Since the virulence of the pathogen was correlated with its sporulating capacity, a technique to maintain the culture in a sporulating condition has been developed. It was also found that this was an easy method for harvesting spores in the preparation of spore suspensions, without mycelial bits.

Fresh, healthy 10 cm long culms of highly susceptible variety, Hamsa, to *Helminthosporium nodulosum* were collected from 60 day-old plants, washed first with tap water and then treated with 0·01% aqueous mercuric chloride for one minute. These culms were then dipped in 250 ppm streptomycin solution for 5 min and buried in sterilized soils infested with *Helminthosporium nodulosum*. The moisture level of the soil was maintained at 50% by adding sterile distilled water[2] at 28° C ± 2° C. The culms were removed after 6 days, washed thoroughly first with tap water and then with sterile distilled water. The culms were then observed under stereo-microscope for the lesions on the culms; such culms were then incubated on moist blotter for 6 days. The fungus sporulated heavily on the culms and at this stage spores can be harvested with the help of a fine brush or by gentle tapping of the culms on a butter paper for inoculation or the culms can be dried and wrapped with butter paper and preserved in a refrigerator as stock culture. This can be maintained for 2 to 3 years without much loss in viability, and on subculturing on potato dextrose agar (PDA) at any time during this period gave sporulation equivalent to the fresh isolates from infected material.

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1. Vidhyasekaran, P., *Indian Phytopath.*, 1971, **24**, 347.
2. Piper, C. S., *Soil and Plant Analysis*, Hans Publishers, Bombay, 1966, p. 82.

DOES SOIL INHIBIT BIOLOGICAL NITROGEN FIXATION?

THE sensitivity and convenience of the acetylene reduction technique for assaying nitrogen fixation has stimulated interest in the study of nitrogen fixing microorganisms and in recent years many a new diazotrophs have been described[1,2]. Excepting perhaps the root-nodule bacteria within the nodules, other diazotrophs live in close contact with soil particles and at the interphase of soil particle and microorganisms many biochemical reactions take place[3]. It has been well realized that the activity of soil microorganisms are tremendously modified by the sorptive interactions between the microbes and soil particles[4]. Recently, Takahashi[5] from Japan observed that nitrogen fixation in *Azotobacter vinelandii* was inhibited considerably by the presence of soil. There appears to be little work so far done on this area and hence the present investigation was undertaken.

Pure cultures of *Azotobacter chroococcum*, *Beijerinckia indica* and *Derxia gummosa* were grown in 100 ml of N-free Waksman broth No. 77 (devoid of calcium carbonate) Becking's broth and Campelo and Doberreiner's broth respectively[6] over a gyrotary shaker at 28° C. The cells were harvested by centrifugation at log phase and dispensed in sterile, 0·1 M phosphate buffer (pH 7·0). Air dried, pound and sieved clay loam soil obtained from rice paddies and coarse sand collected from river bed were used. Two ml of the cell suspension (Ca 87 × 10^7, 2·5 × 10^7 and 25 × 10^7 cells/ml respectively of *A. chroococcum*, *B. indica* and *D. gummosa*) was taken in 20 ml vials fitted with needle puncture rubber stoppers. Weighed quantities of soils were added to the vials and mixed thoroughly. Air in the vessels was replaced by nitrogen. Maintaining an internal O_2 pressure at 0·02 atm, 10% (V/V) of pure acetylene was injected into the vial. After 6 h of incubation at 28° C, 1 ml of gas sample was withdrawn from the vial and fed to a Perkin Elmer (Model F. 15) Gas Chromatograph fitted with Porapak (80–100 mesh) column and flame ionization detector. Nitrogenase activity was computed by measuring the ethylene peaks[7]. For the other experiment designed to study the effect of different adsorbants like resins and activated charcoal on nitrogenase activity, one g of the adsorbant after suitable regeneration was mixed with 2·0 ml of the bacterial cell suspension. Assay conditions and methods were the same as detailed above. For finding out the minimum number of viable cells required for detecting N-ase activity, the cell suspension was diluted manifolds and the assay performed. Cell counts were made following plate count method[8]. Wherever needed the biomass was also determined.

