Schreven *et al*, 1970) and particularly on rootnodule bacteria (Kaszubiak, 1968) the available knowledge on the effect of these 'synthetic poisons' on the antibiotic resistant mutants of rhizobia is rather fragmentary The effect of different groups of insecticides on the CAM^r and St^r mutants of *Rhizobium* spp *in vitro* is presented in this paper

Material and Methods

Organism The wild type *Rhizobium* spp (Cowpea group) and CAM^r and ST^r (100 μg/ml) mutants were obtained from the culture collection of the Department of Biology, Agricultural College and Research Institute, Madurai

Insecticides All the insecticides *viz*, Lindane (gamma B H C 1, 2, 3, 4, 5, 6 hexachloro cyclohexane), B H C (Benzene hexachloride), carbofuran (2, 3-dihydro-2-2 dimethyl-7 benzofuranyl methyl carbamate) Sevin (naphthyl-N-methylcarbamate), thimet (0,0-diethyl-S (ehylthiomethyl) phosphorothiolonate) and phosphomidon [0,0-dimethyl, 0-(2-chloro-2-N-N-diethyl carbamyl-1-1-methylvinyl) Phosphate] used in the present studies were of technical grade Required concentrations of the insecticides were prepared by dissolving the insecticides in 80 per cent acetone

Growth studies To 100 ml of Waksman 79 medium without calcium carbonate in 250 ml Erlenmeyer flasks one ml of wild type or St^r or CAM^r mutant cells (Ca 1×10^8/ml) experiencing log phase of growth was inoculated, particular concentration of the insecticides added and incubated on a rotary shaker for 72 hrs at room temperature ($28 \pm 2°C$) The cells were harvested by centrifuging at $3000 \times g$ for 10 min and the biomass determined after drying in hot air oven at 90°C for 24 hrs

Polysaccharide production From the cells harvested after 72 hrs of incubation, the alkali stable polysaccharides (ASP) were extracted following the method of Hassid and Abraham (1957) and quantified using anthrone method (Salewski *et al*, 1974) Water-soluble polysaccharides (WSP) were determined by precipitating the polysaccharides present in the cell-free centrifugate by the addition of propan-2-01 (3 1 v/v) (Couperwhite and McCallum, 1975) Qualitative analysis of the ASP was done by descending paper chromatography using n-butanol acetic acid water 4 1 5 (v/v) solvent system and benzidine-TCA reagent served as the spray solution (Block *et al*, 1958) The sugars were identified by co-chromatography with authentic Sugar samples

Results and Discussion

The data revealed that all the insecticides at 1 μg/ml concentration stimulated the growth of St^r mutant and wild type while lindane alone at 1 μg/ml concentration inhibited the growth of CAM^r mutant However, at higher concentrations (5 and 10 μg/ml) all the insecticides inhibited the growth of both the mutants and wild type (Table I)

Influence of different insecticides on the ASP and WSP produced by CAM^r and St^r mutants and wild type *Rhizobium* spp is given in Tables II and III Although all the insecticides at the three concentrations inhibited the ASP

TABLE I

Effect of different insecticides on the growth of CAM^1, St^1 mutants and wild type Rhizobium *spp*

Treatment	Control	Biomass (mg/100 ml)																	
		Lindane (μg/ml)			B H C (μg/ml)			Carbofuran (μg/ml)			Sevin (μg/ml)			Thimet (μg/ml)			Phosphomidon (μg/ml)		
		1	5	10	1	5	10	1	5	10	1	5	10	1	5	10	1	5	10
St^r	820	865	765	650	860	780	635	850	810	625	845	790	695	825	805	695	865	795	675
CAM^r	630	610	605	535	635	590	560	620	550	530	720	645	600	710	690	605	745	700	620
Wild type	725	725	665	625	755	750	725	765	725	715	805	750	690	755	700	620	800	705	650

TABEL II

Effect of different insecticides on the alkali-stable polysaccharides produced by CAM^1, St^1 mutants and wild type Rhizobium *spp*

Treatment	Control	Alkali-stable polysaccharides (mg/g dry weight of cells)																	
		Lindane (μg/ml)			B H C (μg/ml)			Carbofuran (μg/ml)			Sevin (μg/ml)			Thimet (μg/ml)			Phosphomidon (μg/ml)		
		1	5	10	1	5	10	1	5	10	1	5	10	1	5	10	1	5	10
St^r	92	90	86	80	91	88	82	90	88	88	92	90	91	90	90	88	90	88	88
CAM^r	85	83	83	82	82	80	79	80	79	76	82	77	76	82	79	74	80	78	74
Wild type	79	78	78	76	78	77	77	78	76	75	77	76	72	77	76	75	76	75	73

TABLE III

Effect of different insecticides on the water-soluble polysaccharides produced by CAM[r], St[r] mutants and wild type Rhizobium *spp*

Treatment	Control	Water-soluble polysaccharides (mg/100 ml)																	
		Lindane (μg/ml)			B H C (μg/ml)			Carbofuran (μg/ml)			Sevin (μg/ml)			Thimet (μg/ml)			Phosphomidon (μg/ml)		
		1	5	10	1	5	10	1	5	10	1	5	10	1	5	10	1	5	10
Str	820	830	800	790	825	810	800	835	800	785	825	800	760	855	825	805	830	800	760
CAMr	715	720	700	700	750	705	675	725	705	645	725	710	680	730	700	640	720	700	685
Wild type	420	420	400	395	420	400	385	430	380	355	430	385	370	420	390	380	420	400	390

TABLE IV

Effect of different insecticides on the qualitative analysis of the polysaccharides produced by CAM^r, St^r mutants and wild type Rhizobium *spp*

Insecticide*	Qualitative differences in the polysaccharides produced by		
	CAM^r mutant	St^r mutant	Wild type
Lindane	Glu, Suc, Rib, Xyl	Glu, Suc, Lact, Ara, Rib	Glu, Suc, Fru, Rib, Xyl
B H C	Glu, Suc, Rib, Ara	Glu, Lact, Xyl, Gal	Fru, Suc, Rib, Ara, Gal
Carbofuran	Glu, Fru, Ara, Mann	Glu, Lact, Rib, Gal	Glu, Lact, Xyl, Ara, Gal
Sevin	Glu, Suc, Fru, Xyl, Rib	Glu, Suc, Fru, Gal, Rib	Glu, Suc, Rib, Xyl, Mann
Thimet	Fru, Suc, Lact, Rib, Gal	Glu, Suc, Ara, Xyl	Fru, Lact, Rib, Ara
Phosphomidon	Glu, Fru, Xyl, Rib	Glu, Lact, Xyl, Ara, Mann	Glu, Lact, Rib, Ara, Xyl
Control	Glu, Suc, Fru, Rib, Xyl	Glu, Suc, Lact, Mann, Ara	Glu, Suc, Fru, Lact

*Incorporated at 1 μg/ml concentration in Waksman 79 medium

Ara—Arabinose Glu—Glucose Rib—Ribose
Fru—Fructose Lact—Lactose Suc—Sucrose
Gal—Galactose Mann—Mannose Xyl—Xylose

production, the inhibition was more pronounced at higher concentrations (5 and 10 μg/ml) All the insecticides at 1 μg/ml concentration promoted the WSP production in both the mutants, however, at the same concentration sevin and carbofuran alone stimulated the production of WSP in wild type All the insecticides at 5 and 10 μm/ml concentration invariably inhibited the WSP in both the mutants and wild type

In the presence of different insecticides (at 1 mg/ml) the monomeric sugar units of the polysaccharides produced by the mutants and wild type consisted of both hexoses and pentoses However, in the absence of any of the insecticides the monomeric sugar units produced by wild type consisted only of hexoses (Table IV)

Rhizobia are known to be greatly influenced by a vast array of pesticides (Gillberg, 1971, Misra and Gaur, 1972) While all the insecticides at 1 μg/ml concentration promoted the growth of St^r mutant and wild type, lindane alone inhibited the growth of CAN^r mutants Magu *et al* (1974) reported that lindane at 1 ppm promoted the nodulation of *Cicer arietinum* while at higher concentrations nodulation was inhibited

The extra-celluar polysaccharides of the rhizobia are immunologically specific biopolymers that permit immunological differentiation between various cross-inoculation groups All the insecticides at 1 μg/ml concentration inhibited the production of ASP but not the production of WSP Sensitivity of different rhizobia in carrying out different metabolic processes are known to be affected by various fungi-static substances (Mukewar and Bhide, 1969) and metabolic inhibitors (Schwinghamer, 1968) Apparently differential inhibition/ stimulation of growth and polysaccharide production of both the mutants and wild type by different insecticides at different concentrations observed in the present study is a reflection of the degree of sensitivity of different rhizobia to different insecticides Further Oblisami *et al* (1974) reported that the activities of rhizobia are greatly influenced by the chemical nature of the insecticides

Albersheim *et al* (1969) reported that the specificity of *Rhizobium* - legume symbiosis could be attributed to extracellular polysaccharides of *Rhizobium* because sugar containing molecules were uniquely sensitive in 'recognizing' different proteins The qualitative differences in the polysaccharides produced by diffirent rhizobia have been elucidated by many workers (Zevenhuizen, 1971, Hepper, 1972) Essentially the constituent monomeric sugar units of the polysaccharides produced by both the mutants and wild type in the presence of any of the insecticides (1 μg/ml) consisted of both hexoses and pentoses However, in the absence of any of the insecticides the monomeric sugar units of the polysaccharides produced by wild type consisted only of hexoses indicating a shift in

the pathway of synthesis of the polysaccharides in the wild type and not in either of the mutants. Jayachandran and Balasubramanian (1977) reported a shift in the pathway of synthesis of polysaccharides in the presence of some phenolic compounds in *Rhizobium leguminosarum*.

Notwithstanding the differences in the growth and polysaccharide production of the two mutants further investigations on the effect of these insecticides on the *Rhizobium*-legume symbiosis *in vivo* will throw more light on the applicability and utility of these two mutants in assessing the contribution of inoculated rhizobia in the soil.

References

Albersheim, P., Jones, T. M. and English, P. D., 1969, Biochemistry of the cell wall in relation to infective processes. *Ann. Rev. Phytopathol.*, 7 : 171.

Block, R. J., Durrum, E. L. and Zweig, C., 1958, *A Manual of Paper Chromatography and Paper Electrophoresis.* Academic Press, New York.

Couperwhite, I. and McCallum, M. F., 1975, Polysaccharide production and the possible occurrence of GDP-D mannose dehydrogenase in *Azotobacter vinelandii. Antonie van Leeuwenhoek*, 41 : 25-32.

Gillberg, B. O., 1971, On the effects of some pesticides on *Rhizobium* and isolation of pesticide-resistant mutants. *Arch. Mikrobiol.*, 75 : 203-208.

Hassid, W. and Abraham, S., 1957, In *Methods in Enzymology*, (Ed.), S. P. Colowick and N. O. Kaplan, Academic Press, New York. 3.

Hepper, C. M., 1972, Composition of extra-cellular polysaccharides of *Rhizobium trifolii. Antonic van Leeuwenhoek.* 38 : 437-445.

Jayachandran, S. and Balasubramanian, A., 1979, Influence of phenolic compounds on the growth and polysaccharide production of *Rhizobium leguminosarum. Curr. Sci.*, 46 : 142-143.

Kaszublak, H., 1968, The effect of herbicides on *Rhizobium.* II. Adaptation on *Rhizobium* to Afalon, Aretit and Lirobetarex. *Aeta microbiol. Pol.*, 17 : 41-50.

Law, I. J. and Strijdom, B. W., 1974, Nitrogen fixing and competitive abilities of a *Rhizobium* strain used in inoculants for *Arachis hypogea. Phytophylactica.* 6 : 221-228.

Magu, S. P., Gaur, A. C. and Sadasivam, K. V., 1974, Effect of lindane on nodulation, grain yield and nitrogen uptake of gram. *(Cicer arietinum)* crop. *Proce. Ind. Natl. Sci. Acad.*, 40 : 526-527.

Misra, K. G. and Gaur, A. C., 1972, Influence of pesticides on root nodulation, growth, yield and nitrogen uptake of groundnut inoculated with *Rhizobium* sp. *Abst. Symb. Legume inoculants-Science and Technology*, p. 28.

Mukewar, P. M. and Bhide, V.P., 1969, Effect of seed treatment with fungicides and antibiotic aureofungin on the efficiency of nodulation by *Rhizobium* strain of groundnut. *Hindustan Antibiot. Bull.*, 11 : 172-176.

Oblisami, G., Balaraman, K., Venkataraman, C. R. and Rangaswami, G., 1973, Effect of three granular insecticides on the growth of *Rhizobium* from redgram. *Madras agric. J.*, 60 : 462-464.

SALEWSKI, A C , SCIBOLD AND FROBSCHLE, H , 1974, Estimation of soluble carbohydrates and cell-wall substances in one operation *Land Forsch* , **27** 112–119

SCHWINGHAMMER, E A , 1967, Effectiveness of *Rhizobium* as modified by mutation for resistance to antibiotics *Antonie van Leeuwenhoek*, **33** 121–136

SCHWINGHAMMER, E A , 1968, Loss of effectiveness and infectivity in mutants of *Rhizobium* resistant to metabolic inhibitors *Can J Microbiol* , **14** 355–367

SRINIVASALU, S AND RANGASWAMY, G , 1973, Studies on the effect of three granular organophosphorus insecticides on soil microflora *Indian J Microbiol* , **13** 89–95

VAN SCHREVEN, D A , LINDERBERG, D J AND KORIDON, A , 1970, Effect of several herbicides on bacterial populations and activity and the persistance of these herbicides in soil *Pl Soil*, **33** 513–532

ZEVENHUIZEN, L P T M , 1971, Chemical composition of exopolysaccharides of *Rhizobium* and *Agrobacterium J Gen Microbiol* , **68** 239–243

Effect of Pesticides on the Growth, IAA Production and Nodulation by a *Rhizobium* Species

A BALASUBRAMANIAN AND T SEKAR
Microbiology Laboratory, Tamil Nadu Agricultural University, Coimbatore-3

ABSTRACT

The effect of aldicarb, fensulfothion (Dasanit) and disulfoton (Disyston) on the growth, indole acetic (IAA) production and nodulation by a species of cowpea *Rhizobium* (P-47) were studied under controlled conditions Aldicarb and disulfoton adversely affected the growth of the *Rhizobium* sp at 5 ppm and 10 ppm concentrations during 72 hr growth period, while stimulation in growth was observed in cells treated with fensulfothion Not only the IAA production potential of the *Rhizobium* isolate was adversely affected in presence of the pesticides but the number of nodules in cowpea plants grown in soil treated with the pesticides (at 10 ppm level) were also considerably reduced, the adverse effect being more with fensulfothion and disulfoton

INTENSIVE agriculture warrants repeated application of large quantities of pesticides for plant protection, the residues of which accumulate in soil These residues, sufficiently persistent in soil, are apt to come into direct contact with soil microorganisms Considerable information is available on the effect of certain pesticides on the metabolic activities of *Rhizobium* (Oblisami *et al* , 1973 , Balasubramanian and Gita Nilakantan, 1975 , 1976 a and 1976 b)

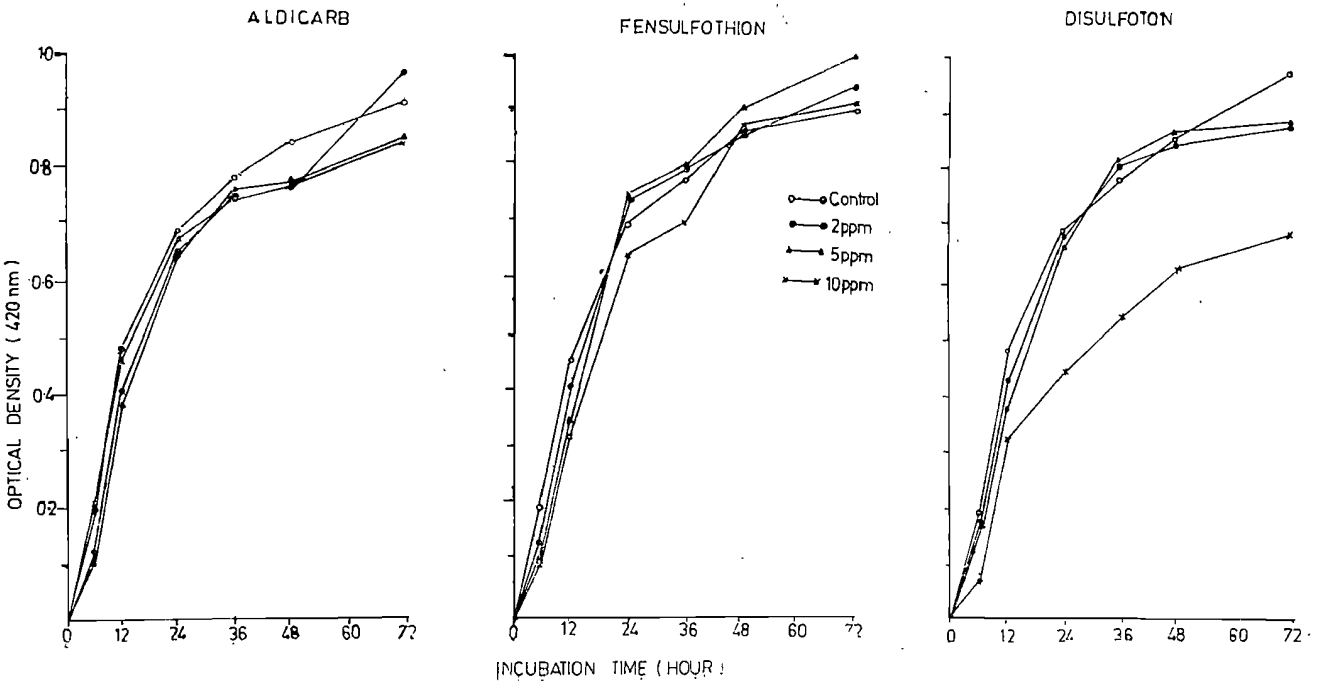
There are also reports to indicate the effect of such residues on the symbiotic effectiveness of the rhizobia in their host plants (Kulkarni *et al*, 1974 and Chendrayan and Prasad, 1976) The present report deals with the effect of three commonly used pesticides *viz*, aldicarb (Temik), fensulfothion (Dasanit) and disulfoton (Disyston) on the growth, indole acetic acid (IAA) production and nodulation by a Rhizobium sp under *in vitro* conditions

