

viding facilities. Thanks are also due to Mr. M. K. Mahajan and A. Qurashi for their help and suggestions.

Bot. Lab. Inst. of Science, M. T. SHEIKH.
Nagpur, October 23, 1970.

1. Chitaley, S. D. and Chitaley, D. V., *Investigation Intelligence*, 1966, 2, 5.

SALT TOLERANCE IN METHI AT GERMINATION STAGE

METHI (*Trigonella foenum-graecum* L.) is an important vegetable crop and is valued for its leafy portion and eaten either raw or cooked. This note reports the results of a study of the salt tolerance in methi under laboratory conditions at germination stage.

TABLE I

Germination percentage in methi as influenced by salt levels

Salt concentrations molar	Hours after sowing							Germination percentage
	24	48	72	96	120	144	168	
Control	21.2	59.6	7.0	7.0	0.5	1.0	0.0	96.45
0.02	2.0	58.3	16.0	16.0	2.0	2.0	0.0	96.45
0.04	0.55	42.9	26.7	7.6	6.0	1.0	0.5	85.45
0.06	0.0	24.2	15.6	19.6	6.7	3.0	0.0	69.30

F test significant : C.D. at 5% = 15.8

C	T ₁	T ₂	T ₃
96.45	96.45	85.4	69.3

Blotting-papers dipped in 0.02% cerasan, to avoid fungus growth, were kept inside petri-dishes. Hundred healthy seeds of local methi variety were used for each treatment and there were two replications. The seeds were pre-treated with 0.002% cerasan solution. Sodium chloride solutions of 0, 0.02, 0.04 and 0.06 molar were prepared in distilled water and were applied to the respective treatments. Data on germination were recorded at intervals of 24 hours and were statistically analysed. The results are shown in Table I.

From Table I it is seen that control and 0.02 molar concentration gave equal germination percentage after 168 hours though there was delay by 24 hours in the case of 0.02 molar. Initial effect of salt is compensated by an improvement in germination after 72, 96, 120 and 144 hours. In the other two cases, there is reduction and delay in germination percentage as the salt concentration increases. Thus methi can tolerate salt concentration up to a level of 0.04 molar but there is reduced germination percentage which is statistically insignificant.

Hence, the delay and fall in germination has been attributed to the osmotic concentration of the media and ionic effect of salts in the substrate. These findings are in agreement with those of several other workers.¹⁻⁴

Univ. of Agric. Sciences, A. F. HABIB.
Dharwar Campus, T. SWAMY RAO.
October 23, 1970. S. W. MENSINKAI.

1. Kaliappan, R. and Rajgopal, A. *Madras Agri. J.*, 1970, 57(4), 231.
2. Patel, A. S. and Dastane, N. G., *Indian J. Agron.*, 1968, 13(4), 280.
3. Richards, L. A., *Agri. Handbook USDA No. 60*, 1954.
4. Seshgiri Rao, T., Achar, H. P. and Hadimani, A. S., *J. Indian Soc. Soil Sci.*, 1969, 17(4), 431.

NOTE ON A STRAIN OF NOSTOC LINCKIA UNABLE TO USE MOLECULAR NITROGEN

ANTIBIOTIC resistance in micro-organisms is well known, which may arise as spontaneous mutations, the drugs acting as a selective factor or as a drug-directed variation,^{1-3,8-10} although both are not mutually exclusive. During the course of studies on antibiotic resistance in blue-green algae, a variant of a nitrogen-fixing strain of *Nostoc linckia* ARM 53 was obtained using streptomycin, which was unable to assimilate molecular nitrogen. The present report deals with this strain.

The parent material (W) was cultivated in a nitrogen-free inorganic medium containing per litre : 0.25 g. MgSO₄.7H₂O, 0.23 g. NaCl, 0.06 g. CaCl₂.6H₂O, 0.36 g. K₂HPO₄, 1 ml. Fe-EDTA and 1 ml. A₆ micronutrient solution (pH 7.5). The filaments were short during the first few days of growth in turbulent cultures. The material was washed with saline and inoculated into fresh medium containing penicillin or streptomycin (0-50 µg./ml.) at an initial

concentration of 25×10^4 filaments per ml. The cultures were incubated on a rotary shaker at $32 \pm 1^\circ \text{C}$. for 12 days under continuous illumination (5,000 lux).

The growth of the alga was found to be inhibited with the increasing concentrations of the antibiotics. While step-wise increase in the resistance to penicillin was found possible upto 1,000 $\mu\text{g./ml.}$, the alga failed to grow at concentrations of streptomycin above 0.2 $\mu\text{g./ml.}$ At 0.2 $\mu\text{g.}$ streptomycin/ml., the growth was abnormally slow. The material from this concentration level was washed with saline and transferred to drug media with and without ammonium sulphate (0.25 g./l). While the alga grew well in the series containing nitrogen, it failed to grow in the nitrogen-free medium. Strain S^+N^+ was isolated from this culture which could not fix nitrogen, as evidenced by its failure to grow in nitrogen-free medium and its inability to bring about an increase in the fixed nitrogen as measured by the kjeldahl method (Table I).

