# SURVIVAL OF RHIZOBIA IN NILGIRIS PEAT

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## INTRODUCTION

ONE of the major causes for poor nodulation in legume crops is the absence of effective strains of rhizobia in the soil. The introduction of effective strains of rhizobia to the soil has been in practice since the close of the last century. In its earliest form the method consisted of growing the bacteria on agar medium, suspending the cells in water, and using the suspension to impregnate either soil directly or to inoculate the seed (Voelcker, 1896). This method is still in vogue in India.

While it is possible to inoculate successfully legume seed using either agar, freeze-dried or peat cultures (McLeod and Roughley, 1961), the peat offers some significant advantages. These include increased protection for the rhizobia when in contact with acid fertilizers (Vincent, 1958), improved survival on the seed and under a lime pellet. Under conditions where germination may be delayed and soil pH unfavourable, peat based inoculants only are recommended (Roughley, 1970).

The comparative ease with which peat base inocula could be prepared, packed and transported has led to the assessment of the suitability of peat base inoculum. The choice of the carrier was based on the tests of its suitability as a medium for the particular strains of rhizobia to be used. The present study was undertaken to assess the survival of rhizobia in a Nilgiris peat, under the conditions of the present experimentation.

## MATERIALS AND METHODS

The peat soil was collected from the State Farm at Kakathope near Ootacamund, at an altitude of 7,500 feet above mean sea level. The peat was spread in layers and shade dried. The dry peat was powdered and passed through a 160 mesh sieve since coarser particles cause balling up when wetted and adhere poorly to the seed coat (Roughley, 1970). As the peat was acidic, finely powdered calcium carbonate, 10 gm per 100 gm dry peat, was added and transferred to sterile screw capped glass jars. Better growth of rhizobia was obtained in sterilized than in non-sterilized peat (Hedlin and Newton, 1948). Hence the glass jars containing the peat were sterilized in an air oven at 110° C for 4 hours.

Yeast extract mannitol medium was used as a broth. A suspension of cells from a pure culture of *Rhizobium* sp. (groundnut), maintained on yeast extract mannitol agar, was introduced into the medium and incubated at room temperature (26°–30° C) for 48 hours. This was used as inoculum for the experiment.

In an effort to find out whether nutrients supplemented with peat base had any prolongation effect on the survival of rhizobial cells, yeast extract mannitol solutions at 0 and 25 ml per 100 gm peat were used. Four initial levels of inoculum were used to compare the trend of survival of rhizobia. As moisture content of peat culture had a marked effect on the number of rhizobia (Van Schreven *et al.*, 1954), two doses of 40 and 50% moisture by weight were tried.

The sterilized peat carrier was aseptically inoculated with the prepared broth culture at 4 levels of 10 ml ($I_1$), 15 ml ($I_2$), 20 ml ($I_3$) and 25 ml ($I_4$). Nutrient solution at 0 ($N_0$) and 25 ml ($N_1$) levels was added. Finally sterile water was added to make up the moisture to 40 ($M_1$) and 50 ($M_2$)%. The contents were mixed with a sterile glass rod introduced into each jar. The jars were incubated at room temperature.

The dilution plate technique was used for taking counts. A known quantity of peat sample was aseptically weighed out from each jar and transferred to dilution flasks. A dilution of 1 in 20 million was used. Plating, in triplicate, was done with yeast extract mannitol agar using Congo red. Enumeration of colonies was done three days after plating. Counts were taken at the end of the first, second, third, fourth, sixth and tenth weeks.