Material and Methods

Measurement of the growth of the Rhizobium *sp* The *Rhizobium* sp an isolate from blackgram (*Phaseolus mungo* L) was grown in malt extract medium (K_2HPO_4 0 5 g, $MgSO_4$ $7H_2O$ 0 2 g, NaCl 0 1 g, Malt extract (Difco) 10 0 g, yeast extract 1 0 g and distilled water 1000 ml with pH adjusted to 6 81, (Iswaran and Jauhri, 1969) for 72 hr in a rotary shaker Calculated quantities of the stock solutions of the technical materials of the pesticides, aldicarb [2–methyl –2 (methyl thio) propinaldehyde–O–methyl carbamoyl oxime], fensulfothion (0,0-diethyl-0-4, methyl sulphinyl phenyl monothiophoshate) and disulfoton [0,0–diethyl-S–2 (ethyl thio) ethyl phosphorodithioate] were transferred to 99 ml of malt extract broth to obtain a final concentration of 2, 5 and 10 ppm of the pesticides, and inoculated with 1 ml of 72 hr old culture of the *Rhizobium* sp before the flasks were incubated at 28 ± 2°C in a rotary shaker Appropriate controls were maintained and the treatments were duplicated The growth of the organism was periodically determined for a period of 72 hr in an Erma Photoelectric colorimeter using a blue filter (420 nm) (Balasubramanian and Gita Nilakantan, 1975)

Estimation of IAA production by the Rhizobium *sp* Malt extract medium (99 ml) containing 0 1 per cent L–tryptophan was inoculated with one ml of a 72 hr old culture of the organism along with 0, 2, 5 and 10 ppm of aldicarb, fensulphothion and disulfoton as described above Each treatment was replicated twice After 3 days incubation on a rotary shaker at 28 ± 2°C the cells were harvested by centrifugation and the cell free supernatant was used for the quantitative estimation of IAA, following the method of Chandramohan and Mahadevan (1968) The IAA in the supernatant was also identified chromatographically using n-butanol acetic acid water (4 1 1 v/v) as the solvent system and Salkowski reagent as spray reagent

Determination of nodulation. Circular mud pots of 12'' diameter, filled with garden land soil and farm yard manure were sterilised at 20 psi for 2 hr Granular formulations of aldicarb (Temik 10 G), fensulfothion (Dasanit 10 G) and disulfoton (Disyston 5G) were added at the rate of 10 ppm of a i of the chemicals and mixed thoroughly Cowpea seeds, coated with equal volume of a thick suspension of the *Rhizobium* sp were sown in the soil at the rate of six seeds per pot and five replications for each treatment were maintained The


ALDICARB
FENSULFOTHION
DISULFOTON
OPTICAL DENSITY (420 nm)
1·0
0·8
0·6
0·4
0·2
0
12
24
36
48
60
72
Control
2ppm
5ppm
10ppm
INCUBATION TIME (HOUR)

pots were watered regularly and the plants were carefully removed 45 days after sowing the roots washed under running tap water and the number of apparently effective nodules were counted

Results and Discussion

Results on the influence of the three pesticides on the growth of the *Rhizobium* sp are presented in Fig 1 Although aldicarb was stimulatory at 2 ppm concentration, the 5 and 10 ppm levels inhibited the growth of the organism While fensulfothion appeared to stimulate the growth of the *Rhizobium* sp at all the three levels, disulfoton adversely affected the growth of the organism, the maximum suppression being recorded with 10 ppm concentration of the pesticide All the three concentrations of aldicarb, fensulfothion and disulfoton decreased the IAA synthesising ability of the organism, the reduction being maximum with 10 ppm of fensulfothion (Table I) The pesticides significantly reduced the number of nodules per plant and the reduction was maximum with dasanit and disyston (Table II)

Table I

Effect of pesticides on indoleacetic acid (IAA) production by the Rhizobium *sp*

Treatment	IAA produced (mg/100 ml medium)		
	Aldicarb	Fensulfothion	Disulfoton
No pesticide (control)	9 188	9 188	9 138
2 ppm	8 125	6 688	7 625
5 ppm	8 813	7 750	7 813
10 ppm	8 750	4 000	7 750

Table II

Effect of soil applied pesticides on nodulation in cowpea plants by the Rhizobium sp

Treatment	*Nodule number/plant
No pesticide (control)	32 54
Temik 10 ppm	28 96
Dasanit 10 ppm	14 40
Disyston 10 ppm	19 46

*Values represent mean of five replications

Inhibition of the growth of the *Rhizobium* sp at 5 and 10 ppm levels, but a stimulation at 2 ppm concentration, is in agreement with the earlier reports of Balasubramanian and Gita Nilakantan (1975, 1976a and 1976b) Stimulatory effect on the growth of the *Rhizobium* by the pesticides, as in the present case of fensulfothion, is not uncommon (Gupta and Sud, 1971) The observations on the increased inhibition of disulfoton with increasing concentrations of the

chemical also corroborated with the earlier reports of Oblisami *et al.* (1973). The reduction caused in the IAA production potential of the *Rhizobium* sp. by all the three pesticides is in conformity with the observations of Balasubramanian and Gita Nilakantan (1976b) with *R. japonicum* and aldicarb. Such an inhibition of the IAA production potential of the organism by the pesticides and a reduction in the mean number of nodules formed in the cowpea plants raised in the pesticides treated soil, suggests a positive relationship between the IAA production potential of the *Rhizobium* and its symbiotic efficiency. A positive correlation between the IAA production *in vitro* and symbiotic efficiency of rhizobia has already been brought out by Vidhyasekaran *et al.* (1973). Similar reduction in the number of nodules in bean roots due to treatment with 30 to 40 ppm of Temik and with 50 ppm of the chemical in clover have been reported by Gawaad *et al.* (1972). Kulkarni *et al.* (1974) observed significant reduction in the number of nodules in groundnut plants raised in soil treated with dasanit. Disyston has also been reported to decrease the nodule number in groundnut plants at 200 ppm, though an increase in the same was reported due to 12 ppm of the chemical (Prasad and Ramani, 1976). However, the mode of action of these pesticide residues in soil on the IAA synthesis *in vitro* of the *Rhizobium* sp. and its symbotic effectiveness, remains to be demonstrated.

The authors are thankful to the International Atomic Energy Agency, Vienna, for their financial assistance.

References

Balasubramaniam, A. and Gita Nilakantan, 1975, Effect of an insecticide on growth and metabolism of some non-target soil microorganisms, *J. Nuclear agric. Biol.*, **4**: 38–40.

Balasubramanian, A. and Gita Nilakantan, 1976a, Effect of aldicarb on the growth and radiocarbon (14C) and radio-phosphorus (32P) assimilation by *Rhizobium japonicum*. *Mysore J. agric. Sci.*, **10**: 59–63.

Balasubrmanian, A. and Gita Nilakantan, 1976b, Effect of aldicarb on the growth and indole acetic acid production by *Rhizobium japonicum* and *Azotobacter chroococcum*. *Madras agric. J.*, **63**: 265–267.

Chandramohan, D. and Mahadevan, A., 1968, Indole acetic acid metabolism in soils. *Curr. Sci.*, **37**: 112-113.

Chendrayan, K. and Prasad, N. N., 1976, Effect of soil application of phorate and disulfoton on *Rhizobium* groundnut symbiosis. *Madras agric. J.*, **63**: 528-530,

Gawaad, A.A.A., El-Minshawy, A. M. and Zeid, M., 1972, Studies on soil insecticides VIII. Effect of some soil insecticides on broad beans and Egyptian clover nodule forming bacteria. *Zent. Bakt. Parasitenkd. Infektionskr. Hyg. Abst. II*, **127**: 172–177.

Gupta, K. C. and Sud, R. K., 1971, *Studies on carbamate bacteria interaction*. Proceedings of the First All India Symposium on Progress and Problems in Pesticide Residue Analysis held at Ludhiana, p. 166.

Iswaran, V. T. and Jauhri, K. S., 1969, Malt-extract as a good source of carbon for large scale production of *Rhizobium* inoculants. *Curr. Sci.*, **38**: 67.

KULKARNI, J. H., SARDESHPANDE, J. S. AND BAGYARAJ, D. J., 1974, Effect of four soil applied in secticides on symbiosis of *Rhizobiom* sp with *Arachis hypogea* Linn. *Pl. Soil.* **40** : 169–173.

OBLISAMI, G., BALARAMAN, K., VENKATARAMAN, C. V. AND RANGASWAMI, G., 1973, Effect of three granular insecticides on the growth of *Rhizobium* from redgram. *Madras agric. J.*, **60** : 462–464.

PRASAD, N. N. AND RAMANI, C.E., 1976, Effect of certain pesticides on nodulation and nitrogen fixation by *Rhizobium* sp. in groundnut. *Madras agric. J.*, **63** : 646–650.

VIDHYASEKARAN, P., BALARAMAN, K., DEIVEEGASUNDARAM, M. AND RANGASWAMI, G., 1973, Relationship between virulence and auxin production by *Rhizobium* sp. isolates from groundnut. *Curr. Sci.*, **42** : 66–67.

Influence of Soil Applied Pesticides on the Translocation of Foliar Applied ^{14}C-Glucose to the Root Nodules of *Vigna sinensis* L.

A. BALASUBRAMANIAN AND T. SEKAR
Microbiology Laboratory, Tamil Nadu Agricultural University, Coimbatore-3

ABSTRACT

The influence of soil applied pesticides, *viz.*, Temik (aldicarb), Dasanit (fensulfothion) and Disyston (disulfoton) at 10 ppm level on the nodulation and translocation of foliar applied ^{14}C-glucose to the root nodules of cowpea (*Vigna sinensis* L.) was examined. Although, the number of nodules per plant and the weight of nodules decreased in presence of the pesticides in the soil, the specific activities of ^{14}C translocated to the nodules were enhanced significantly, as compared to the untreated control. Soil application of dasanit (at 10 ppm) mobilised maximum ^{14}C-radio activity in the root nodules. While a major portion of the absorbed radio activity remained in the shoot, the percentage of ^{14}C-radioactivity translocated to the root nodules was more than that incorporated in the root tissue, in all the cases.

LARGE quantities of pesticides find their way into the soil due to intensive plant protection schedules for the control of pests and diseases. Influence of such soil applied insecticides and nematicides on the symbiotic, nitrogen fixing rhizobia and the host plant have been brought out by several workers (Gawaad *et al.*, 1972; Pareek and Gaur, 1969 and 1970; Balasubramanian *et al.*, 1974; Kulkarni *et al.*, 1974). Information on the effect of these pesticidal residues in soil on the translocation of photosynthates to the root nodules in leguminous crops is very meagre. The present paper gives a preliminary account on the influence of certain soil applied insecticides *viz.*, Temik (aldicarb), Dasanit

(fensulfothion) and Disyston (disulfoton) on the translocation of foliar applied radio active (^{14}C) glucose to the root nodules of cowpea (*Vigna sinensis* L.)

Material and Methods

Cowpea plants (*Vigna sinensis* L.) were raised in mud pots with sterilised gardenland soil containing 10 ppm concentration of Temik (10 G), Dasanit and Disyston and the treatments were replicated thrice. To 70 day plants, uniformly labelled (^{14}C) d-glucose was applied on the leaves. An aliquot of 0.5 ml of radioactive solution of 0.5 microcurie per ml was applied to five plants in each pot using an automatic microsyringe. The plants were left for 48 hr after the foliar treatment and then removed without damaging the root system and washed under running tap water.

Determination of nodulation and dry matter production: The root nodules of each plant from different treatments were counted and the fresh weight of the nodules was recorded. The shoots and roots of the plants were separated and the dry weights were recorded.

Determination of radioactivity in the plant samples: Two to three large pinkish nodules were selected at random and placed in an aluminium planchet, crushed well with a glass rod and dried under infra red heat. The radioactivity was monitored in a gas flow Proportional Counting System (Electronics Corporation of India Ltd.,) and the specific activity was calculated based on the dry weight of the nodules from each treatment.

The dried root and shoot samples from each treatment were powdered and sieved through 1 mm sieve. A known quantity of the root or shoot sample was placed on a planchet to which few drops of distilled water were added and then dried under infrared heat so that the powdered sample formed a cake on the planchet. The radioactivity was monitored and the specific activity calculated as described above.

Results and Discussion

The residues of Temik, Dasanit and Disyston were found to reduce both the number of nodules per plant and the fresh weight of nodules (Table I). Significant reduction in nodulation of bean roots was noticed due to 30 to 40 ppm of Temik and due to 50 ppm of Temik on clover roots (Gawaad *et al.*, 1972). Kulkarni *et al.* (1974) reported significant reduction in the number of nodules in groundnut plants raised in soil treated with Dasanit. Soil incorporation of the pesticides resulted in reduction of dry weight of roots, except in dasanit, where an enhancement in the root weight was observed (Table II); however, all the pesticides increased the dry weight of shoot.

TABLE I

Translocation of foliar applied (^{14}C) glucose to root nodules as influenced by soil application of the pesticides

Treatment	*Nodule number per plant	*Nodule weight (mg/plant)	^{14}C-specific activity in root nodules (Counts/ 100 sec/100 mg dry tissue)
No pesticide (Control)	56.66	266.41	120±11.7
Temik 10 ppm	44.58	209.91	115±15.7
Dasanit 10 ppm	40.50	179.50	321±10.6
Disyston 10 ppm	36 25	163.00	261± 7.3

*Data represent the mean of 12 plants

TABLE II

Specific activity of ^{14}C-glucose translocated to root and shoot of cowpea plants as affected by the pesticides application

Treatment	*Dry wt. of root (mg/plant)	C^{14}-specific activity in root (Counts/100 sec/ 100 mg dry tissue)	*Dry wt. of shoot (mg/plant)	C^{14}-specific activity in shoot (Counts/100 sec/ 100 mg dry tissue)	% radioactivity translocated to		
					Nodules	Root	Shoot
No pesticide (Control)	311.80	24±11.3	1236.3	355±14.3	6.69	1.57	91.74
Temik 10 ppm	248.00	55± 4.0	1330.0	298±22.4	7.33	3.07	89.60
Dasanit 10 ppm	368.10	35± 4.0	2186.8	254±14.9	9.20	2.06	88.74
Disyston 10 ppm	294.80	20±15.9	1472.1	278±14.1	9.33	1.29	89.38

*Data represent the mean of 12 plants

The results indicated that a major portion of the ^{14}C-glucose applied on the foliage remained in the shoot of the cowpea plants. However, considerable portion of the radioactivity translocated to the roots was incorporated into the root nodules. The pesticide residues caused marked variation in the radioactivity translocated to both the nodules and roots of cowpea plants. Temik and Dasanit increased the translocation of ^{14}C-glucose to root system while Disyston decreased the same. Accumulation of more radioactivity in the nodules of the treated plants possibly indicates an enhanced metabolic activity in the nodular tissues. The significance of such enhanced assimilation of foliar applied glucose in the nodular tissues on the nitrogen fixation is yet to be explored.

The authors are thankful to the International Atomic Energy Agency, Vienna, for financial support.