The results presented in Table I revealed that the N-ase activity of the three organisms has been considerably inhibited by the addition of soil. The inhibition was strickingly more with clay loam than coarse sand. When 2 g of the clay loam was added there was practically no N-ase activity. The clay loam contained 35·6% of clay and 9·3% silt with a CEC of 38·5 me/100 g which caused the adsorption of of the cells whereas in coarse sand only a small fraction was fine sand and hence poor adsorption. That microbial cells in an adsorbed state exhibit

TABLE I

Nitrogenase activity in bacteria as influenced by soils

Quantity of soil added (mg)	Nitrogenase activity*					
	Azotobacter chroococcum		*Beijerinckia indica*		*Derxia gummosa*	
	Clay loam	Coarse sand	Clay loam	Coarse sand	Clay loam	Coarse sand
100	416·0	980·0	630·0	870·0	736·0	1420·0
200	234·0	920 0	446·0	830·0	360·0	1260·0
300	176·0	670·0	226·0	720·0	196·0	1100·0
400	37·8	450·0	134·0	665·0	42·0	936·0
500	trace	410·0	118·0	410·0	36·0	916·0
1000	No activity	380·0	18·0	325·0	No activity	886·0
2000	No activity	270·0	No activity	312·0	No activity	824·0
Control (without soil)	1020·0	1020·0	930·0	930·0	1618·0	1618·0

* Nitrogenase activity is expressed as nmoles of C_2H_4 formed/mg dry wt of the cell/hr.

TABLE II

Effect of certain adsorbants on nitrogenase activity

Adsorbant	Nitrogenase activity*		
	A. chroococcum	*B. indica*	*D. gummosa*
Dowex–50 (Cl)	48·0	14·8	26·8
Amberlite–1R–128 (Cl)	12·8	19·0	22·0
Activated charcoal	42·6	62·0	38·0
Control	1020·0	930·0	1618·0

* Nitrogenase activity expressed as nmoles of C_2H_4 formed/mg of cells/hr.

poor metabolic activity was amply demonstrated by Hattori and Furusaka[9] in the case of *E. coli*.

The different adsorbants used in this study revealed a profound inhibition of N-ase activity (Table II). It follows therefore, that in soil, the natural habitat of microorganisms, a sizable proportion of them exists in adsorbed state due to which phenomenon like N-fixation is not well expressed. This is perhaps the reason why soil samples and cores when assayed for acetylene reduction recorded low activity as compared to samples of rhizosphere and rhizoplane[10].

Table III presents another set of data where for even detecting N-ase activity *in vitro* the minimum counts of viable cells required are provided. Only when the cell count was Ca 5000–6000/ml the activity became detectable. Despite high populations/ml clay loam soil inhibited N-ase activity to the extent of even 100%. In soil the populations of the nitrogen-fixing

TABLE III

Minimum counts of diazotrophs for detectable N-ase activity

Organisms	Nitrogenase activity*						
	100–120 cells/ml	550–600 cells/ml	2800–3000 cells/ml	6500–7000 cells/ml	$3·5–3·8 \times 10^4$ cells/ml	$17·5–17·8 \times 10^6$ cells/ml	$9·00 \times 10^8$ cells/ml
Azotobacter chroococcum	No activity	No activity	Trace	36·00	96·00	168·00	268·00
Beijerinckia indica	No activity	No activity	No activity	6·50	24·00	112·00	346·00
Derxia gummosa	No activity	No activity	2·50	128·00	153·40	245·00	673·00

* Nitrogenase activity expressed as nmoles of C_2H_4 formed/24 hr/ml of cell suspension.

bacteria often reach over several thousands'g; however, the sorptive phenomenon modifies the activites of these organisms. Therefore in making precise measurement of nitrogen fixation it is better one takes into consideration the "adsorption" of cells to soil particles whereby much of the activities are kept at lower ebb if not completely inhibited.