## RESULTS AND DISCUSSION

The rhizobial population of the inoculum at the time of inoculating the peat base was found to have 600 million cells per millilitre. The initial population of the four levels of inoculum thus worked out at 60, 90, 120 and 150 million viable cells per gm of dry peat. Table I gives the survival of the rhizobial population in peat base carrier with the different treatments at different intervals of time. Table II gives the summary of results and Figs. 1 and 2 the trend curves for the survival of rhizobia at different levels of inoculum,

**Table I**

*Influence of a Nilgiris peat on the growth and survival of* Rhizobium sp. *(groundnut) held at 26° to 30° C*

| Treatments | No. of viable rhizobial cells × $10^4$ per gm dry peat (average of 3 plates) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial | Counts at the end of (in weeks) | | | | | |
| | | 1st ($P_1$) | 2nd ($P_2$) | 3rd ($P_3$) | 4th ($P_4$) | 6th ($P_6$) | 10th ($P_{10}$) |
| $I_1 M_1 N_0$ | 60 | 140 | 223 | 110 | 40 | 30 | 30 |
| $I_1 M_1 N_1$ | 60 | 186 | 196 | 196 | 53 | 37 | 36 |
| $I_1 M_2 N_0$ | 60 | 132 | 180 | 140 | 50 | 35 | 10 |
| $I_1 M_2 N_1$ | 60 | 146 | 163 | 150 | 50 | 45 | 40 |
| $I_2 M_1 N_0$ | 90 | 166 | 146 | 125 | 73 | 55 | 80 |
| $I_2 M_1 N_1$ | 90 | 280 | 183 | 140 | 70 | 60 | 53 |
| $I_2 M_2 N_0$ | 90 | 260 | 230 | 156 | 46 | 36 | 66 |
| $I_2 M_2 N_1$ | 90 | 360 | 293 | 185 | 46 | 36 | 30 |
| $I_3 M_1 N_0$ | 120 | 252 | 206 | 156 | 80 | 63 | 33 |
| $I_3 M_1 N_1$ | 120 | 346 | 290 | 190 | 60 | 53 | 50 |
| $I_3 M_2 N_0$ | 120 | 252 | 243 | 140 | 76 | 55 | 53 |
| $I_3 M_2 N_1$ | 120 | 372 | 286 | 150 | 80 | 63 | 53 |
| $I_4 M_1 N_0$ | 150 | 286 | 200 | 113 | 113 | 83 | 33 |
| $I_4 M_1 N_1$ | 150 | 392 | 273 | 163 | 123 | 83 | 53 |
| $I_4 M_2 N_0$ | 150 | 292 | 246 | 100 | 116 | 76 | 50 |
| $I_4 M_2 N_1$ | 150 | 332 | 276 | 250 | 136 | 93 | 56 |