References

Balasubramanian, A Shantaram M V, Emmimath, V S and Siddaramappa, R 1974, Lindane increases nodulation and yield in soyabean plants *Pesticides*, **8** 35

Gawaad, A A A, Minshway, A M E and Zeid, M, 1972, Studies on soil insecticides VIII Effect of some soil insecticides on broad beans and Egyptian clover nodule forming bacteria *Zent Bakt Parasitenkd Infektionskr Mv 8 Abst II*, **127** 172–177

Pareek, R P and Gaur, A C, 1969, Effect of DDT on nodulation, growth yield and nitrogen uptake of *Pisum sativum* inoculated with *Rhizobium leguminosarum* *Indian J Microbiol*, **9** 93–99

Pareek, R P and Gaur, A C, 1970, Effect of DDT on symbiosis of *Rhizobium* sp with *Phaseolus aureus* *Pl Soil*, **33** 297–304

Kulkarni J H, Sardeshpande, J S and Bagyaraj, D J, 1974, Effect of four soil applied insecticides on symbiosis of *Rhizobium* sp with *Arachis hypogea* Linn *Pl Soil*, **40** 169–173

Use of Difolatan and Nitrofix R[1] Culture as a Seed Treatment on Groundnut and their Effect on Germination, Nodule Formation and Yield

V Agnihotrudu and S C Tripathi

Rallis India Limited Fertilizers and Pesticides Division, R and D Laboratories, Bangalore-560025

Difolatan (Captafol) as an effective seed treatment for controlling the root rot caused by *Macrophomina phaseoli* on groundnut under field conditions is reported by Shanmugam and Govindaswamy (1973) and alfa root disease of groundnut caused by *Aspergillus flavus* by Aujla *et al* (1975) Increase in germination as well as higher yields of kernel was noticed by these investigators by using Difolatan Jackson (1965) reported that Difolatan was one of the best amongst the fungicides tried for controlling the develoment of pod infecting organisms on groundnut Papavizas and Lewis (1975) reported captafol seed treatment having given effective control of *Fusarium solani* on bean The effect of Difolatan captafol Cis-N-(1, 1, 2, 2-tetrachloroethyl thio)-4-cyclohexene-1, 2-dicarboximide) seed dresser on root nodule formation in groundnut was studied in a field trial

Groundnut variety Sulamit (60 days old seed) received from Pondicherry and from Gujarat (140 day old seed) and S-203 were used in this trial Sulamit frequently gave poor germination because of infection caused by *Aspergillus* and

[1] Rallis Nitrofix R is the trade name for *Rhizobium* incorporated in lignite base produced by Rallis India Ltd, R and D Laboratories

Rhizoctonia sp. Before sowing the seeds were dry treated first with Difolatan 80W at 3 g/kg and pelleted with $CaCO_3$ and the pelleted seed was inoculated with Nitrofix R (*Rhizobium*) culture. Ten grams of inoculant was used per kg of groundnut seed. Germination and nodule counts were taken on 15th and 60th day after sowing respectively.

Variety S-203 recorded better germination than Sulamit. Difolatan treated plots recorded better germination than untreated plots especially in variety Sulamit where seed rot was more. Irrespective of varieties, all *Rhizobium* treated plots recorded more number of nodules than untreated. Sulamit recorded 3 times higher pod yield than S-203, whereas the latter had not shown any remarkable difference in yield between the treatments. Sulamit (new seed) had recorded more yield in a case where Difolatan was used. *Rhizobium* treatment in Sulamit (old seed) gave more yield than untreated control (Table I). Groundnut which

TABLE I

Effect of Difolatan and Nitrofix on groundnut

Treatment	Germination %	Av.No. of nodules/ plant	Yield in q/acre
Sulamit new seed+Difolatan (seed treatment)	95	52	32.0
Sulamit new seed+Difolatan+Nitrofix R (seed treatment)	71	41	22.7
Sulamit new seed+Nitrofix R (seed treatment)	54	17	21.4
Sulamit new seed (no treatment)	64	16	23.9
Sulamit old seed+Difolatan (seed treatment)	21	37.5	22.0
Sulamit old seed+Difolatan+Nitrofix R (seed treatment)	59	24.5	16.0
Sulamit old seed+Nitrofix R	16	19	33.1
Sulamit old seed (no treatment)	15	16	26.7
S-203+Difolatan (seed treatment)	90	19	8.8
S-203+Difolatan+Nitrofix R (seed treatment)	97	23	10.8
S-203+Nitrofix R (seed treatment)	95	28	10.4
S-203 (no treatment)	91	25	11.5

belongs to the cowpea cross inoculation group nodulates well in almost all the soils surveyed for nodulation so far (Gaur *et al.*, 1976). Groundnut also shows promiscuity towards *Rhizobium* for nodulation and even if the nodulation noticed in these trials was due to native *Rhizobium* strains, the results showed that *Rhizobium* was not adversely affected by Difolatan treatment.

The authors are thankful to Mr. K. S. Anantharaman, General Manager, Fertilizers and Pesticides Division, Rallis India Limited, for permission to publish this note.

References

Aujla, S. S., Chohan, J. S. and Kang, M. S., 1975, Field occurrence and chemical control of 'Aflaroot' disease of groundnut caused by *Aspergillus flavus* Link. ex Fries. *J. Res.* **12**: 52–55.

Gaur, Y. D., Sen, A. N. and Subba Rao, N. S., 1976, Problem regarding groundnut (*Arachis hypogaea* L.) inoculation in tropics with special reference to India. *INSA Bull.* No. **51**: 561–575.

Jackson, C. R., 1965, Laboratory evaluation of fungicides for control of some fungi found on peanuts. *Pl. Dis. Reptr.*, **49**: 928–931.

Papavizas, G. C. and Lewis, J. A., 1975, Effect of seed treatment with fungicides on bean roots. *Pl. Dis. Reptr.*, **50**: 24–28.

Shanmugam, N. and Govindswami, C. V., 1973, Control of *Macrophomina* root rot of groundnut. *Madras agric. J.*, **60**: 500–503.

A Note on the Effect of Fungicidal Seed Treatment on Rhizobial Inoculation of Peas (*Pisum sativum* L.)

H. R. Raghava Reddy, Gita Mahalingan and R. B. Patil

Department Agril. Microbiolgy, University of Agril. Sciences, GKVK, Banglore-560 024

Pea is susceptible to several fungal diseases such as downy mildew, leaf rust and damping off. The fungicides recommended for the control of these diseases are captan, Dithane-M45, Morestan and sulphur. Mukewar and Bhide (1969) have shown that fungicides did not affect the nodulation to any significant extent when used at normal doses in case of groundnut. Balaraman and Prasad (1973) and Sardeshpande *et al.* (1973) also reported the similar effects of fungicides on nodulation in groundnut.

The influence of fungicidal treatment with Captan and Dithane M-45, and rhizobial inoculation of peas on nodulation and nitrogen fixation is presented in this note.

Commercial grade captan (N-trichloro-methyl-thiotetrahydropthahimide) and Dithane M-45 (Mn Zn ethylene 1.2 bisdithiocarbamate) were employed. Two rhizobial inoculants UASB-101 and UAS composite (consisting of four local isolates of rhizobia and UASB-101) were used for seed treatment. The field trial was laid out in randomised block design with three replications in each treatment at G.K.V.K. farm of University of Agricultural Sciences, Bangalore. The plots of 3m × 4 m size were prepared. Basal dressing of 60 kg P_2O_5 and

45 kg K_2O per ha was given. Bounville variety of pea which does not require staking was selected and after seed treatment it was sown with a spacing of 30×8 cm. The seeds were first treated with fungicides and then with the *Rhizobium* control consisted of seed not treated with either the fungicide or *Rhizobium*. Observations on the number and dry weight of nodules and dry weight of plant tops were taken six weeks after sowing. Harvesting of green pods was done in three pickings starting from nineth week at weekly intervals.

Maximum nodultion was obtained with composite culture followed by UASB–101 in combination with captan, while the dry weight of nodules was more with composite culture and UASB–101 *plus* fungicide combinations. There was not much significant difference in dry weight of plate tops. Here again the composite culture proved to be superior, while UASB–101 gave lesser weight than the control (Table I). The yield data showed that there was a reduction in the yield when UASB–101 was used alone. Of the two fungicides used, Captan was more efficient as compared to Dithane M–45 as it increased the pod yield by 36 per cent in combintion with both UASB–101 and the composite, inoculants (Table II).

TABLE I

Effect of fungicidal seed treatment and rhizobial inoculation on nodulation of pisum sativum

Treatment	No. of nodules	Dry weight of nodules (mg)	Dry weight of plant tops (g)
Cotrol	88	139	3.493
UASB–101	85	112	2.810
UASB–101 + Captan	109	149	3.103
UASB–101 + Dithane	94	168	3.163
Composite	130	159	4.190
Composite + Captan	63	91	3.507
Composite + Dithane	65	106	3.430

TABLE II

Effect of fungicidal seed treatment and rhizobial inoculation on the pod yield of Psium sativum

Treatment	Pod yield (q/ha)	% increase over uninoculated control
Control	29.49	—
UASB 101	24.48	—
UASB–101 + Captan	40.26	36.52
UASB–101 + Dithane	35.27	19.59
Composite	32.27	9.42
Composite + Captan	40.18	36.24
Composite + Dithane	36.73	24.55

Nodule number was not related to the yield. The dry weight of plant tops was related to the yield. The present result are in conformity with the earlier reports of Mukewar and Bhide (1969), Balaraman and Prasad (1973) and Sardeshpande *et al.* (1973).

References

Balaraman, K. and Prasad, N. N., 1973, Influence of wet ceresan seed treatment on nitrogen fixation by *Rhizobium* in groundnut. *Pesticides.* 7 (**1**) : 11

Mukewar, P. M. and Bhide, V. P. 1969, Effect of seed treatment with fungicides and antibiotic aureofungin on the efficacy of nodulation by *Rhizobium* strain of groundnut. *Hindustan Antiboit. Bull,* **11** : 172–176.

Sardeshpande, J. S., Kulkarni, J. H. and Bagyaraj, D. J., 1973, Influence of fungicidal seed treatment on the nodulation of groundnut inoculated with *Rhizobium*. *Pesticides*, **7 (11)** : 11–13.

Effect of Lasso and Tok E-25 on *Rhizobium*-Greengram Symbiosis

G. Jeganathan, S. Jayachandran and C. V. Govindaswamy

Department of Biology, Agricultural College and Research Institute, Madurai–625 104 Tamil Nadu

Abstract

Of the two herbicides Lasso and Tok E-25 tested at three different concentrations only Lasso at 0.5 kg ai/ha stimulated rhizobial population, N_2 content of soil and yield of pods. All the other treatments in general exerted inhibitory effect on all the above mentioned characters.

Agricultural soils are the ultimate recipient and depository of most applied foliar and soil pesticides and the pesticides thus used for plant protection influence both microbial and plant proclivities (Kearney *et al.*, 1967 ; Morris and Penney, 1971 ; Sahrawat, 1975). Notwithstanding the abundance of information on the establishment nodule formation and N_2 fixation of different rhizobia (Vincent, 1974) the effect of different herbicides on various biological processes of 'native' and 'introduced' rhizobia is poorly understood. More often the herbicides proved to be deleterious to the soil microorganisms and particularly to the root nodule bacteria (Fletcher *et al.*, 1957 ; Lakshmikumari

et al , 1975) The effect of two herbicides, Lasso and Tok E-25 at different concentrations on *Rhizobium* greengram symbiosis is reported in this paper

Material and Methods

Mud pots of 45 cm diameter at the top and 45 cm height were filled with 10 kg of air dried red sandy loam soil Two herbicides *viz* , Lasso and Tok E-25 were applied in the form of a fine mist at the rate of 0 5, 1 0 and 1 5 kg ai/ha The soil moisture was kept at approximately 60 per cent water holding capacity with tap water Six CO2 greengram seeds either treated or untreated with appropriate *Rhizobium* were sown in each pot On the 9th day they were thinned to three plants per pot Three replicates were kept in each treatment.

The most probable number (MPN) of *Rhizobium* from each treatment on 48th day was estimated from the serially diluted suspension of rhizosphere soils by plate dilution count method (Clark, 1965) All plants in different treatments were removed when they were 48 days old taking care to retain maximum number of nodules intact with the roots The total number of nodules in each plant was counted and recorded The dry weight of nodules and plants were determined after oven drying at 101°C for 24 hrs The yield of pods in each plant under different treatments were also recorded Total nitrogen of plant and soil was determined by micro-kjeldahl (Bremner, 1960) and macro-kjeldahl (Piper, 1950) methods respectively

Results and Discussion

The data on the effect of two different herbicides at varying concentrations on nodulation and other characters of greengram treated with rhizobium ('introduced') are set out in Table I All the treatments except Lasso at 0 5 and 1 0 kg

Table I

Effect of herbicides on nodulation and other plant characters of greengram treated with Rhizobium '*introduced*'

Treatment	Concentration (kg/ai/ha)	*Rhizobium* population ($x10^5$/g of soil	No of nodules/plant	Wt of nodules (mg/plant)	Dry wt of plants (g/plant)	Nitrogen content of plants (%)	Nitrogen content of soil (%)	No of pods/plant
Lasso	0 5	14 05	18 00	216 67	2 24	2 18	0 95	13 66
Lasso	1 0	12 93	15 33	171 67	1 98	2 07	0 73	11 33
Lasso	1 5	12 37	14 33	150 00	1 61	1 95	0 71	10 66
Tok E-25	0 5	12 66	11 67	149 33	2 10	2 40	0 87	11 00
Tok E-25	1 0	11 88	11 00	150 00	1 84	2 02	0 84	9 66
Tok E-25	1 5	11 00	9 00	77 33	1 68	2 00	0 16	10 00
Control	—	13 27	14 67	255 00	2 13	2 19	0 91	12 33
C D at 5%	—	0 205	0 65	6 25	0 08	0 04	0 01	1 15

ai/ha inhibited the number of nodules formed The weight of nodules was found to be less than that of the control in all the treatments Excepting Lasso at 0 5 kg ai/ha all other treatments inhibited dry matter production, N_2 content of soil and pod yield However, Tok E-25 at 0 5 kg ai/ha exerted a stimulatory effect in respect of N_2 content of plants only

The effect of two herbicides at different concentrations on nodulation and other plant characters of greengram untreated with *Rhizobium* ('native') is given in Table II All the herbicides at different concentrations tested significantly

TABLE II

Effect of herbicides on nodulation and other plant characters of greengram untreated with Rhizobium *'native'*

Treatment	Concentration (kg/ai/ha)	No of nodules/ plant	Wt of nodules (mg/plant)	Dry wt of plants (g/plant)	Nitrogen content of plants (%)	Nitrogen content of soil (%)	No of pods/ plant
Lasso	0 5	4 00	111 67	2 07	1 81	0 72	10 66
Lasso	1 0	3 00	48 67	1 95	1 82	0 55	10 00
Lasso	1 5	1 00	15 00	1 50	1 76	0 51	9 66
Tok E-25	0 5	4 67	108 33	2 08	2 02	0 72	10 33
Tok E-25	1 0	2 00	46 00	1 84	1 96	0 62	10 00
Tok E-25	1 5	1 00	12 00	1 62	1 90	0 57	9 66
Control	—	6 33	107 00	2 10	1 85	0 75	10 66
C D at 5%	—	0 65	6 25	0 08	0 04	0 01	1 15

inhibited nodulation and N_2 content of soil However, Lasso and Tok E-25 at 0 5 kg ai/ha neither stimulated nor inhibited the weight of nodules, dry matter production and pod yield and were on par with control Tok E-25 at all the three concentrations increased the N_2 content of plants

Most often the effectiveness of the herbicides applied to the soil are governed by many instrinsic factors *viz* , concentration of the herbicides, time of application, agro-climatic conditions *etc* (Alexander, 1961) Some of the herbicides that give very good control of weeds when applied in legumes in addition to exerting hazardous effects on the rhizobia, often prove to be phytotoxic either on the standing crop or on the succeeding crop or on both However Rankov *et al* (1966) and Yogeswara Rao *et al* (1973) reported that herbicides if applied at lower concentrations exhibit 'auxin-like' effects both on the *Rhizobium* as well as on legume plant

Differential stimulation or inhibition of *Rhizobium* to various herbicides is directly correlated with their sensitivity or resistance to respond to the herbicides in question (Kearney *et al* 1967) Lasso alone at 0 5 kg ai/ha stimulated the *Rhizobium* population in soil (Table I) Lakshmikumari *et al* , (1974) and Tewfik *et al* 1975 also reported that application of Dalapan and DBCB at lower

concentrations stimulated *Rhizobium meliloti* and *leguminosarum*, respectively. The stimulation of nodule formation, increase in the dry matter production, enhanced N_2 content of soil and increase in pod yield observed in the present study when Lasso was applied at 0.5 kg ai/ha is in conformity with the findings of Rethinam *et al.*, (1974). With respect to Tok E-25 the results from the present studies corroborated the findings of Rethinam *et al.*, (1974).

More often than not the 'native' rhizobia present in the soil prove to be less efficieient in fixing atmosphere N_2 (Brockwell and Dudman, 1968) and more susceptible to different herbicides (Avrov, 1966) and this might explain the poor performance of the 'native' rhizobia in the presence of different herbicides on nodulation and other parameters of greengrams.

The production of indoleacetic acid (IAA) by *Rhizobium* both *in vitro* and *in vivo* from an exogenous tryptophan has been unequivocally proven by many workers (Rovira and Mc Dougall, 1967). The interaction of Lasso (0.5 kg ai/ha) with the inoculated *Rhizobium* has significantly yielded better nodulation, more dry matter production and N_2 content of soils than the interaction of Lasso (0.5 kg ai/ha) with 'native' *Rhizobium*. As suggested by Bovey (1971) the observed differences might be due to 'hormone-like' activities of Lasso at lower concentrations on the 'introduced Rhizobium'. The production of 'hormone-like' substances in the 'introduced' rhizobia may perhaps be more than in the 'native' rhizobia which in turn is reflected on rhizobial population and other plant characters. However, further work on the qualitative and quantative differences in the growth promoting substances produced due to interaction of Lasso with the 'introduced' and 'native' *Rhizobium* is essential.

References

Alexander, M., 1961, *Introduction to Soil Microbiology*, John Willey and Sons, Inc., New York p. 472.

Avrov, O. E., 1966, Effect of herbicides on nodule bacteria and nodule formation in legumes. *Dokl. Vses. Akad. Scl. Khuz. Nauk.*, **3**: 16–19.

Bovey, R. W., 1971, Hormone - like herbicides in weed control. *Econ. Bot.*, **25**: 288–400.

