herbarium records, it is shown that *S. spirale* is restricted in distribution to the North-Eastern region of India and the contiguous parts of Bangladesh and Burma. The present results indicate that *S. spirale* can hardly be treated as endemic to the North-Eastern region. It is reported[6] that 17% plant species described in Nilgiri and Pulney hill-top occur in Khasia hills also, about 2200 km away. These two areas are sufficiently distant to envisage dissemination of the species from one area to the other. On the other hand it may be suggested that the species also occupied the intervening land in the past and during evolution got localized in the North-Eastern hilly areas and temperate climatic zones at high altitudes in South India. The chromosome number $n = 12$ and $2n = 24$ in this species conforms to the reports on other non-tuber bearing species of *Solanum*.

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DILUTION OPTIMA OF BROTH CULTURES FOR MAXIMIZING PRODUCTION OF CARRIER-BASED INOCULANTS

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MANY developed countries produce rhizobial broth cultures in automatic and semiautomatic fermentors. However, for a developing country like India, fermentors are expensive not only to acquire and maintain but to install and operate. Special skills are required for their operation. Often they get contaminated if proper precautions are not taken during their operation resulting in loss of time and expensive nutrients. Most of the commercial units in India, therefore, use rotary shakers despite limited output of the broth cultures. Therefore, any method which improves production of broth cultures from shakers is welcome.

The present study was undertaken to test whether or not carrier-based inoculants prepared from prediluted broth culture of a very slow growing strain of *Rhizobium* cowpea sp. (ARS-112) can attain and sustain an acceptable number, during the incubation period. The utility of cheaper substitutes in place of yeast extract in diluent solution was also examined.

The broth culture of *Rhizobium* cowpea sp. (ARS-112) was prepared by inoculating yeast-extract-mannitol (YEM)[1] broth with a 10-day-old agar culture and incubating the same on a rotary shaker for 7 days at 30 ± 2°C. The rhizobial population of the broth culture after 7 days incubation was 96×10^9 viable cells/ml. Glass distilled water, 10% solution of commercial grade molasses and malt extract and YEM medium (full strength) were prepared and sterilized at 121°C (15 p.s.i.) for 30 min and used for diluting the broth culture of *Rhizobium* cowpea sp. (ARS-112) in the ratio of 1:0, 3:1, 1:1, 1:3 and 1:9 before blending it with the carrier. Finely powdered (100 mesh) and air-dried charcoal and soil (sandy-loam) were mixed in the ratio 3:1. Calcium carbonate (0.02%) and K_2HPO_4 (0.05%) were added to this mixture along with 10% of moisture. A sample of 200 g of this carrier mixture was packed in autoclavable polypropylene bags ($6 \times 10''$ size, 75 μ thick) and heat sealed. The carrier material in sealed bags was sterilized in an autoclave at 121°C (15 p.s.i.) for 3 h. On cooling, an aliquot of 60 ml of undiluted and diluted broth cultures with each diluent was injected aseptically into separate bags containing 200 g of sterilized carrier using a syringe. This gave 40% moisture content into the inoculant packet. Each treatment was replicated thrice. The bags were kept unmixed overnight to allow the mixture to get absorbed into the carrier. Next day, the contents of the bags were mixed thoroughly by massaging from outside. These packets were incubated in a BOD incubator at 30°C for 180 days. The rhizobial population in carrier was enumerated at regular intervals of storage by dilution-plate count method using YEMA medium containing congored[1].

Table 1 *Influence of dilution of broth culture on the survival of Rhizobium cowpea sp. (ARS-112) in carrier*

Dilution ratio (culture : diluent)	Log number of viable rhizobia/(g) of carrier				
	Days after inoculation				
	0	30	60	180	Mean
1:0	10.50	9.10	7.00	4.95	7.89
3:1	10.03	9.51	8.47	6.18	8.55
1:1	9.54	9.42	8 13	6 58	8.42
1:3	9.04	9.18	7.48	6.55	8.06
1:9	8 60	8.82	7.44	6.47	7.83
Mean	9.54	9.21	7.70	6.15	
	Dilution (D)		Period (P)		$D \times P$
S.E.M.	0 05		0.04		0.10
CD at 5%	0.13		0.12		0.27

S.E.M.—Standard error of mean.