**Table II**

*Summary of results*

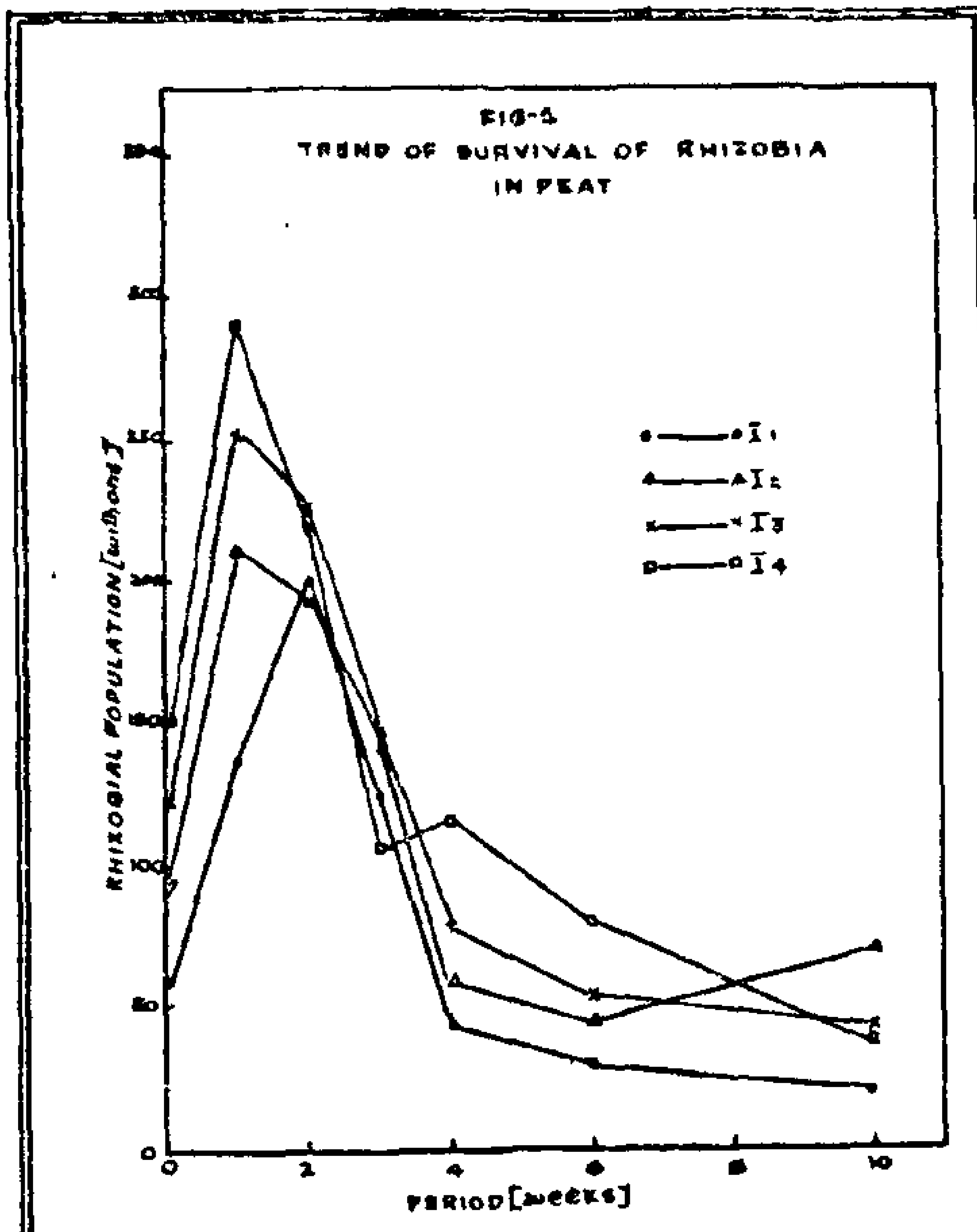
| Treatment | Conclusions | |
|---|---|---|
| Periods | $P_1 P_2 P_3 P_4 P_6 P_{10}$ | |
| Inoculum | $I_4 I_3 I_2 I_1$ | |
| Nutrients | $N_1 N_0$ | |
| Periods × Nutrients | $P_1 - N_1 N_0$ | |
| | $P_2 - N_1 N_0$ | |
| | $P_3 - N_1 N_0$ | |
| | | $N_0 - P_1 P_2 P_3 P_4 P_6 P_{10}$ |
| | $P_4 - N_1 N_0$ | |
| | | $N_1 - P_1 P_2 P_3 P_4 P_6 P_{10}$ |
| | $P_6 - N_1 N_0$ | |
| | $P_{10} - N_1 N_0$ | |
| Inoculum × Periods | $I_1 - P_3 P_2 P_1 P_4 P_6 P_{10}$ | $P_1 - I_4 I_3 I_2 I_1$ |
| | $I_2 - P_1 P_2 P_3 P_4 P_{10} P_6$ | $P_2 - I_3 I_4 I_2 I_1$ |
| | $I_3 - P_1 P_2 P_3 P_4 P_6 P_{10}$ | $P_3 - I_3 I_4 I_2 I_1$ |
| | $I_4 - P_1 P_2 P_3 P_4 P_6 P_{10}$ | $P_4 - I_4 I_3 I_2 I_1$ |
| | | $P_6 - I_4 I_3 I_2 I_1$ |
| | | $P_{10} - I_2 I_4 I_3 I_1$ |