Bremner, J. M., 1960, Determination of nitrogen in soil by Kjeldahl method. *J. Ag. Sci.*, **55**: 11–33.

Brockwell, J. and Dudman, W. F., 1968, Ecological studies of root nodule bacteria introduced into field environments. II Initial competition between seed inocula in the nodulation of *Trifolium subterraneum* seedlings. *Aust. J. agri. Res.* **19**: 749–757.

Clark, F. E., 1965, In: *Methods of soil analysis* part II (Eds.) C. A. Black, D. D. Evans, J. L. White., L. E. Ensminger and F. E. Clark, Amer. Soc. of Agron., Inc., Madison, Wisconsin, U.S.A. pp. 1487.

FLETCHER, W. W., DICKENSON, P. G., FORREST, J. D. AND RAYMOND, R. C., 1957, The effect of soil applications of certain substituted phenoxy acetic and phenoxy butyric acids on the growth and nodulation of *Trifolium repens sylvestre*. *Phyton.*, **9**: 41–46.

KEARNEY, P. C., KAUFMAN, D. D. AND ALEXANDER, M., 1967, Biochemistry of herbicide decomposition in soils. pp: 318–342. In: *Soil Biochemistry* (Ed.) A. D. McLaren and G. H. Peterson, Marcel Dekker, Inc., New York.

LAKSHMIKUMARI, M., BISWAS, A., VIJAYALAKSHMI, K., NARAYANA, H. S. AND SUBBA RAO, N. S., 1975, Effect of certain water soluble herbicides on legume-*Rhizobium* symbiosis. *Proc. Ind. Natl. Sci. Acad.*, **40** B: 528–534.

MORRIS, R. F. AND PENNEY, B. G., 1971, Persistance of Linuron residues in soils at Kelligrews, Newfoundland. *Can. J. pl. Sci*, **51**: 242–245.

PIPER, C. S., 1950, *Soil and plant Analysis*. Inter Science Publishers, New York.

RANKOV, V., ELANKOV, E., AND SURELKOV, P., 1966, Effects of some herbicides on development of nitrogen fixing bacteria *Agrokhimiya*, **4**: 115–120.

RETHINAM, P., KESAVAN, R. AND PURUSHOTHAMAN, D., 1974, Effect of application of pre-emergence herbicides on nodulation of greengram (*Phaseolus aureus* Roxburg). *Indian J. Microbiol.*, **14**: 149–151.

ROVIRA, A. D. AND MCDOVGALL, M., 1967, Microbial and biochemical aspects of the rhizorsphere. In: *Soil Biochemistry*. (Eds.) A. D. McLaren and G. F. Person Marcel Dekker, Inc., New York pp. 417–463.

SAHRAWAT, K. L., 1975, Effect of pesticides on nitrogen fixation. *Pesticides*, **9**: 21–23.

TEWFIK, M. S., EMBADI, M. S. AND HAMDI, Y. A., 1975, Efficiency of *Rhizobium leguminosarum* as affected by certain herbicides and nematicides. *Soil Fertilizers*, **39**: 424.

VINCENT, J. M., 1974, Root nodule symbiosis with *Rhizobium* In: *The Biology of nitrogen fixation* (Ed.), A. Quispel North-Holland Publishing Company, Amsterdam. pp. 265-341.

YOGESWARA RAO, G. H., SANKARA REDDY AND VENKATARAMA REDDY M. V., 1973, Weed control in greengram (*Phaseolus aureus* Roxb) under rainfed conditions. Third All India Weed Control Seminar, Hissar, 1973.

Effect of Certain Pesticides on the Establishment of Rhizobia in the Spermosphere and Rhizosphere Regions of Leguminous Crops

K Singaravadivel and N N Prasad
Microbiology Division, Faculty of Agriculture, Annamalai University, Annamalainagar-608 101, Tamil Nadu

Abstract

The effect of certain fungicides, insecticides and herbicides on the establishment of rhizobia in the spermosphere and rhizosphere regions of groundnut (*Arachis hypogaea* L), blackgram (*Phaseolus mungo* L) and greengram (*P aureus* Roxb) was studied The organophosphorus pesticides, disyston and phorate did not reduce the populations of rhizobia in spermosphere and rhizosphere regions of the three crop species On the other hand, the fungicides, Brassicol and Benlate and the herbicides, TOK-E 25 and Matchet have slightly reduced the population levels in the spermosphere and rhizosphere regions of the three crop species However, such a reduction in the rhizobial colonization due to fungicides and herbicides was relatively less marked in the spermosphere and rhizosphere regions of groundnut compared to those of either blackgram or greengram

The colonization and multiplication of *Rhizobium* in the spermosphere and rhizosphere regions are of considerable importance in bacterial inoculation The effect of plant protection measures on the efficiency of legume–*Rhizobium* symbiosis in fixing nitrogen has been studied by several workers (Leonard, 1936, Ebbels, 1967, Pareek and Gaur, 1969, Balaraman and Prasad, 1973, Oblisami *et al*, 1973) The effects of certain fungicides, insecticides and herbicides on the survival and multiplication of rhizobia in the spermosphere and rhizosphere of groundnut (*Arachis hypogaea* L), blackgram (*Phaleosus mungo* L) and greengram (*P aureus* Ro × b) are reported in this paper

Materials and Methods

The seeds of groundnut (TMV 4), blackgram (Co 2) and greengram (Anjugam) were surface sterilized with 0 1 per cent mercuric chloride solution and washed with several changes of sterile water They were then treated for 12 hr with respective rhizobial suspension The treated seeds were dried in shade on filter paper The seeds were sown in sterile soil incorporated with the following pesticides

Fungicides: Brassicol 60WP (Pentachloronitrobenzene), Benlate 50 WP (methyl–1–(Butyl carbamoyl)–2–Benzimidazole carbamate).

Insecticides: Disyston 5G (0,0–diethyl–0–quinoxalinyl (2)–thionophosphate, Phorate 100 (0,0–diethyl–5–(ethyl thio methyl phosphorodithioate)

Herbicides : Matchet–(Butuchloro) = 3 – chloro-2'6' – diethyl–N – (Butoxy–methyl)–acetamide. TOK–E. 25–2,4–Dichlorophenyl 4–nitrophenyl ether.

The pesticides were applied at their respective field rates. Brassicol, Benlate and Matchet were applied at 11 ppm and disyston, phorate and TOK–E–25 at 12.9 and 7 ppm respectively.

The spermosphere soil samples were collected at the end of 24 hrs and 72 hrs after sowing. Rhizosphere soil samples were collected at the end of 7th day and 9th day after sowing. The *Rhizobium* populations were estimated by serial dilution method employing congo red agar medium (Allen, 1953).

Results and Discussion

The results on the effect of certain pesticides on the multiplication of *Rhizobium* in the spermosphere and rhizosphere regions of groundnut, blackgram and greengram are presented in Tables I to III.

Table I

Effect of certain pesticides on the establishment and multiplication of rhizobia in the spermosphere and rhizosphere regions of groundnut*

Treatment	Spermosphere		Rhizosphere	
	1st day	3rd day	7th day	9th day
Control (*Rhizobium* alone)	3.60	4.80	7.20	7.84
Brassicol + *Rhizobium*	3.50	4.65	6.80	7.20
Benlate + *Rhizobium*	3.32	4.64	6.90	6.80
Disyston + *Rhizobium*	3.50	4.90	7.25	7.00
Phorate + *Rhizobium*	3.80	4.78	7.10	6.60
Matchet + *Rhizobium*	3.40	3.48	7.10	7.20
Tok-E-25 + *Rhizobium*	2.40	3.12	6.00	6.72

*Population in 10^6/g of moisture free soil.

In all the three crop species the seed inoculated *Rhizobium* sp. multiplied and colonized in the spermosphere and rhizosphere regions. The organophosphorus pesticides disyston and phorate treatments, did not significantly reduce the colonization of inoculated rhizobia in spermosphere and rhizosphere regions of the three crop species. Moreover, phorate treatment slightly increased the colonization of inoculated rhizobia in blackgram and greengram. Similar observations were reported by Ramani (1974), when groundnut seeds were

TABLE II

Effect of certain pesticides on the establishment and multiplication of rhizobia in the spermosphere and rhizosphere regions of blackgram*

Treatment	Spermosphere		Rhizosphere	
	1st day	3rd day	7th day	9th day
Control (*Rhizobium* alone)	2 00	8 82	10 52	9 05
Brassicol+(*Rhizobium*)	1 78	8 71	8 28	7 99
Benlate+(*Rhizobium*)	2 00	7 19	8 25	8 53
Disyston+(*Rhizobium*)	1 89	8 55	10 86	9 63
Phorate+(*Rhizobium*)	1 58	9 20	11 05	9 81
Matchet+(*Rhizobium*)	1 05	6 66	8 95	8 07
Tok-E-25+(*Rhizobium*)	1 40	6 08	8 07	7 56

*Population 10^6/g of mosture free soil

TABLE III

Effect of certain pesticides on the establishment and multiplication of rhizobia in the spermosphere and rhizosphere regions of greengram*

Treatment	Spermosphere		Rhizosphere	
	1st day	3rd day	7th day	9th day
Control (*Rhizobium* alone)	8 50	13 05	15 08	9 57
Brassicol+(*Rhizobium*)	8 08	11 50	13 82	8 05
Benlate+(*Rhizobium*)	8 92	11 85	8 99	7 58
Disyston+(*Rhizobium*)	8 60	13 28	15 32	8 27
Phorate+(*Rhizobium*)	8 48	15 02	16 55	9 60
Matchat+(*Rhizobium*)	6 92	9 52	10 23	7 50
Tok-E-25+(*Rhizobium*)	5 69	9 23	9 55	6 92

*Population in 10^6/g of moisture free soil

treated with *Rhizobium plus* Disyston or D D T Oblisami *et al* (1974) reported that the nodulation, root length and dry weight of the groundnut plant was not affected by the application of four granular insecticides *viz*, Dasanite, Ekalux, Furadan and Solvirex

In the present study the Brassicol treatment did not markedly reduce the colonization of inoculated rhizobia in spermosphere and rhizosphere regions of the three crop species Benlate and two herbicides, Matchet and TOK-E 25, were found to significantly reduce the colonization of rhizobia in the spermosphere and rhizosphere regions of the three crop species Vrany (1975) reported that Benlate application brought about a fall in the bacterial population in the rhizosphere region However, significant reduction was not found with Benlate treatment in groundnut Balaraman and Prasad (1972) reported that groundnut seeds when treated with *Rhizobium plus* wet ceresan or captan or thiram did

not affect, the multiplication of rhizobia in the rhizosphere region of young plant Similar finding was also reported by Ramani (1974) when groundnut seeds were treated with *Rhizobium plus* Brassicol or wet ceresan or Lasso or TOK-E 25

References

Balaraman, K and Prasad, N N , 1972, Compatibility of certain seed dressing chemicals with *Rhizobium* *Curr Sci* , **41** 344

Balaraman, K and Prasad, N N , 1973, Influence of wet ceresan seed treatment on nitrogen fixation by *Rhizobium* in groundnut *Pesticides*, 7 11

Ebbls, D L , 1967, Effect of soil fumigants on *Fusarium* with and nodulation of peas (*Pisum sativum* L) *Ann Appl Biol* , **60** 391–398

Leonard, L J , 1936, Pea rhizobia and red copper oxide *Proc Soil Sci Soc Amer* , **1** 219

Oblisami, G , Balaraman, K , Venkataramanan C V and Rangaswami, G , 1973, Effect of three granular insecticides on the growth of *Rhizobium* from redgram. *Madras agric J* , **60** 462–464

Oblisami, G , Balaraman, K , Natarajan, T and Kulandaivelu, R , 1974, Effect of granular insecticides on growth and nodulation of groundnut *XV Annual Conf Assocn Microbiologists of India*, (*Abs*) p 86

Pareek, R P and Gaur, A C , 1970, Effect of Dichloro-diphenyl-trichloro-ether (D D T) on nodulation, growth, yield and nitrogen uptake of *Pisum sativum* inoculated with *Rhizobium leguminosarum* *Ind J Microbiol* , **9** 93–100

Ramani, C E , 1974, *Studies on the effect of certain fungicides, insecticides and boron and molybdenum on the symbiotic nitrogen fixation of Rhizobium* sp *in groundnut* M Sc (Ag) Thesis, Annamalai University, Annamalainagar, India

Effect of Yellow Mosaic Virus Disease on Nodulation and Yield of Horsegram

P Muthukrishnan, M Rangarajan, P Padmanabhan and P V Marappan
Agricultural College and Research Institute, Tamil Nadu Agricultural University
Madurai

Abstract

Eleven varieties of horsegram (*Dolichos biflorus* L) were examined for the incidence of yellow mosaic and its effect on growth nodulation and yield All the eleven varieties of horsegram were susceptible to the disease having lot of variation in the degree of susceptibility Varieties such as BGM, Co 1, HPK 1 were less susceptible having infection up to 10 per cent, whereas two varieties *viz*, HPK 4 and HPK 2 were highly susceptible having the yellow mosaic infection of more than 25 per cent The virus disease reduced the dry weight of the plant by 9 to 11 per cent, total number of nodules by 44 4 to 87 6 per cent and grain yield by 16 to 21 6 per cent in highly susceptible varieties

Incidence of yellow mosaic virus disease in different pulse crops such as greengram, blackgram and horsegram is reported to be widely prevalent in Tamil Nadu (Nariani, 1960, Williams *et al*, 1968) It affects horsegram (*Dolichos biflorus)* and seriously reduces the growth and yield of the plants (Nariani, 1960) Nagaich and Singh (1972) reported that sesbania mosaic reduced the growth, nodulation and yield of *Sesbania* and French-beans Nene *et al* (1972) reported that the yellow mosaic disease caused serious losses to blackgram and greengram thus reducing the grain yield of the crop by about 90 per cent under natural conditions Sivaprakasam *et al* (1974) observed that all the varieties of greengram and blackgram were susceptible to the yellow mosaic and the growth was stunted in legumes In horsegram the yellow mosaic virus disease appears on young leaves in the form of mild scattered yellow specks or spots. Irregular yellow and green patches are found alternating each other The green areas are slightly raised and the leaves show slight reduction in size Irregular leaf lamina is found and the plant is greatly dwarfed due to reduction in leaf size

The effect of the yellow mosaic disease under natural conditions on growth, nodulation and yield of eleven varieties of horsegram is reported in the present paper

Material and Methods

A field trial was laid out in *rabi* season of 1976–77 under irrigated conditions in a randomised block design with four replications having plot size of 5 × 2 5 m The trial comprised of eleven treatments in which the eleven different varieties of horsegram such as BGM, Co 1, HPK 1, HPK 3, HPK 4, HPK 5, Hebbal 1, Hebbal 2, Hebbal 3, HG 76, HG 93, were grown The plants were allowed at the rate of one plant per hole with spacing of 30 × 20 cm The crop was cultivated by adopting all the recommended package of practices The incidence of yellow mosaic was first observed on 35th day after sowing and later, the observations were recorded on 65th day The number of infected plants and healthy plants in a plot were first counted and then the percentage of infection was assessed Healthy and diseased plant samples were collected from each plot and observations were recorded to assess the effects on growth and nodulation Dry weight of the whole plant was taken as a parameter to assess the effect on growth Total number of nodules per plant and the dry weight of the nodular tissue were considered as parameters to assess the effect on nodulation The grains extracted from the pods harvested regularly, were finally pooled and weighed and the grain yield per plant was estimated

Table I

Effect of yellow mosaic disease on nodulation and yield of horsegram

Horsegram varieties	Yellow mosaic infection (%)	Dry wt of plant (g)		Total nodules per plant (No)		Dry wt of nodular tissue (g)		Grain yield/ plant (g)	
		Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Less Susceptible (0–10%)									
BGM	6 8	23 1	22 9	35 8	35 8	0 4249	0 4113	12 1	12 1
Co 1	7 0	7 7	7 5	16 5	15 8	0 1695	0 1672	10 1	10 0
HPK 1	8 4	4 8	4 8	38 8	38 8	0 4373	0 4369	4 6	4 5
Moderately Susceptible (10–25%)									
HG 76	10 3	14 4	14 4	63 5	63 0	0 6815	0 6789	5 1	5 1
HG 93	10 9	18 6	18 6	30 8	30 5	0 3636	0 3371	5 6	5 5
HPK 5	14 3	5 0	4 9	20 3	20 0	0 2517	0 2518	4 1	4 0
Hebbal 1	19 7	5 3	5 3	24 0	23 3	0 2635	0 2623	12 2	12 0
Hebbal 3	21 0	5 4	5 3	18 3	18 3	0 2291	0 2260	5-9	5 8
Hebbal 2	23 7	8 9	8 4	27 0	26 8	0 3267	0 3263	4 7	4 6
Highly Susceptible (Above 25 %)									
HPK 4	25 0	8 3	7 6	32 0	18 0	0 3735	0 2494	6 9	5 5
HPK 2	28 0	6 1	5 4	36 0	20 0	0 3410	0 2495	8 8	9 9
S E	1 92	0 71	0 71	1 21	1 01	0 018	0 013	0 034	0 035
CD at 5%	5 54	2 06	2 05	3 48	2 91	0 051	0 038	0 100	0 100