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1. Mulder, E. G. and Brotonegoro, S., In : *The Biology of Nitrogen Fixation*, A. Quispel (ed.), North Holland, Amsterdam, Oxford, 1974. p. 37.
2. Hardy, R. W. F. and Silver, W. S. (eds.) *A Treatise on Dinitrogen Fixation, Section III. Biology*, John Wiley Sons, New York, p. 675.
3. Russel, J. R., *Soil Conditions and Plant Growth*, Longmans, Green and Co., Ltd., London.
4. Marshall, K. C., In: *Soil Biochemistry*, A. D. McLaren and J. Skujins (eds.), Marcel Dekker, Inc., New York, 1971, **2**, 409.
5. Takahashi, H., In *Nitrogen Fixation and Nitrogen Cycle*, H. Takahashi (ed.), JIBP Synthesis, Tokyo, 1975, **12**, 61.
6. Subba Rao, N. S., *Soil Microorganisms and Plant Growth*, Oxford and IBH Publishing House, New Delhi, 1977, p. 289.
7. Purushothaman, D., Dhanapal, N. and Rangaswami, G., *Curr. Sci.*, 1977, **46**, 863.
8. Pramer, D. and Schmidt, E. L., *Experimental Soil Microbiology*, Burgess Publishing Co., Minneapolis, Minn., 1965.
9. Hattori, T. and Furusaka, C., *J. Biochem.*, 1960, **48**, 831.
10. Tjepkema, J. and Von Berkum, P., *Appl. Env. Microbiol.*, 1977, **33**, 626.

OVULE AND SEED OF *MORINGA CONCANENSIS* NIMMO

As there has been no previous account on the development of the ovule and seed in *Moringa concanensis*, some of the more important details are described here.

The ovule starts as a small protuberance and the outer integument is initiated either before the differentiation of the hypodermal archesporial cell or after it has cut off a parietal cell (Figs. 1, 2). The primary parietal cell divides anticlinally and periclinally resulting in two parietal layers (Fig. 3). The megaspore mother cell undergoes the two meiotic divisions to give rise to either a linear or a T-shaped tetrad of megaspores (Figs. 4,5). In one case the upper dyad cell has not divided in consequence of which a triad is formed (Fig. 6). At the megaspore tetrad stage both the integuments are three-cell thick. The chalazal megaspore is functional and its nucleus undergoes three free nuclear divisions resulting in an 8-nucleate embryo sac of the Polygonum type (Figs. 7, 8, 9). At this stage the outer integument is six cells and the inner is four cells in thickness. A point of especial interest is the presence of abundant chloroplasts in the cells of the integuments (Fig. 10). The anatropous ovule is borne on parietal placenta and the micropyle is formed by both the integuments (Fig. 16). The mature embryo sac shows no unusual features except that the antipodal cells indicate signs of early degeneration.

The endosperm development is of the Nuclear type and many free nuclei are formed prior to the first division of the zygote (Fig. 11). In some preparations a persistent pollen tube could be seen (Fig. 15) and a re-examination of *M. oleifera* also revealed it in one preparation. The nuclei of the endosperm showed some variation in size and several nuclei accumulate around the developing proembryo. In late globular stage of the proembryo, cell wall formation commences in the endosperm in the micropylar region of the embryo sac and extends to the entire chalazal region and each cell is usually uninucleate. The statement of Datta and Mitra[2] that "it is first nuclear but cell-formation begins at a very late stage in the *micropyle* and is confined to that region" in *M. oleifera* is erroneous. A hypostase is organized and as the cells of the nucellus are absorbed, the inner epidermal cells of the inner integument differentiate as endothelium (E) which extends upwards from the chalazal region (Fig. 12).

Regarding the formation of seed coat in *M. oleifera* there are conflicting statements. Narayana[4] states (p. 69) "the mature seed coat is constituted by the outer integument only". And the same author[5] states elsewhere (p. 38) that it is "formed by both integuments". As such the development of the seed coat in *M. concanensis* is described below.

During post-fertilization stages, the embryo sac enlarges and elongates considerably and one or two additional layers of cells are usually formed in the inner integument the cells of which are ultimately crushed and absorbed and only their remnants could be observed in the mature seed. As in *M. oleifera*[4] the outer integument becomes highly multiplicative because of the differentiation and activity of the two meristems, one located in the hypodermal region and the other in the innermost region, forming upto 40 layers of cells all around and some more layers in the