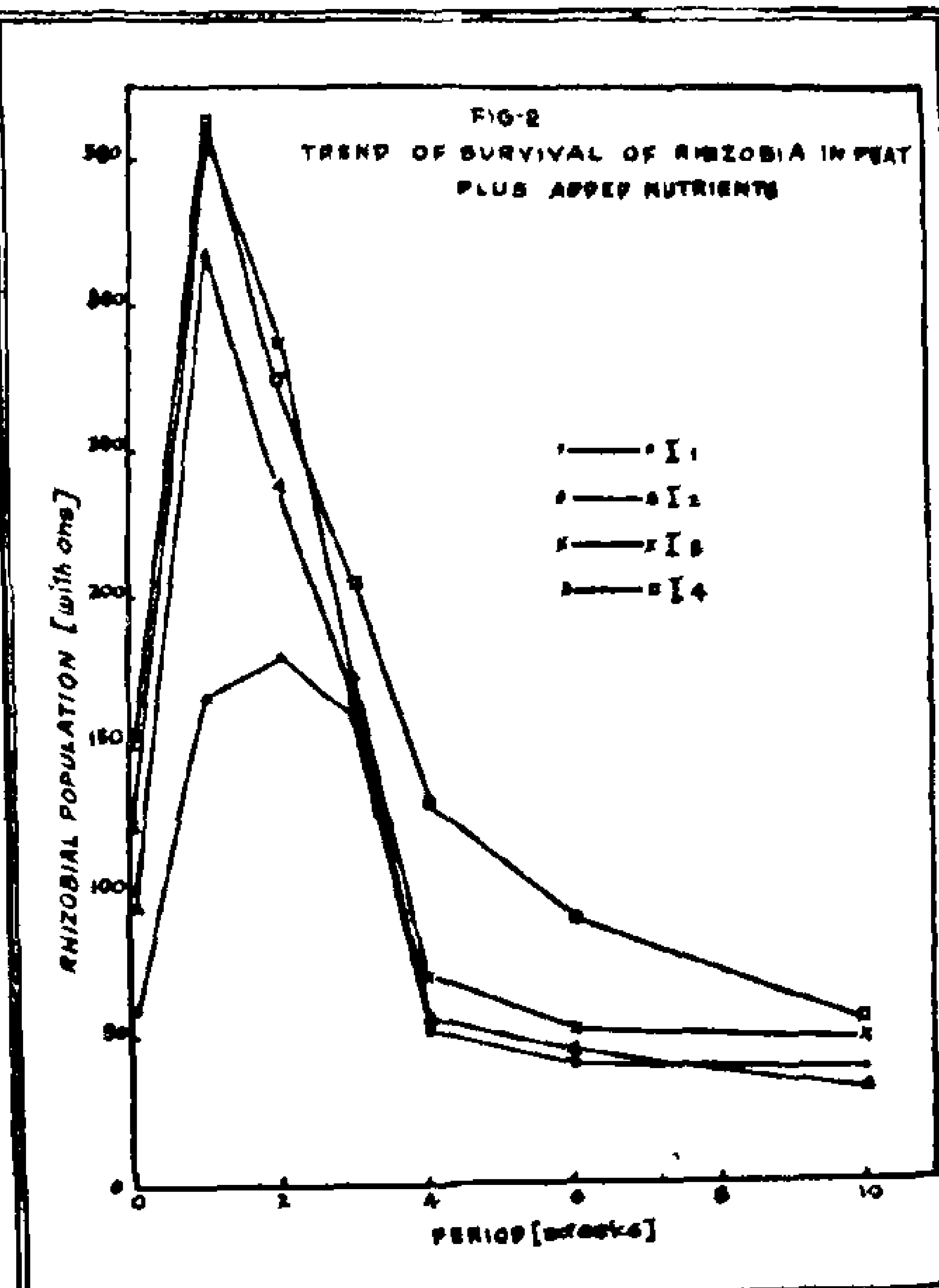
*Periods.*—The population of rhizobia at various intervals indicated that the highest was at the end of the first week. Thereafter a gradual continuous decline was seen. The steady decline could be attributed to the rapid depletion of the nutrients in the peat.