Results and Discussion

All the eleven varieties of horsegram were susceptible to yellow mosaic disease However, the horsegram varieties such as BGM, Co 1 and HPK 1 were comparatively less susceptible having the infection up to 10 per cent The varieties such as HG 76, HG 93, HPK 5, Hebbal 1, HPK 3 and Hebbal. 2 were moderately susceptible having the infection ranging from 10 to 25 per cent But the varieties such as HPK 4 and HPK 2 were highly susceptible showing the infection above 25 per cent It was found that the dry weight of the whole plant, total number of nodules per plant, dry weight of nodular tissue and the grain yield per plant did not vary very much between the diseased and healthy plants of the less susceptible and moderately susceptible varieties But varieties of highly susceptible group, such as HPK 4 and HPK 2 were affected very much in growth, nodulation and yield (Table I) In the case of HPK 4 the reductions in growth and nodulation were 9 per cent and 87 6 per cent, respectively while in case of HPK 2 reductions in growth and nodulation were 11 per cent and 44 4 per cent, respectively Similarly, there was significant reduction in the dry weight of nodular tissue Yield of grain was also reduced by 16 per cent in HPK 4 and by 21 6 per cent in HPK 2 These observations are in conformity with the findings of Nagaich and Singh (1972)

References

Nagaich, B B and Singh, R A , 1972, Effect of sesbania mosaic on growth and nodulation of *Sesbania* and French-bean XIII Ann Conf AMI, Ludhiana, P 3

Nariani, T K , 1960, Yellow mosaic of mung (*Phaseolus aureus* Roxb) *Indian Phytopath* , **13** : 25-29

Nene Y L , Srivastava, S K and Naresh, J S , 1972, Evaluation of blackgram (*Phaseolus mungo* Roxb) and greengram (*P aureus* Roxb) varieties and germplasms for resistance to yellow mosaic virus of greengram *Indian J Agric Sci* , **42** 251-254

Sivaprakasam, K , Pillayar Samy, K , Rajamani, and Soumini Rajagopalan, C K , 1974, Evaluation of blackgram *(Phaseolus mungo L)* and greengram (*P aureus* Roxb) Varieties for resistance to yellow mosaic virus of greengram *Madras agric J* **61** 1021-1022

Williams F J , Grewal, J S and Amin, K S , 1968, Serious and new disease of pulse crops in India in 1966 *Pl Dis Reptr* **52** 300-304

Effect of Different Insecticides on *Azotobacter* Isolates Obtained from the Rhizsophere of C_4 Plants

S. JAYACHANDRAN, G. JEGANATHAN AND C. V. GOVINDASWAMY
Department of Biology, Agricultural College and Research Institute
Maduri-625 104, Tamil Nadu

ABSTRACT

The effect of different technical grade insecticides *viz.*, lindane, thimet and carbofuran at 1 and 10 μg/ml concentrations on the growth, polysaccharide production, pigmentation and nitrogen fixation of three isolates of *Azotobacter* obtained from *Brachiaria mutica* (Azo. 1), *Mollugo* sp. (Azo. 2) and *Portulaca oleraceae* (Azo. 3) was investigated. At 1 μg/ml concentration all the insecticide stimulated the growth of the three isolates. However at 10 μg/ml concentration while all the three insecticides stimulated the growth of Azo. 2, thimet and carbofuran stimulated the growth of Azo. 1. All the insecticides at 1 and 10 μg/ml concentration inhibited the alkali-stable polysaccharide production ; all the insecticides at 10 μg/ml exerted inhibilitory effect on water soluble polysaccharide production. Although at 1 μg/ml concentration all the insecticides and at 10 μg/ml concentration of lindane stimulated pigment production, in all the isolates tested. None of the insecticides at 1 μg/ml concentration exhibited any marked difference with respect to nitrogen fixation. However, all the insecticides at 10 μg/ml concentration inhibited nitrogen fixation of all the isolates.

ALTHOUGH it has become axiomatic that the plants fix CO_2 by Calvin cycle, recent studies have revealed yet another pathway–C_4 dicarboxylic acid pathway in some tropical plants that accumulate more carbohydrates (Zelitch, 1971 ; Bhagwat and Sane, 1976). Dobereiner *et al.* (1972a, 1972b) reported that *Azotobacter* associated with such C_4 plants are more efficient in fixing molecular nitrogen presumably by utilising the 'ready-made' carbohydrate meterials provided by the C_4 plants in the rhizosphere region. Soil, ultimately being the repository of various pesticides, detergents etc., the microorganisms prevalent in the soil are tremendously influenced by such chemicals (Van Schreven *et al.*, 1970 ; Sreenivasalu and Rangaswami, 1973) and more so the heterotrophic, free living nitrogen fixing bacteria – *Azotobacter* (MacKenzie and MacRae 1972 ; Sahrawat, 1975). The effect of some insecticides and the activities of *Azotobacter* isolates obtained from different C_4 plants are reported in this paper.

MATERIAL AND METHODS

Organism : From the rhizosphere of three C_4 plants *viz.*, *Brachiaria mutica*, *Mollugo* sp. and *Portulaca oleraceae*, *Azotobacter* isolates Azo. 1, Azo 2·

and Azo 3, respectively were obtained following standard microbiological techniques (Pramer and Schmidt, 1966)

Growth studies The various insecticides *viz*, lindane (gamma BHC 1,2,3, 4,5,6, hexachloro cyclohexane), thimet [0,0-diethyl-S-(ethylthiomethyl) phosphorothiolothionate] and carbofuran (2,3,-dihydro-2-2-dimethyl-7 benzo furanyl methylcarbomate) at 1 and 10 μg/ml concentration were incorporated into Waksman 77 medium and dispersed in 100 ml quantities into 250 m Erlenmeyer flasks One ml of *Azotobacter* isolates in log phase of growth (ca 7×10^9 cells/ml) was inoculated and incubated on a rotary shaker 72 hr at $28 \pm 2°C$ The growth of the organism was assessed by determining the biomass

Estimation of polysaccharides Cells were harvested by centrifugation at $3000 \times g$ for 15 min and the alkali-stable polysaccharides (ASP) were extracted following the method of Hassid and Abraham (1957) and quantified using anthrone method (Selewski *et al*, 1974) Water-soluble polysaccharides (WSP) were estimated by a modified procedure of Damery and Alexander (1969) To 100 ml of cell-free centrifugate 200 ml of acetone were added and after 2 hr at room temperature ($28 \pm 2°C$) the crude polysaccharide was filtered throgh Whatman No 42 filter paper and the residue was dried at 70°C to constant weight

Pigment production From the cells harvested after centrifugation at $3000 \times g$, pigments were extracted and estimated by boiling the cells in 5 ml of 1l M KOH for 1 hr and adjusting the pH to 3 0 by the add tion of 1 N HCl The volume was made up to 20 ml and the optical density measured at 600 nm

Nitrogen estimation The total nitrogen in the broth was estimated by micro Kjeldahl method (Bremner, 1960)

Results and Discussion

All the insecticides at 1 μg/ml concentration promoted the growth of the three isolates of *Azotobacter* However at 10 μg/ml concentration while all the insecticides stimulated the growth of Azo 2, thimet and carbofuran stimulated the growth of Azo 1 also (Table I) All the insecticides at 1 μg/ml concentration inhibited the ASP production but not the WSP production However at 10 μg/ml concentration all the insecticides inhibited both the ASP and WSP production in all the isolates (Table II)

With regard to the influence of different insecticides on pigment production (Table III) all the insecticides at 1 μg/ml concentration stimulated pigment production in the three isolates However at 10 μg/ml concentration while lindane stimulated pigment production in all the isolates, thimet stimulated pigment production in Azo 1 alone The effect of different insecticides on

TABLE I

Effect of different insecticides on the growth of Azotobacter *isolates*
(Expressed as biomass mg/100 ml)

Isolate	Control	Lindane (μg/ml)		Thimet (μg/ml)		Carbofuran (μg/ml)	
		1	10	1	10	1	10
Azo 1	515	780	465	660	540	800	620
Azo 2	860	1060	920	910	880	935	875
Azo 3	765	905	715	785	655	960	745

TABLE II

Effect of different insecticides on the production of polysaccharides by different Azotobacter *isolates*

Isolate	Water soluble polysaccharides (mg/100 ml)							Alkali stable polysaccharides (mg/g dry weight)						
	Control	Lindane (μg/ml)		Thimet (μg/ml)		Carbofuran (μg/ml)		Control	Lindane (μg/ml)		Thimet (μg/ml)		Carbofuran (μg/ml)	
		1	10	1	10	1	10		1	10	1	10	1	10
Azo 1	1825	1830	1710	1855	1725	1825	1805	125	120	105	115	95	110	85
Azo 2	1915	1950	1805	1930	1860	1920	1825	110	100	85	105	75	100	80
Azo 3	1405	1505	1300	1480	1305	1420	1365	105	90	65	95	60	95	75

TABLE III

Effect of different insecticides on pigment production by Azotobacter *isolates*
(Expressed as pigment produced in OD/g dry weight)

Isolate	Control	Lindane (μg/ml)		Thimet (μg/ml)		Carbofuran (μg/ml)	
		1	10	1	10	1	10
Azo 1	4 05	4 25	4 15	4 20	4 15	4 15	4 05
Azo 2	3 75	3 95	3 80	3 90	2 75	4 15	2 25
Azo 3	5 15	5 20	5 20	5 25	3 25	5 00	3 10

nitrogen fixation of the three isolates is quite revealing (Table IV) in that none of the insecticides at 1 μg/ml concentration exhibited any marked difference in their nitrogen fixing capacity However, at 10 μg/ml concentration marked inhibition of nitrogen fixation was observed

TABLE IV

Effect of different insecticides on nitrogen fixation by Azotobacter *isolates*
(Expressed as mg of N_2 fixed/g of mannite)

Isolate	Control	Lindane (μg/ml)		Thimet (μg/ml)		Carbofuran (μg/ml)	
		1	10	1	10	1	10
Azo 1	24 0	24 0	20 5	23 5	20 5	23 5	20 5
Azo 2	23 5	24 0	20 0	23 0	19 0	23 0	20 0
Azo 3	25 0	25 0	23 0	24 5	20 0	24 5	22 5

Sensitivity of *Azotobacter* to various groups of insecticides has been reported by many workers (Gaur and Misra, 1970, MacRae and Celo, 1974 Mallikarjunaiah and Bhide, 1975) The differential inhibition or stimulation of growth of three isolates in the presence of the insecticides at the concentrations tested could be attributed either to the time lag in the adaptation of bacteria (Sahrawat, 1975) or to the degradation and utilization of the insecticides respectively (Kearney *et al*, 1967) However further investigations on the enzymatic analysis and the nature of various intermediates of pesticide degradation are warranted

The production of polysaccharides is an important character of aerobic nitrogen fixing *Azotobacter* At 1 μg/ml concentration while all the insecticides stimulated the WSP production, the production of ASP was inhibited Sutherland (1972) reported that the quantity and quality of the polysaccharides synthesised are governed by many factors *viz*, nutritional conditions, presence of certain trace elements, genetic character of the strains etc Thus the differences in the quantity of ASP and WSP produced by the three isolates in the presence of different insecticides is probably due to the change in the

physiology of the organisms brought about by the insecticides The degree of inhibition or stimulation of growth by different insecticides at various concentrations does not bear any correlation on the production of ASP or WSP in all the isolates and this is in conformity with the findings of Jayachandran *et al* (1977) who reported that growth and polysaccharide production were not related with each other in *A chroococcum*

The fact that polysaccharide production occurs only with those nitrogen fixers found in soil or plant surfaces but not with those in water lends further support to the view that polysaccharides serve in impeding the entry of oxygen into *Azotobacter* cells and thus protect the 'oxygen labile' nitrogenase enzyme (Postgate, 1974) Hill *et al* (1972) also reported that *Derxia* cells devoid of slime could not fix molecular nitrogen However, no conclusive evidence is available on the role and function of polysaccharides on nitrogen fixation

The production of a specific pigment by *Azotobacter* is a genetically controlled character (Mulder and Brotonegoro, 1974) Purushothaman *et al* (1977) reported that the intensity of pigmentation in different *Azotobacter* isolates obtained from C_3 and C_4 plants were different from each other Further, the introduction or deletion of some chemicals like copper was reported (Mulder and Brotonegoro, 1974) to affect pigment production in *A chroococcum*

One of the fringe consequences of intensive study on N_2 fixation in *Azotobacter* is the recognition of various ecological, edaphic and agro-climatic factors on the magnitude of N_2 fixed in soil The inhibition of N_2 fixation by all the insecticides at 10 μg/ml concentration is in conformity with the findings of Sahrawat (1975) who reported the inhibition of N_2 fixation in *Azotobacter* by 2, 4, 5 T Although lindane, thimet and carbofuran inhibited the growth, polysaccharide production, pigmentation and N_2 fixation to varying levels on the *Azotobacter* isolates tested there existed no correlation between (i) growth and polysaccharide production (ii) growth and pigmentation and (iii) growth polysaccharide production, pigmentation and N_2 fixation

References

Bhagwat, A S and Sane, P V , 1976, Studies on enzymes of C-4 pathway Part II–Regulation of CO_2 fixation by malic enzyme in C-4 plants *Ind J Exptl Biol*, **14** 691-693

Bremner, J M , 1960, Determination of nitrogen in soil by Kjeldahl method *J Ag Sci*, **55** 11–33

Damery, J T and Alexander, M , 1969, Physiological differences between effective and ineffective strains of *Rhizobium* *Soil Sci* **108** 209

Dobereiner, J , Day, J M and Dart, P J , 1972a, Nitrogenase activity and oxygen sensitivity of the *Paspalum notatum* - *Azotobacter paspali* Association *Can J Microbiol*, **71** 103–116

DOBEREINER, J DAY, J M AND DART, P J, 1972b, Nitrogenase activity in the rhizosphere of sugarcane and some other tropical grasses *Pl Soil*, **37** 191–196

GAUR, A C AND MISRA, K S, 1970, Effect of insecticides on soil microorganisms *Pesticides*, **4** 24–27

HASSID, W AND ABRAHAM, S, 1957, In *Methods in Enzymology*, (Ed) S P COLOWICK, AND N O KAPLAN, Academic Press, New York

HILL, S, DROZD, J W AND POSTGATE, J R, 1972, Environmental effects on the growth of the nitrogen - fixing bacteria *J Appl Chem Biotechnol*, **22** 541–558

JAYACHANDRAN, S, PURUSHOTHAMAN, D AND BALASUBRAMANIAN, A, 1977, Influence of phenolic compounds on the growth, polysaccharide production and nitrogen fixation in *Azotobacter Chroococcum* Beij *Plant Biochem, J*, (In Press)

KEARNEY, P C, KAUFMAN, D D AND ALEXANDER, M, 1967, Biochemistry of herbicides decomposion in soils pp 318–342 In *Soil Biochemistry* (Ed) A D Mc Laren and G H Peterson Marcel Dekkar Inc New York

MACRAE, I C AND CELO, J C, 1974, The effects of organophosphorus pesticides on the respiration of *A vinelandii Soil Biol Biochem*, **6** 109–111

MACKENZIE, K A AND MACRAE, I C, 1972, Tolerance of the nitrogen fixing system of *A vinelandii* for four commonly used pesticides *Antonie van Leeuwenhoek* **38** 529-535

MALLIKARJUNAIAH, P R AND BHIDE, V P, 1975, Stimulation of *Azotobacter* by some seed disinfectants *Ind J Microbiol*, **15**, 92

MULDER, E G AND BROTONEGORO, S, 1974, Free-living heterotrophic nitrogen - fixing bacteria, pp 37–85 In *The Biology of Nitrogen Fixation* (Ed) A Quispel, North-Holland publishing company, Amsterdam

POSTGATE, J R, 1974, Evoluation within nitrogen fixing systems *Symposium Society for General Microbiology*, **24** 263–292

PRAMER, D AND SCHMIDT, E. L, 1965, *Experimental Soil Microbiology* Burgess Pub Co Minneapolis, Minn, p 107

PURUSHOTHAMAN, D, KASIRAJAN, C. AND RANGASWAMI, G, 1977, Nitrogen fixation of *Azotobacter* occurring in the rhizosphere of C_4 plant species *Curr Sci*

SAHRAWAT, K L, 1975, Effect of pesticides on nitrogen fixation *Pesticides*, **9** 21–23

SALEWSKI, A C, CIBOLD, C S AND FROBSCHLE, H, 1974, Estimation of soluble carbohydrates and cell-wall substances in one operation *Landw Forsch*, **27** 112–119

SREENIVASAI U, S AND RANGASWAMI, G, 1973, Studies on the effect of three granular organophosphorus insecticides on soil microflora *Indian J Microbiol*, **13** 89–95

SUTHERLAND, I W, 1972, Bacterial Exopolysaccharides *Adv Microbiol Physiol*, **8** 143–213

VAN SCHREVAN, D A, LINDENBERG, D J AND KORIDON, A, 1970, Effect of several herbicides on bacterial populations and activity and the persistance of these herbicides in soil *Pl Soil*, **33** 513–532

ZELITCH, I, 1971, In *Photosynthesis, Photorespiration and Plant Productivity* Academic Press, New York

Effect of Insecticides on Growth, IAA Production and Nitrogen Fixation of *Azotobacter chroococcum*

A Balasubramanian and R Narayanan
Microbiology Laboratory, Tamil Nadu Agricultural University, Coimbatore-641 003