High rhizobial population densities in carrier-based inoculants are of vital importance for effective nodulation of the cultivated legumes. Dilution of broth cultures has been suggested to increase production of carrier-based inoculants as they attain high population optima in carrier within a week of incubation[2]. The present observations however reveal that this method has limitations for preparing high quality inoculants with slow growing rhizobia. The inoculants prepared from the prediluted broth

Table 2 *Influence of diluent of broth culture on the survival of Rhizobium cowpea sp. (ARS-112) in carrier*

Diluent	Log number of viable rhizobia/(g) of carrier				
	Days after inoculation				
	0	30	60	180	Mean
Distilled water	9.54	8.68	7.44	5.89	7.89
YEM broth	9.54	8.67	7.54	5.88	7.91
Molasses solution (10%)	9.54	10.41	8.27	6.67	8.72
Malt extract solution (10%)	9.54	9.07	7.56	6.14	8.08
Mean	9.54	9.21	7.70	6.15	
	Diluent (D)		Period (P)		$D \times P$
S.E.M.	0.04		0.04		0 09
CD at 5%	0.12		0.12		0.24

S E.M.—Standard error of mean.

Table 3 *Influence of the interaction of dilution and the diluent on the survival of Rhizobium cowpea sp. (ARS-112) in carrier*

Diluent	Log number of viable rhizobia/(g) of carrier					
	Dilution ratio (culture : diluent)					
	1:0	3:1	1:1	1:3	1:9	Mean
Distilled water	7.89	8.39	8.17	7.68	7.33	7.89
YEM broth	7.89	8.64	8.19	7.68	7.13	7.91
Molasses solution (10%)	7.89	8 73	8.83	8.84	9.32	8.72
Malt extract solution (10%)	7.89	8.43	8.48	8 04	7.56	8.08
Mean	7.89	8.55	8.42	8.06	7.83	
	Dilution (D)		Diluent (Dt)		$D \times Dt$	
S.E.M.	0.05		0.04		0.10	
CD at 5%	0.13		0.12		0.27	

S E.M.—Standard error of mean.

cultures (from 3:1 to 1:9) attained an acceptable number of viable rhizobia in carrier within a month of incubation (table 1). Maximum rhizobial population in carrier was attained which survived with minimum dilution of the broth culture (i.e. 3:1 dilution). Further dilution, however, brought down the rhizobial number in carrier. The chemical nature of the diluent had a significant effect on the survival of *Rhizobium* in the carrier (table 2). Molasses solution as a diluent supported maximum number of *Rhizobium* followed by malt extract solution, YEM broth and distilled water. The rhizobial number in carrier increased with the quantity of molasses solution added to the broth culture (table 3). This suggests that molasses has an intrinsic nutritional value for the slow growing strain of *Rhizobium* cowpea sp. A similar effect of molasses on rhizobial growth was also reported to obtain high yields of *Rhizobium* for preparing cell concentrates[3,4]. It may be concluded that a good quality carrier-based inoculant can be prepared from diluted broth cultures of even very slow growing strains of *Rhizobium*. The success of this method, however, depends on the growth rate of *Rhizobium*, the dilution optima and the nature of diluent used for the broth culture. This method offers vast scope to scale up production of inoculants in India where shakers are used for production of broth cultures.

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ANNOUNCEMENT

INTERNATIONAL SYMPOSIUM ON THE STRUCTURE AND DYNAMICS OF THE INDIAN LITHOSPHERE

The above three day symposium sponsored by the Inter-Union Commission on the Lithosphere (ICL) and the International Association of Seismology and Physics of the Earth's Interior (IASPEI) will be held at the National Geophysical Research Institute, Hyderabad, India, during February 1–3, 1989. Research contributions in the following areas are invited: 1. Structure and tectonics (geophysical, geological, and geochemical), 2. Intraplate stress regime, 3. Kinematics of the Indian plate and Indian continent, 4. Lithosphere — asthenosphere interactions, 5. Mantle convection and thermal history.

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