*Inoculum.*—Statistically, among the levels of inocula $I_4$ (150 million cells per gm peat) and $I_3$ (120 million cells per gm peat) were on a par and better than $I_2$ (90 million cells per gm peat) and $I_1$ (60 million cells per gm peat). However, the trend curves (Figs. 1 and 2) indicate that with the lower level of initial inoculum $I_1$ (60 million cells per gm dry peat), the rhizobial population increased for two weeks before declining. Whereas with the other levels of inoculum, the population rose only during the first week and thereafter steadily declined. This suggests that the lower initial population is better than a higher one, as the period of multiplication is enhanced, thereby the survival prolonged.

*Nutrients.*—Addition of nutrients to the peat base, generally, recorded an increased population than the treatments without added nutrients. This again could be attributed to the availability of more nutrients for rapid multiplication.

*Moisture.*—Though moisture plays an important part in the microbial activity, in the present experiment 40 and 50% moisture levels were statistically non-significant as also their interactions. Moisture




at 40 to 50% by weight could thus be used without affecting the survival of the rhizobia.

*Period* × *Inoculum*.—With the $I_1$ level of inoculum, the rhizobial population for the first three weeks was on a par, while the other levels recorded the highest population at the end of the first week only. This further indicated the superiority of the lower initial level of inoculum over others, as the population could be maintained for three weeks in contrast to only one week by higher levels of inoculum. However, after three weeks all the levels were on a par.

*Periods* × *Nutrients*.—Treatments with added nutrients recorded, for the first three weeks, a higher population than treatments without added nutrients. After three weeks the differences in population were not, statistically outstanding. In the initial periods, additional presence of nutrients probably aided in rapid increases in population. After the period of maximum growth, nutrients being depleted, all treatments behaved alike.

## Summary and Conclusions

To test the suitability of a Nilgiris peat as carrier for strains of rhizobia, under our conditions, the present experiment was conducted. Four levels of inoculum, two levels of nutrients and two levels of moisture in various combinations formed the different treatments. Lower initial inoculum of 60 million rhizobial cells per gm dry peat was found to be better than higher initial levels. Addition of nutrients was beneficial while moisture levels were non-significant. With lower initial level of inoculum the rhizobial population in peat could be maintained for three weeks, and for only one week where higher initial population was introduced.

The organic carbon content of the peat under study was rather very low, *viz.*, only 11·12%. This had probably influenced the rather short period of multiplication and had limited the growth to only two weeks after inoculation. Further experiments, with better peats or with supplementation of other sources of organic carbon, on the prolongation of the survival of rhizobia in peat base carrier have to be conducted. The trend of survival, under our conditions, had indicated that peat can be used as carriers for the rhizobial bacteria for short period only.

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# SOLUTION OF LINEAR IMMISCIBLE DISPLACEMENT PROCESS IN A FINITE POROUS MEDIUM WITH RANDOMLY ORIENTED FRACTURES

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ABSTRACT

In this paper a specific problem of immiscible displacement through a porous medium containing randomly oriented fractures is discussed. An analytical expression for the phase saturation distribution is obtained by adopting a perturbation procedure.