Abstract

The effect of Aldicarb (2 methyl-2 [methyl thio] thio) propionaldehyde-O methyl carbamoyl oxime), Disyston (disulfoton) (0,0-diethyl-S-2 (ethylthio) ethyl phosphorodithioate) and Dasanit (fensulfothion) (0,0 dimethyl 0-4, methyl sulphinyl phenyl monothiophosphate) on the growth, indole acetic acid (IAA) production and nitrogen fixation of *Azotobacter chroococcum* Beij were studied *in vitro* Significant reduction in the growth of the cells treated with 5 ppm (normal dose) of the pesticides was observed while the effect at 2 ppm level varied with the pesticide Not only the IAA production but the *in vitro* nitrogen fixation was also adversely affected by increasing concentration of the pesticides

Intensive plant protection methods necessitate application of large quantities of pesticides for the control of pests and diseases of crops Eventhough most of these chemicals are known to be degraded fairly quickly by several soil microorganisms, a considerable portion of them remain in soil for a long period as recalcitrant molecules and they are known to accumulate in soil in due course These insecticide residues on coming into direct contact with the soil microorganisms are likely to affect the microbiological processes of the soil Although several workers have shown that these pesticides, at normal concentrations, do not affect the population of *Azotobacter chroococcum* Beij , they have not elucidated the effect of these chemicals on the activities of this organism at the cellular level (Eno, 1958, Gaur and Mishra, 1970 and Sreenivasalu and Rangaswamy, 1973) The effect of three common soil insecticides on the growth and certain metabolic activities of *A chroococcum* are reported in this paper

Material and Methods

Growth measurements The original inoculum was prepared by transferring the authentic culture from an agar slope to 100 ml of Waksman-77 broth contained in a 250 ml Erlenmeyer flask and incubated for 72 hr on a rotary shaker at 28°C±2°C For growth studies, one ml of this uniform suspension was inoculated into 99 ml of the same liquid medium Calculated quantities of sterilised, technical grade insecticides, aldicarb (2 methyl-2 (methyl thio) propionaldehyde-0-methyl carbamoyl oxime), Dasanit (fensulfothion) (0,0-dimethyl 0-4-methyl sulphinyl phenyl phosphorothioate) and Disyston (disulfoton) (0,0-diethyl S-2-ethyl thio ethyl phosphoro-dithioate) solutions were injected into the medium

to obtain final concentrations of 2, 5 and 10 ppm (a i), just before inoculating the *A chroococcum* cells Each treatment was replicated twice The flasks were inoculated at 28°C on a rotary shaker and at periodical intervals the growth was measured with an Erma colorimeter at 420 nm (blue filter) (Balasubramanian and Gita Nilakantan, 1976) The optical density (O D) was computed and the growth curve was plotted

Estimation of indole acetic acid (IAA) production One ml of 72 hr old culture was inoculated into 99 ml of Waksman-77 broth containing 10 mg of L-tryptophan along with 0,2,5 and 10 ppm of the insecticides and incubated for 15 days at 28°C The indole acetic acid content was estimated at 4, 8 and 15 days intervals following the procedure of Chandramohan and Mahadevan (1968) The total quantity of IAA produced per 100 ml of mediam was calculated from a standard curve prepared from authentic samples of IAA

Estimation of nitrogen content One ml of 72 hr old inoculum was transferred to 99 ml of Waksman-77 broth along with 0, 2, 5 and 10 ppm of the insecticides and incubated for 15 days at 28°C Nitrogen content of the cells was estimated at 5, 10 and 15 day intervals in a microkjeldhal's distillation unit following the procedure described by Humphries (1956)

Results and Discussion

While the lower concentration of aldicarb (2 ppm) treatment showed slight stimulation in the growth of *A chroococcum* cells, the normal (5 ppm) and the higher (10 ppm) levels inhibited the growth, with disulfoton and fensulfothion, there was not much variation in growth at 2 ppm level, whereas at 5 and 10 ppm levels growth was considerably affected (Fig 1)

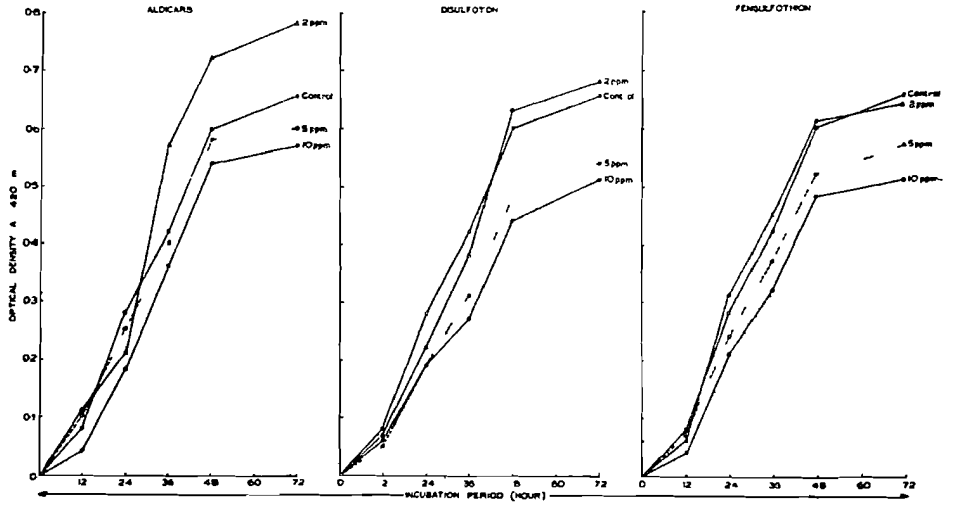


Fig 1

That the lower concentration of the pesticides did not affect the growth of *Azotobacter* have been reported by earlier workers (MacKenzie and MacRae, 1972) Inhibition of growth of *A chroococcum* at higher concentrations of pesticides has also been reported earlier (Kandasamy *et al*, 1975, Balasubramanian and Gita Nilakantan, 1975 and 1976, Garg, 1977) The results indicate that at very low concentrations the insecticides have no significant effect on *A chroococcum* cells, while at the higher concentrations affected the growth of the organism It may be due to toxicity of the chemicals or inhibition on the assimilatory processes and conseqently on growth

The IAA production potential of *A chroococcum* cells was maximum on the 4th day of incubation which declined thereafter All the three insecticides considerably reduced the IAA production potential of the organism *in vitro* at 5 and 10 ppm concentrations, with little effect at the 2 ppm level (Table I)

TABLE I

Effect of the insecticides on indole acetic acid (IAA) production by A chroococcum

Treatment	*IAA produced in micrograms/100 ml culture		
	4th day	8th day	15th day
Control (No insecticide)	331 56	128 57	34 50
Aldicarb 2 ppm	349 98	162 71	20 00
,, 5 ppm	299 33	121 27	23 10
,, 10 ppm	242 53	96 71	26 70
Disulfoton 2 ppm (Disyston)	345 38	132 01	30 70
,, 5 ppm	287 05	127 41	31 50
,, 10 ppm	217 97	101 31	23 80
Fensulfothion 2 ppm	328 49	135 85	38 40
(Dasanit) 5 ppm	305 47	118 97	35 30
,, 10 ppm	205 69	85 54	34 50

* values represent mean of duplicates

Balasubramanian and Gita Nilakantan (1976) have shown that aldicarb at the recommeded level adversely affected the *in vitro* production of IAA by both *A chroococcum* and *Rhizobium japonicum*

The *in vitro* nitrogen fixation of *A chroococcum* was maximum on the 10th day of incubation, however, the nitrogen fixation in presence of different concentrations of the insecticides, decreased significantly with increase in the insecticide concentration (Table II) Considering the reduced growth and IAA production by the insecticide treated cells, the decrease in the quantity of nitrogen fixed is quite understandable In view of the adverse effect of the insecticides on the growth, IAA production and nitrogen fixation of *A chroococcum* at the normal (5 ppm) and higher (10 ppm) levels of the insecticides it is

TABLE II

Effect of the insecticides on the in vitro *nitrogen fixation by* A chroococcum

Treatment	*Nitrogen content in mg/100 ml culture		
	5th day	10th day	15th day
No insecticide (control)	11 90	14 48	11 03
Aldicarb 2 ppm	10 90	10 90	10 10
,, 5 ppm	11 20	11 29	8 84
,, 10 ppm	8 40	9 15	8 49
Disulfoton 2 ppm (Disyston)	9 50	10 68	9 19
,, 5 ppm	8 80	9 49	8 58
,, 10 ppm	7 70	7 98	8 05
Fensulfothion 2 ppm	9 80	10 15	9 36
(Dasanit) 5 ppm	7 70	8 75	7 96
10 ppm	8 80	7 84	7 00

*values represent mean of duplicates

likely that the beneficial effects of this organism on plant growth may also be affected adversely by these presence of the insecticides in soil (Balasubramanian and Gita Nilakantan, 1976)

The authors gratefully acknowledge the financial assistance of the International Atomic Energy Agency, Vienna, in carrying out these studies

REFERENCES

BALASUBRAMANIAN, A AND GITA NILAKANTAN, 1976, Effect of aldicarb on the growth, indole acetic acid production by *Rhizobium japonicum* and *Azotobacter chroococcum* *Madras agric J* 63 265-267

CHANDRAMOHAN, D AND MAHADEVAN, A , 1968, Indole-acetic acid metabolism in soils *Curr Sci* 37, 112-113

ENO, C F , 1958, Insecticides and the soil *J Agr Food Chem* , 6 348

GARG, F C , 1977, Effect of systemic fungicides on the growth of *Azotobacter* sp *Pesticides*, 11 41

GAUR, A C AND MISHRA, K C , 1970, Effect of insecticides on soil microorganisms *Pesticides*, 4 24-27

HUMPHRIES, E C , 1956, Mineral components and ash analysis *Modern Methods of Plant Analysis* Springer Verlag, Berlin, 1 468-502

MACKENZIE, K A AND MACRAE, I C , 1972, Tolerance of the nitrogen fixing system of *Azotobacter virelandii* for four commonly used pesticides *Antonnie Van Leeuwenhoek* 38 529-535

POSTGATE, J R , 1971, *The Chemistry and Biochemistry of nitrogen fixation* Plenum Press, London, New York, p 326

SREENIVASULU, S AND RANGASWAMI, G , 1973, Studies on the effect of three granular organophosphorus insecticides on soil microflora *Indian J Microbiol* , 13 89-95

Variations in the Degradation of Disyston by *Azotobacter chroococcum* and *Aspergillus niger*

D KANDASAMY, K CHENDRAYAN, G OBLISAMI AND M BALASUBRAMANIAN
Tamil Nadu Agricultural University, Coimbatore-641 003

MUCH of current research is concerned with the fate of pesticides in soil and their effect on microorganisms and their activities As inhibitors of biological systems, the pesticides may exert deleterious effect on soil microorganisms, the result of which may be detrimental to many soil microbiological processes Microorganisms, however, play a major role in the degradation of pesticides (Ahmed and Cassida, 1958, Matsumura and Boush, 1971) Although extensive evidences are available on the biodegradation of insecticides by microorganisms in general, not much information is at hand, in particular on the extent of degradation by *Azotobacter chroococcum* and *Aspergillus niger*

Disyston (100 ppm) was added to (a) sterile red sandy loam soil, (b) sterile red sandy loam soil inoculated with a suspension of *Azotobacter* or *Aspergillus* and (c) Normal (unsterile) soil The residue of disyston was estimated colorimetrically as per the procedure described by Schumann and Olson (1964) at weekly interval Total microbial populations in normal soil and *Azotobacter* and *Aspergillus* populations from the inoculated soils were estimated at weekly interval following the standard procedures

The results revealed that the dissipation of disyston increased with incubation time (Table I) In sterile soil eventhough there are no biological

TABLE I

Dissipation of disyston in soil inoculated with microorganisms (initial level of insecticide 100 ppm)

Treatment	Days of incubation			
	7	14	21	28
Sterile soil	91 78	89 39	79 10	68 45
Normal soil	84 22	66 60	54 22	34 15
	(91 76)	(74 50)	(68 54)	(49 89)
Aspergillus niger	88 41	74 66	69 00	57 18
inoculated soil	(96 32)	(83 52)	(87 23)	(83 53)
Azotobacter chroococcum	58 35	49 82	30 41	9 38
inoculated soil	(63 57)	(55 73)	(38 44)	(13 70)

Figures in the parentheses denote the percentage of residue in soil

system there was about 30 per cent dissipation at the end of 4 weeks which might be due to photo-decomposition of the chemical or evaporation into the atmosphere by dust particles and water vapour (Matsumura and Boush, 1971) In normal soil the dissipation was rapid when compared to sterile soil indicating the role of soil microflora in the degradation Among the two organisms studied, *Azotobacter* degraded the insecticide very rapidly The percentage of residue left in *Azotobacter* inoculated soil was very low at all stages of sampling when compared to the level of residue in *Aspergillus* inoculated soil The difference is quite distinct at the end of fourth week when the quantity of residue was very less in *Azotobacter* inoculated soil The reason may perhaps, be due to its capacity to absorb the insecticide as reported by Kandasamy *et al* (1976)

TABLE II

Changes in microbial population in soil added with disyston (population × 10^x per g of moisture free soil)

Treatment	Population				
	Before application	After application (days)			
		7	14	21	28
Normal soil (Total population) ($\times 10^7$)	12 35	15 48	18 30	21 55	26 06
Aspergillus niger inoculated soil ($\times 10^5$)	50 71	51 02	56 21	36 41	37 20
Azotobacter chroococcum inoculated soil ($\times 10^4$)	61 44	58 17	63 15	71 47	108 14

In *Aspergillus*-inoculated soil, there was an increase in the residue level during the third and fourth weeks although it was decreasing during the initial stages The fact that a depression in *Aspergillus* population at these stages (Table II) suggested the possible absorption and slow release of insecticide from the dead cells into the medium (Ahmed and Cassida, 1958)

REFERENCES

AHMED, M K AND CASSIDA, J E 1958, Metabolism of some organophosphorus insecticides by microorganisms *J Econ Ent*, **51** 59–63

KANDASAMY, D, CHENDRAYAN, K, OBLISAMI, G, RAJUKKAN, K, RAGHURAJ, R AND SUBRAMANIAN, T R, 1976, Absorption of an organophosphorus insecticide by *Azotobacter* cells *Madras agric J*, 63 527-528

MATSUMURA, F AND BOUSH, G M, 1971 Metabolism of insecticides by microorganisms In *Soil Biochemistry*, (Ed) A D McLaren and J Skujins, Marcel Dekker Inc, New York, U S A, pp 320–326

SCHUMANN, S AND OLSON, T, 1964, Colorimetric determination of disyston residues in soil Report No 13059 of Chemagro Crop, Res Rept

In Vitro Screening of *Azotobacter* for Tolerance towards Certain Fungicides, Granular Insecticides and Herbicides

R Prakash and N N Prasad

Microbiology Division, Faculty of Agriculture, Annamalai University, Annamalainagar–608 101

Abstract

The effect of Benlate, Dexon, Demosan, Brassicol and Wet Ceresan on the *in vitro* growth of *Azotobacter* was studied at different concentrations *viz*, 100, 250, 500 and 1000 ppm Benlate and Brassicol did not markedly inhibit the growth at all the concentrations tested Demosan and Dexon slightly inhibited the growth up to 500 ppm, but 1000 ppm concentration inhibited the growth markedly Wet Ceresan affected the growth very pronouncedly at higher concentrations Ekalux, Disyston, Thimet and Furadan exerted only a slight inhibitory effect on growth Machete and Lasso inhibited the growth only at 100 ppm

The improved yield and soil fertility due to inoculation with *Azotobacter* have been reported by many workers Various agro-chemicals applied to the soil are likely to affect the population of *Azotobacter* in the soil The effect may be stimulatory or harmful (Deshmukh and Shrikhande, 1974, Siddaramegowda *et al*, 1973, and Mallikarjunaiah and Bhide, 1975) The results on the effect of certain fungicides, granular insecticides and herbicides at different concentrations on the *in vitro* growth of *Azotobacter* are presented in this paper

Material and Methods

The following fungicides, granular insecticides and herbicides were used in this study

Fungicides

i) Benlate 50 WP (methyl–1–(Butyl Carbamoyl)-2–Benzimidazole carbomate),

ii) Brassicol 60 WP (Pentachloronitrobenzene),

iii) Demosan 60 WP (1, 4-dichloro 2–5 dimethoxy benzene)

iv) Wet Ceresan (Methoxy ethyl mercuric chloride)

GRANULAR INSECTICIDES

i) Ekalux 5 G (0, 0-diethyl-0 (Quinoxalinyl (2)-thionophosphate),

ii) Disyston 5 G (0, 0-diethyl -S-2 (ethylthio) Phosphorodithioate),

iii) Thimet 10 G (0, 0-diethyl-5, 5 (ethyl thio methyl phosphorodithioate),

iv) Furadan 3 G (2, 3-dihydro-2, 2-dimethyl-7-benzofuranylmethyl Carbamate)

HERBICIDES

i) Machete (2-chloro-2'-6'' - diethyl - N -(Butoxy methyl) - acetanilide)

ii) Lasso (2-chloro -2'-6'' - diethyl -N- (methoxy methyl) - acetanilide)

The fungicides, insecticides and herbicides were incorporated aseptically into 50 ml of sterilized yeast extract mannitol broth at different concentrations Uniform inoculation potential was added into these flasks and the growth of *Azotobacter* isolate (AU 3) was measured at 24hr interval up to 72hr by reading the absorbance at 610 mμ in a Spectronic - '20 Colorimeter

The fungicides were tested at 100, 250, 500 and 1000 ppm concentrations The insecticides and herbicides were tested at 10, 20, 50 and 100 ppm levels

RESULTS AND DISCUSSION

The results on the effect of different fungicides on the *in vitro* growth of *Azotobacter* are presented in Table I All the fungicides tested adversely

TABLE I

Effect of certain fungicides on the in vitro *growth of* Azotobacter

Treatment (ppm)	Benlate	Dexon	Demosan	Brassicol	Wet Ceresan
Control	0 270	0 270	0 110	0 195	0 195
100	0 240	0 205	0 075	0 190	0 187
250	0 195	0 195	0 070	0 172	0 170
500	0 185	0 125	0 065	0 150	0 160
1000	0 122	0 058	0 035	0 132	0 077
C D (P=0 01)	0 007	0 006	0 019	0 020	0 007

Absorbance at 610 mμ

affected the *in vitro* growth of *Azotobacter* The inhibitory effect increased with increase in the concentration of the fungicide However, none of the fungicides tested have inhibited the growth markedly up to 100 ppm Even at a concentration of 1000 ppm, Benlate and Brassicol did not reveal pronounced inhibition of the growth of the culture Siddarame Gowda *et al* (1973) reported an increase in *Azotobacter* population when soil was treated with Benlate

at concentration of 10,000 ppm. Mallikarjunaiah and Bhide (1975) also reported stimulation of *Azotobacter* growth by Benlate and Brassicol.

The effect of insecticides on the *in vitro* growth of *Azotobacter* are presented in Table II. With Disyston and Thimet the growth increased with

TABLE II

Effect of certain insecticides on the in vitro *growth of* Azotobacter

Treatment (ppm)	Ekalux	Ditsyston	Thimet	Furadan
Control	0.152	0.180	0.180	0.172
10	0.135	0.227	0.145	0.152
20	0.125	0.165	0.137	0.142
50	0.120	0.137	0.127	0.112
100	0.107	0.115	0.110	0.112
C.D.	(P=0.05)=0.026	(P=0.05)=0.008	(P=0.01)=0.026	(P=0.05)=0.029

Absorbance at 610 mμ

increase in concentration up to 10 ppm. Thereafter the growth decreased with increase in concentration. Pochan and Lajudie (1948) reported stimulation of *Azotobacter* by BHC at the recommended doses. On the other hand, in the case of Ekalux and Furadan the growth decreased steadily with increase in concentration. However, the growth was not markedly lowered with these two insecticides even up to 20 ppm. Jones (1956) and Duda (1958) reported that non-symbiotic nitrogen fixing bacteria were not inhibited by excessive amounts of Chlordane, Aldrin, Dieldrin, Endrin and Methoxychlor.

The effect of herbicides on the *in vitro* growth of *Azotobacter* are presented in Table III. No inhibitory effect of Machete was observed up to 100 ppm and that of Lasso up to 20 ppm. Even at 50 ppm of Lasso considerable growth was present. Arthur (1953), Magee and Colmer (1955) and Goarin and

TABLE III

Effect of herbicides on the in vitro *growth of* Azotobacter

Treatment (ppm)	Machete	Lasso
Control	0.160	0.160
10	0.220	0.187
20	0.205	0.165
50	0.182	0.130
100	0.162	0.087
C.D. (P=0.01)	0.016	0.020

Absorbance at 610m μ

Armand (1957) stated that herbicides like 2, 4-D, MCPA Dalapon, triethanolamine and dinitrocresol applied at recommended doses were without any effect on the soil *Azotobacter* The results of the present investigation have revealed that the fungicides, insecticides and herbicides tested are not likely to inhibit *Azotobacter* in soil at the usual field rate application

References

Arthur, R C, 1963, The action of 2, 4-D upon *Azotobacter* of some sugarcane soils. *Appl Microbiol*, **1** 184–187

Deshmuk, V A and Shrikhande, J G, 1974, Effect of some herbicides on *Azotobaeter* and non-symbiotic nitrogen fixation in the soil *Indian J Microbiol*, **14** 81–82

Duda, J, 1958, Wplyw srodkow owadotojczych Hchi Chlordaru na mikroflore gleby *Acta Microbiol Polon*, **7** 237–244

Goarin, P and Armand, R D D S, 1957, Influence des herbicides sur la vie microbienne d'un Sol de rizıere *Agron Trop Nogent*, **12** 508–519

Jones, L W, 1956, Effects of some pesticides on microbial activities of the soil *Utah Agr. Exp Sta Bull*, **390** 17

Magee, L A and Colmer, A R, 1955, The effect of herbicides on soil microorganisms III The effect of some herbicides on the respiration of *Azotobacter Appl Microbiol*, **3** 288–293

Mallikarjunaiah, R R and Bhide, V P, 1975, Stimulation of *Azotobacter* by some seed disinfectants *Indian J Microbiol*, **15** 92–93

Pochan, J. and Lajudie, J, 1948, Action de certains substances nematocides et uisecticides sur la microflore normale du sol *Ann Agron*, **18** 449–451

Siddarame Gowda, T K, Shivappa Shetty, K, Patil, R B and Rai, P V, 1973, Effect of Benlate on biological activity of red sandy loam soil *Fourteenth Ann Conf. Assoc Microbiologists of India* Poona, Abstr of papers, p 59.

Behaviour of *Azotobacter chroococcum* in the Presence of Certain Herbicides

K Chendrayan, D Kandaswamy and P, Rajasekaran
Microbiology Laboratory, Tamil Nadu Agril University, Coimbatore-641 003

Several workers have reported a decline in the number of *Azotobacter* species in soil following herbicide applications and inferred that nitrogen fixation would, therefore, be affected (Galler and Khariton, 1961 Babak, 1968) However the effect of herbicides on the metabolic activities of specific soil microorganism especially of agricultural importance is little understood This note deals with the results on the effect of three soil applied, pre-emergence herbicides *viz*, Karmex [3, (3, 4–dichlophenyl)–1, 1–dimethyl urea (Diuron), simazine (2, 4–Bis] (ethylamino)–6(chloro)–5–triazine) and Tok–E–25 (2 4–Dichlorophenyl), 4–nitrophenyl ether) (Nitrofen) on biomass, respiration and extracellular water-soluble polysaccharide production of *Azotobacter chroococcum*

The organism was grown in the nitrogen free Waksman No 77 broth The medium was dispensed in 250 ml Erlenmeyer flasks and sterilized One ml of uniformly suspended *Azotobacter* cells, experiencing stationary phase of growth, was inoculated into the flask containing the medium Calculated quantities of sterile commercial grade herbicides solution were added into the medium just before inoculating the bacterial cells to obtain final concentrations of 1 ppm, 5 ppm and 10 ppm active ingredient

After inoculation, the culture were incubated in a rotary shaker for 48 hr at 28°C±1°C Duplicates were maintained for each treatment with appropriate control

The endogenous respiratory activity was measured in a Warburg respirometer The biomass production and extracellular water-soluble polysaccharide production was estimated following the method detailed by Couperwhite and McCallum (1975)

The results are presented in Table I The growth (Biomass production) of the organism was reduced in the presence of higher concentrations (5 and 10 ppm) of Karmex and Tok–E–25 Babak (1968) found that pure cultures of *Azotobacter* were sensitive to some extent to variety of herbicides at levels approximating normal dosage for weed control However, Chandra (1964) and Bounds and Colmer (1964) reported that some herbicides did not inhibit microbial growth for any significant period Similarly, Mackenzie and MacRae (1972)

TABLE I

Influence of pre-emergence herbicides on Azotobacter chroococcum

Treatment	Biomass (mg/100 ml)	Respiratory activity (μm of O_2/g/hr)	Water soluble polysaccharide (mg/100 ml)
Karmex	—1 00 ppm	100 00	367 45	190 0
,,	—5 0 ,,	50 0	123 60	165 0
,,	—10 0 ,,	40 0	103 30	130 0
Simazine	—1 0 ,,	250 0	390 53	270 0
,,	—5 0 ,,	120 0	320 76	255 0
,,	—10 0 ,,	100 0	310 20	185 0
TOK-E-25	—1 0 ,,	120 0	405 00	250 0
,,	—5 0 ,,	50 0	114 61	160 0
,,	—10 0 ,,	30 0	101 82	145 0
Control		100 0	372 50	235 0

also reported that at field application rate and fifty times the levels of herbicides had no effect on *Azotobacter vinelandii*

Endogenous respiratory activity of the organism, in general, was reduced by all the three herbicides, although 1 0 ppm concentration of simazine and TOK–E–25 were slightly stimulatory Extracellular water-soluble polysaccharide production was inhibited in the presence of Karmex and higher concentration of Tok–E–25 (5 and 10 ppm and simazine (10 ppm)

Interestingly enough, there exists an apparent positive relationship between respiratory activity, biomass and polysaccharide production A reduction in respiratory activity reflects, in general, on less biomass and polysaccharide indicating an altered metabolism of the organism in the presence of the herbicide

REFERENCES

BABAK, N M , 1968, On *Azotobacter* sensitivity to some antibiotics and herbicides *Microbiologiya* **37** 338-44

BOUNDS, H C AND COLMER, A R , 1964, Resistance of *Streptomyces* to herbicides *Sugar Bull New Oval* , **42** 274-276

CHANDRA, P , 1964, Herbicidal effects on certain soil microbial activities in some brown soils of Saskatchewan *Weed Res* , **4** 54-63.

COUPERWHITE, I AND MCCALLUM, M F , 1975, Polysaccharide production and the possible occurrence of GDP-D-mannose dehydrogenase in *Azotobacter vinelandii*, *Antonie van Leeuwenhoek*, **41** 25-32

GELLER, I A AND KHARITON, E G , 1961, The effect of herbicides on soil microflora *Microbiologiya*, **30** 494 98

MACKENZIE, K A AND MACRAE, I C , 1972, Tolerance of the nitrogen-fixing system of *Azotobacter vinelandii* for four commonly used pesticides *Antonie van Leeuwenhoek*, **38** 529-35

SESSION IV

Problems in the Production and Quality Control of Inoculants

A Note on Cheap Substitute for Mannitol in *Rhizobium* Inoculant Production Medium

J V D K Kumar Rao, H R Raghava Reddy, Gita Mahalingam and R B Patil
Department of Agricultural Microbiology, University of Agricutural Sciences
Bangalore–560 065

In India large scale production of legume inoculants on commercial scale is of recent origin and is gaining importance Major factors contributing to the cost of the inoculant are availability and price of mannitol which is a commonly used carbon substrate in the production medium of yeast extract mannitol broth

Earlier reports by Vangnai *et al* (1976) have shown that is case of *Rhizobium japonicum*, good growth could be obtained with sucrose as carbon and energy source and reducing the cost of production by 70 per cent Nandi and Sinha (1975) have shown mollasses to be a superior carbon source to malt extract, sucrose and mannitol mixture or mannitol alone Their modified medium, comprised of 10 g sucrose, 5 g mannitol and 200 ml yeast extract solution per litre It proved to be the best among the various formulations tried

In the present study two isolates of *Rhizobium viz* , UASB-94 and UASB-126, and media containing one per cent mannitol, glucose, sucrose, malt extract, sorbitol or commercial grade sugar, as the case may be were used. Two sets were maintained with two replications each One set was incubated in the shake culture while the other was kept under stationary conditions Samples were removed at periodic intervals and plated on yeast extract mannitol agar with congo red for viable count.

The results (Table I) indicated that under stationary culture conditions the isolate, UASB-126 attained the maximum growth on sixth day with glucose as carbon source

Under shake culture conditions all the carbon sources gave higher cell yield per ml than control containing mannitol alone Among these sorbitol was the best followed by sucrose On ninth day of incubation malt extract gave the highest cell yield followed by mannitol

In case of UASB-94, (Table II) shake culture proved better than stationary culture On the fourth day all the sugars other than mannitol gave higher cell yield Of these, commercial grade sugar was the best followed by sorbitol On the sixth day mannitol gave higher cell yield than the other carbon sources

TABLE I

Cheap substitute for mannitol in mass culture medium with *Rhizobium japonicum* (USAB–126)

Treatment	Rhizobium count per ml (X 10^8) on		
	3rd day	6th day	9th day
	Standing Culture		
Mannitol	1 63	13 0	5 82
Glucose	2 45	31 0	2 64
Sucrose	0 72	12 0	8 40
Malt Extract	0 20	15 0	2 80
Sorbitol	1 12	7 0	6 56
Sugar	0 80	11 0	4 88
	Shake Culture		
Mannitol	5 0	9 0	28 0
Glucose	18 0	17 0	19 0
Sucrose	10 0	23 0	15 0
Malt Extract	0 8	12 0	110 0
Sorbitol	8 0	47 0	11 0
Sugar	8 0	23 0	8 0

TABLE II

Cheap substitute for mannitol in mass culture medium with *Rhizobium* sp (USAB–94)

Treatment	Rhizobium count per ml (X 10^8) on		
	2nd day	4th day	6th day
	Standing Culture		
Mannitol	0 046	0 040	0 095
Glucose	0 031	0 030	Contaminated
Sucrose	0 150	0 090	0 195
Malt Extract	0 066	0 120	0 032
Sorbitol	0 150	0 060	0 215
Sugar	0 013	0 063	0 370
	Shake Culture		
Mannitol	0 110	0 800	36 00
Glucose	0 115	1 000	29 00
Sucrose	0 250	2 400	21 00
Malt Extract	0 170	2 100	27 00
Sorbitol	0 140	4 300	18 00
Sugar	0 090	6 000	28 00

Malt extract in general was superior to other sugars Nandi and Sinha (1975) also arrived at the same conclusion

REFERENCES

NANDI, P AND SINHA, P , 1975, Mass scale production of *Rhizobium* culture Proc Indian Natl Sci Acad , **40B** 479–481

VANGNAI, S , SINGHBOOTRA, N , VIJARNSORN, C , INTAKANAN, P , HONSPRAYOD, C AND MALO, T , 1976, *Rh Newsletter*, **21** 45

Effect of Sorbitol on the Growth of *Rhizobium* for Inoculant Production

K. Balaraman and G. Oblisami
Department of Biology, Tamil Nadu Agricultural University, Coimbatore-641 003.

Rhizobia are not highly fastidious in their nutrient requirements; they utilize mono-di and poly-saccharides readily, although some slow growing strains prefer pentoses as carbon source (Graham and Parker, 1964; Burton, 1967). Mannitol is employed in culturing cowpea rhizobia for large scale production (Burton, 1967). A high count broth is required to ensure a high quality inoculant. For this, a rapid growth period is desirable to reduce the risk of contamination and also to reduce the running costs of the equipment under large scale production of legume inoculants. Due to scarcity and increased cost of mannitol, the possibility of replacing the same with comparatively cheaper carbon sources like sucrose and sorbitol was attempted and the results are presented in this note.

The mannitol content of the yeast extract mannitol broth medium was replaced by different carbon sources like dextrose, sucrose and sorbitol and the sterilized medium was introduced with the standard uniform load of inoculum (*Rhizobium* sp – Ah6 strain) and kept under shake culture with sufficient numbers of replication. Samples were removed at periodical intervals and the optical density was recorded employing Spectronic 20 colorimeter using 470 nm. The rhizobial cells were removed by filtration and with repeated washings and dry weight of the biomass was estimated at 36 hour after the growth.

Maximum O.D. was recorded with sucrose followed by mannitol and sorbitol. The biomass as recorded by cell weight was maximum in the medium containing sucrose (291 mg/l) followed by mannitol (242 mg/l) and sorbitol (237 mg/l). However, very poor biomass production (125 mg/l) was recorded with dextrose (Table I).