## INTRODUCTION

A POROUS medium consisting of extensively developed system of randomly oriented fractures is called a fractured porous medium. In such a medium the entire porous domain is made up of a large number of porous blocks each surrounded by the fractures that may form an interconnected network of narrow passages imbedded in the porous medium (*see* Fig. 1). The physics of oil water flow in fractured media is described by

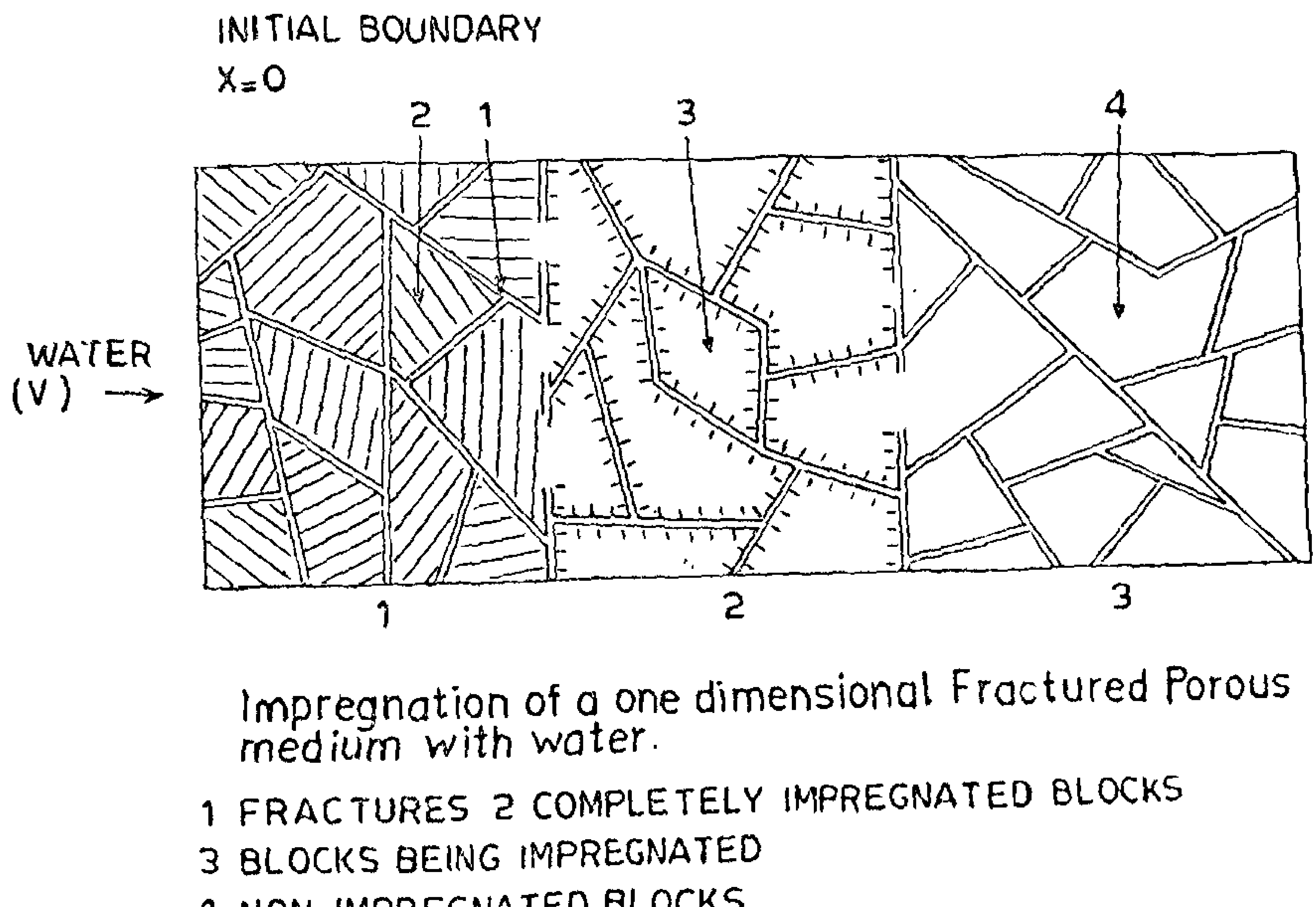


FIG. 1