In another experiment, sorbitol and sucrose at 1 : 1 ratio gave the maximum growth followed by mannitol and sorbitol, which were superior over the individual carbon sources. The present results indicated the possibility of replacement of mannitol by the combination of sucrose and sorbitol to give the sufficient load of rhizobial cells in the growth medium. The present results confirmed the earlier works of Nandi and Sinha (1975) and the possibility of

TABLE I

Effect of different carbon sources on the growth of Rhizobium

Carbon source	Growth (O D)			Cell weight at 36th hr (mg/100 mg)
	12th hr	24th hr	36th hr	
Dextrose	0 25	0 41	0 45	12 5
Sucrose	0 25	0 44	0 54	29 1
Mannitol	0 24	0 45	0 51	24 2
Sorbitol	0 24	0 44	0 51	23 7
S E		0 0163		
C D (at p 0 05)		0 0355		

TABLE II

Effect sorbitol in combination with other carbon sources on the growth of Rhizobium

(Growth recorded in O D value)

Treatment	Growth at different time internal		
	12th hr	24th hr	36th hr
Mannitol	0 210	0 483	0 563
Sorbitol	0 193	0 520	0 570
Sucrose	0 193	0 457	0 500
Mannitol + Sorbitol (1 1)	0 237	0 547	0 617
Mannitol + Sucrose (1 1)	0 237	0 500	0 510
Sorbitol + Sucrose (1 1)	0 190	0 450	0 673
S E		0 020	
C D (at p 0 05)		0 040	

manufacture of rhizobial inoculants required for pulse crops by employing comparativity cheaper ingredients

REFERENCES

BURTON, J C , 1967, Rhizobium culture and use In *Microbial Technology,* (Ed) H J Pepler Reinhold Publishing Corp , New York, pp 1-33

GRAHAM, P H AND PARKER, C A , 1964 Diagnostic features in the characterization of the root *Pl Soil,* **20** 383-396

NANDI, P AND SINHA, P , 1975, Mass scale production of *Rhizobium* culture *Proc Indian Natl Sci Acad* , 40 B 479-481

ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ
ವಿಶ್ವವಿದ್ಯಾನಿಲಯ ಗ್ರಂಥಾಲಯ
ಬೆಂಗಳೂರು - 65
0 8 DEC 2022
Acc No: 19904

A Study on Carriers for *Rhizobium* sp

D B Godse[1], B B More and P L Patil[2]

Department of Plant Pathology and Agril Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, District-Ahmednagar

Abstract

Survival and efficiency of *Rhizobium* sp of greengram (*Phaseolus aureus* Roxb) for six months at 10°C in locally available material *viz* , press-mud,ŝ penicillin waste, groundnut husk and their soil combinations were studied in comparison with peat, lignite and soil+F Y M +sand Lignite was the most superior carrier to all other caeriers followed by press-mud alone Soil combinations *viz* , saw dust+ soil, groundnut husk+soil and soil+F Y M ± sand gave better results Penicillin waste alone and in combination with soil was significantly inferior to all carriers

Introduction

Commercial production and extensive use of peat based inoculants have been profitable in many countries like U S A , Newzealand, U S S R etc In India, however, a large scale production of legume inoculants on commercial basis is of recent origin

Sankaram (1960) and Iswaran *et al* (1969) showed the peat soil as carrier for the *Rhizobium* sp on account of its richness in organic matter and good water-holding capacity Limited availability of peat soil in Nilgiri Hill ranges, that too of low grade quality and high charges involved in transporting it from the source are limiting factors in its extensive use

Kandasamy and Prasad (1971) showed that *Rhizobium* spp multiplied well in lignite Pugashetti, *et al* (1971) reported the use of cellulose powder as a satisfactory carrier Iswaran (1971) reported that coir+soil could serve as a good carrier Iswaran *et al* (1972) tested artificially decomposed organic material as a carrier Pugashetti *et al* (1972) studied survival and multiplication of *Rhizobium* spp in coffee husk compost and forest soil as carrier Sharma and Verma (1973) showed the possibility of use of lignite as carrier and found it suitable for *Rhizobium leguminosarum R trifoli* and *R meliloti* Khatri *et al* , (1973) indicated that the rice husk and sand supported the growth and multiplication of different rhizobia

[1] Department of Agril Microbiology, University of Agril Sciences, Bangalore-560 065
[2] Agril Bacteriologist, College of Agriculture, Pune-5

In view of anticipated increase in demand for bacterial inoculants, which is estimated to be as high as 75 million culture packets per year (Subba Rao, 1973) in next few years, it is imperative to locate readily available and cheap source of organic materials, which can be used as a substitute for peat and lignite and other carriers being used in commercial production of inoculants

Material and Methods

Materials collected from the various sources were made fine and passed through a seventy mesh sieve Materials alone and in combination with soil were used as carriers The treatments were press-mud, groundnut husk, penicillin waste, peat, lignite, saw dust + soil (1 1), groundnut husk + soil (1 1), penicillin waste + soil (1 1) press-mud + soil (1 1) and soil + farm yard manure + sand (2 1 1). The carriers were analysed for various properties Maximum water-holding capacity was determined by taking 25 g sample of the material in a dish and measuring the water just required to wet the material Hydrogen ion concentration was determined on Beckman's zeromatic pH meter Carriers tending to acidic range were adjusted to pH 7 with the help of calcium carbonate Organic carbon was determined by potassium dichromate method of Allison (1969) while total nitrogen content was estimated by macro-kjedahl method Effective strain of *rhizobium* sp of greengram (*Phaseolus aureus* Roxb) was obtained from the Agricultural Bacteriologist, College of Agriculture, Pune and multiplied on yeast extract mannitol broth The contents were shaken for eight hours a day on rotary shaker at 29°C for nine days

The carrier material was sterilised for 30 minutes at 20 lbs pressure on two consecutive days Three hundred and fifty ml of broth culture of *Rhizobium* sp having population of 1×10^{13} cells/ml were mixed with 800 g of amended carrier separately Water was added to the material so as to bring moisture level to 50–60 per cent of maximum water-holding capacity (Roughley, 1967, Sharma and Verma, 1973)

Inoculated material was immediately transferred to shallow trays, lined with plastic sheet, at 20°C for ten days (Iswaran, 1971), polythene bags of 0 40 gauge were used for filling ripened carriers after pre storage Each bag was filled in with fifty grams of ripened carriers, separately Bags were incubated at 10°C Sampling of carriers was done for counting number of viable rhizobia One bag from each carrier was randomly selected and used at a time Monthly counts were taken after four months storage Prior to storage plate count after pre storage was taken by using yeast extract mannitol agar containing congo red (Hahn, 1966)

The efficiency of root nodule bacterium to infect host plant is the only test for determining its agricultural value (Vincent, 1954) The nodule forming

TABLE I

Chemical analysis of carrier materials (data represent mean of two samples)

Carrier material	pH	Water-holding capacity (%)	Organic carbon (%)	Nitrogen (%)
Press-mud	6 05	232	19 40	1 025
Groundnut husk	6 80	168	19 60	1 237
Penicillin waste	6 10	88	25 10	3 458
Peat	3 85	75	11 60	0 559
Lignite	5 05	96	35 40	0 538
Saw dust + Soil	5 90	96	37 19	0 370
Groundnut husk + Soil	6 75	84	9 60	0 651
Penicillin waste + Soil	6 25	66	13 50	1 688
Press-mud + Soil	6 25	96	9 40	0 552
Soil + F Y M + sand	6 70	78	1 80	0 258

ability of *Rhizobium* grown in different carriers was tested in sterile soil The soil was collected from the field, passed through 2 mm sieve and amended with 850 g of farm yard manure, 7 5 g of single superphosphate and 1 5 g of muriate of potash per 15 kg of soil The mixture was sterilised for an hour at 30 lbs pressure for two successive days Pots of six inches diameter sterilised with 5 per cent $CuSO_4$ solution were filled in with 7 5 kg of sterilised soil Pot culture experiment was laid out in a completely randomized design with above treatments using uninoculated as control and replicated thrice

Seeds of greengram variety Jalgaon 781 was surface sterilized using the method of Dadarwal (1968) Sterilized seeds were inoculated with carriers stored at 10°C for 6 months using slurry method of seed inoculation described by Roughley (1961) Number of nodules, nitrogen content in plant and soil and dry weight of plants were recorded at flowering stage

RESULTS AND DISCUSSIONS

All carrier materials under study were acidic in reaction Taking into account favourable pH for *Rhizobium* sp all carriers except groundnut husk alone and in combination with soil and soil + farm yard manure and sand required pH adjustment to 7 All carriers having enough organic matter except soil + farm yard manure + sand could hold good deal of water The penicillin waste was the only carrier havin highest nitrogen content

High population of *Rhizobium* sp soon after per storage was observed in press-mud and groundnut husk and their soil combinations followed by peat and lignite No rhizobial growth was observed in penicillin waste and its soil combination Soil + farm yard manure + sand had a lowest rhizobial cell count

TABLE II

Plate counts of Rhizobium *sp in various carriers at 10°C*

Treatment	*Rhizobia* × 10^9 at different periods			
	Interval in months			
	0*	4	5	6
Press-mud	350	250	230	90
Groundnut husk	450	60	40	30
Penicillin waste	—	—	—	—
Peat	200	142	134	96
Lignite	210	75	58	30
Saw dust + soil	87	90	64	50
Groundnut husk + soil	140	180	80	120
Penicillin waste + soil	—	—	—	—
Press-mud + soil	100	37	30	140
Soil + F Y M + sand	80	57	40	130

* Count after pre storage

per gram of a carrier (Table II) There was not much influence of storage period on survival of *Rhizobium* sp in various carriers at 10°C A gradual decrease in rhizobial population was observed in all carriers Even after six months storage at 10°C-counts were quite high in all carriers except penicillin waste and its soil combination as per Australian standard (Date, 1970, Roughley, 1970)

Results regarding nodule forming ability and efficiency of greengram *Rhizobium* after six months storage at 10°C, presented in Table III, revealed that soil + farm yard manure + sand, groundnut husk + soil, saw dust + soil, peat and lignite produced significantly higher dry matter over control The highest dry matter was recorded in lignite whereas lowest was in groundnut husk

Soil + farm yard manure + sand, saw dust + soil, groundnut husk + soil, press-mud and lignite showed significantly higher nodulation over control Lignite produced significantly highest number of effective nodules over other carriers Saw dust + soil, groundnut husk + soil and press-mud were of same order with each other, but differed significantly over soil + farm yard manure + sand in effective nodulation Brockwell (1954) reported that over a period of six months, eleven peat culture inoculants for subterranean clover contained non-infective bacteria Moreover, in groundnut husk, pressmud + soil complete loss of the nodule forming ability was observed probably due to loss of virulence of rhizobial cells Highest survival of root nodule bacteria and also nodulation after six months storage at 10°C was noted in the lignite based inoculant Sharma and Verma (1973) also reported that very satisfactory number of rhizobia retained their viability up to storage of sixteen weeks at low temperatures in lignite

594.926
(AS/B)

2022
1990

PRINTED AT UAS PRESS

W. O. No. 288, 500/23-5-80

Preface

There has been an increasing impetus to research efforts on biological nitrogen fixation in the context of worldwide shortage of non-renewable sources of energy The spiralling costs of petroleum products used in fertilizer industry has added to cost of chemical fertilizer and in consequence resulted in pushing up the cost of cultivation of many a chemical fertilizer dependent high-yielding variety of commercial and food crops

A possible solution to the intense fertilizer deficit particularly in relation to nitrogen nutrition of crops, is to utilize the natural biological nitrogen fixing systems The optimization of nitrogen fixing activity in soils depends on several factors such as presence of the proper kind of microorganisms involved and management of soil to intensify their activity In India, particularly with its varying climates types of soils, varieties of crops cultivated and the different farming systems, there is a need to study the effects of varying meteorological factors and edaphic conditions on the capabilities of nitrogen fixing microorganisms, to sustain the nitrogen needs of crop plants With a view to bring together the active groups involved in biological nitrogen fixation research in particular and the development of other microbial inoculants in general, a regional conference was convened for the first time in 1975 at the Tamil Nadu Agricultural University, Coimbatore and during 1976 at the Annamalai University Annamalainagar

The third regional conference on Microbial Inoculants in crop production was held at the University of Agricultural Sciences, Dharwad campus during April 1977 where over forty research papers on various aspects of microbial inoculant research was presented and discussed

The delegates from Agricultural Universities of Karnataka, Kerala, Andhra Pradesh, Tamil Nadu and Maharashtra and representatives from other research institutes and commercial organisations involved in the development and production of microbial inoculants participated in the deliberations Most of the work presented in these papers have a bearing on problems related to nitrogen fixation through legumes and non-legumes in soil in agroclimatic situations prevailing in Karnataka and other adjoining southern Indian States The papers presented have been brought out in this publication which hopefully, may help in bringing to light the areas of interest and the recent findings on aspects of biological nitrogen fixation and other problems related to the development, production and quality control of microbial inoculants, being pursued in the region

The arduous task of collection and editing of the manuscripts was done by Dr. M. V. Shantaram, Microbiologist, Department of Agricultural Microbiology, University of Agricultural Sciences, G.K.V.K. Campus, Bangalore and Dr. G. Oblisami, Professor and Head, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. They were ably and willingly assisted by Dr. Suhas, P. Wani, Mr. H. N. Chanakya, Mr. V. B. Kanvi and Mr. M. R. Jagannath, graduate students and the other members of the staff and graduate students of the department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore The keen interest evinced in this effort by Dr. R. B. Patil, Director of Instruction (Agri), University of Agricultural Sciences, Dharwad Campus, deserves special mention.

—B. V. VENKATA RAO

TABLE III

Influence of six months storage at 10°C in various carriers on efficiency of Rhizobium *sp. in pot culture*

Treatment	Parameters of efficiency				
	Mean dry matter weight (g/plant)	Mean No. of nodules plant	Mean dry weight of nodules (mg/plant)	Mean per cent nitrogen in plant	Mean per cent nitrogen in soil
Press-mud	1.295	7.80	1.170	3.720	0.043
Groundnut husk	0.906	10.00	2.420	2.756	0.028
Penicillin waste	0.950	0.00	0.000	2.473	0 025
Peat	1.290	0.21	0.790	3.256	0.047
Lignite	1.308	13.80	3.433	3.910	0.049
Saw dust + soil	1.285	6.96	1.503	3.750	0.046
Groundnut husk + soil	1.280	7.80	1.096	3.836	0.047
Penicillin waste + soil	0.996	0.00	0.000	2.386	0.027
Press-mud + soil	0.936	5.0	1.5	2.503	0.022
Soil + F.Y.M. + sand	1.205	2.79	1.300	3.303	0.044
Uninoculated (control)	0.966	0.00	0.00	2.320	0.020
S.E.	0.07215	0 628	0.48	0.1042	0.0015
C.D.	0.2127	1.8420	1.4079	0.3057	0.0040

Dry matter weight of nodules per plant indicated that size of nodules (Wilson, 1971) in saw dust + soil and lignite significantly increased the weight of nodules over control. Lignite significantly increased nodules size over other carriers. Press-mud, soil + farm yard manure + sand and saw dust + soil were of same order as regards weight of nodules. Nitrogen content in plant was significantly increased in all treatments over control except penicillin waste alone and in combination with soil and press-mud + soil.

In all treatments except soil combinations of penicillin waste and press-mud increased soil nitrogen level. Although penicillin waste and groundnut husk increased just significant nitrogen in soil over control but they were statistically inferior to other treatments.

The senior author is grateful to the Indian Council of Agricultural Research for providing him with a Junior Fellowship during the tenure of this work.

REFERENCES

ALLISON, L. E., 1969. *Methods of Soil Analysis* in Part 2, American Society of Agronomy, Inc., Madison, Wisconsin, U.S.A.

BROCKWELL, J., 1954. Noninfective nodule bacteria in peat cultures. *Aust. Int. Agri. Sci.*, **20**: 243.

Rpt: 19904

DADARWAL, R , 1968, Influence of seed diffusates on bacterization of leguminous crops. M.Sc. Thesis, IARI, New Delhi.

DATE, R. A., 1970, Microbiological problems in the inoculation and nodulation of legumes. *Pl. Soil*, **32**: 703.

HAHN, N. J., 1966, The congo red reaction in bacteria and its usefulness in identification of rhizobia. *Can. J. Microb.*, **12**: 725.

ISWARAN, V., 1971, Effect of aeration on survival of *Rhizobium japonicum* in peat contained in polythene bag. *Mysore J. agric. Sci.*, **5**: 493.

ISWARAN, V , SUNDAR RAO, W. V. B. AND MAGER, S. P., 1969, Indian peat as a carrier of *Rhizobium*. *Curr. Sci.*, **38**: 468.

ISWARAN, V., SEN, A. AND APTE, R., 1972, Plant composts as a substitute for peat for legume inoculation. *Curr. Sci.*, **41**: 20.

KANDASAMY, R. AND PRASAD, N. N., 1971, Lignite as a carrier of rhizobia. *Curr. Sci.*, **40**: 496.

KHATRI, A. A., Choksey, M. AND D'SILVA, E., 1973, Rice husk as the medium for legume inoculants. *Sci.* and *Cult.*, **31**: 194.

PUGASHETTI, B. K., GOPALGOWDA, H. S. AND PATIL, R. B., 1971, Cellulose powder as legume inoculants base. *Curr. Sci.*, **40**: 494.

PUGASHETTI, B. K., PATIL, R. B., BAGYARAJ, D. J. AND SHRINI[illegible], H. P., 1972, Symposium on legume inoculants. Science and Technology. Abstr. 10.

ROUGHLEY, R. J., 1961, Inoculation of legumes with nitrogen [illegible] bacteria. *Agri. Gaz*, (N.S.W.), **72**: 40.

ROUGHLEY, R. J., 1967, Growth and survival of *Rhizobium* spp. in p[illegible]ulture. *J. app. Bacil.* **30**: 362.

ROUGHLEY, R. J., 1970, The preparation and use of legume seed inoculants. *Pl. Soil*, **32**: 675.

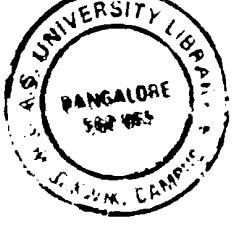
SANKARAN, A., 1960, Survival of rhizobia in peat based legume inoculant. *Curr. Sci.*, **29**: 97.

SHARMA, C. R. AND VERMA, J., 1973, Lignite based legume inoculants. 14th Annual Conference, Association Microbiologist of India, Abst. p. 62.

SUBBA RAO, N. S., 1973, Lets help crop plants produce their own nutrients. *Science Today*, **7**: 13.

VINCENT, J. M., 1954, The control of the quality of legume seed inoculants. *J. Aust. Inst-Agri. Sci.* **20**: 247.

WILSON, J. R., 1971, Comparative nodulation, nitrogen fixation and growth of *Glycine weihti*. Cooper and *Phaseolus atropurpureus* Sirato seedlings. *Aust. J. Agri. Res.* **23**: 1.



UAS LIBRARY GKVK

R-19904