TABLE I

Specific growth rates of penicillin (P^+N^-) and streptomycin (S^+N^+) resistant strains of *Nostoc linckia*

Strains	k
Wild	0.11
P^+N^- (1,000 $\mu\text{g.}$ penicillin/ml.; N-free)	0.04
S^+N^+ (0.02 $\mu\text{g.}$ streptomycin/ml.; N-free)	0
S^+N^+ (0.02 $\mu\text{g.}$ streptomycin/ml.; with N)	0.066

Repeated transfers in media with and without the drugs and nitrogen source were carried out to determine the stability of the strains with respect to their resistance to the drugs and the inability to assimilate molecular nitrogen.

The strains isolated were of the following types:

(a) P^+N^- , resistant to 1,000 $\mu\text{g.}$ penicillin/ml. and could grow in nitrogen-free medium (Fig. 1), and

(b) S^+N^+ , resistant to 0.2 $\mu\text{g.}$ streptomycin/ml., but unable to use molecular nitrogen (Fig. 1). Nitrogen fixation by this strain could not be stimulated by the addition of a variety of substances like vitamins, bases and casein hydrolysate. But it grew well when exogenous ammoniacal nitrogen was provided. The block appears to be between N_2 and NH_3 .

Figure 1 shows the growth of P^+N^- and S^+N^+ strains in their respective antibiotic containing media, after several transfers in drug-free media.

Drug resistance in these two strains has also been found to be accompanied by a fourfold increase in their resistance to ultra-violet irradiation (2537 Å; distance 16") (Fig. 2). The LD_{50} dose of UV-irradiation for the wild strain was about 1.2 min., while for the P^+N^-

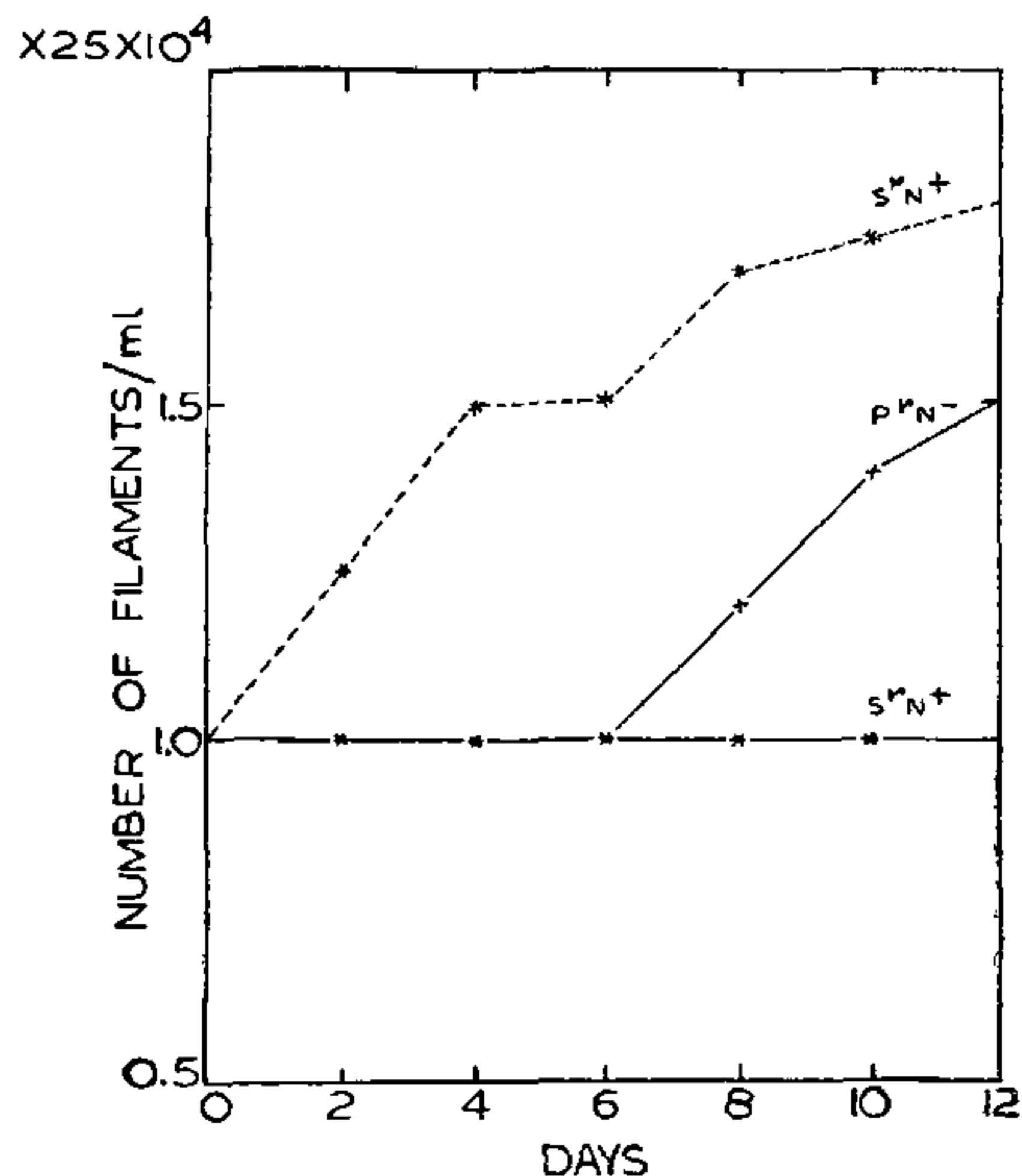


FIG. 1. Growth curves of P^+N^- and S^+N^+ strains of *Nostoc linckia* (----- nitrogen supplemented medium; ——— nitrogen-free medium).

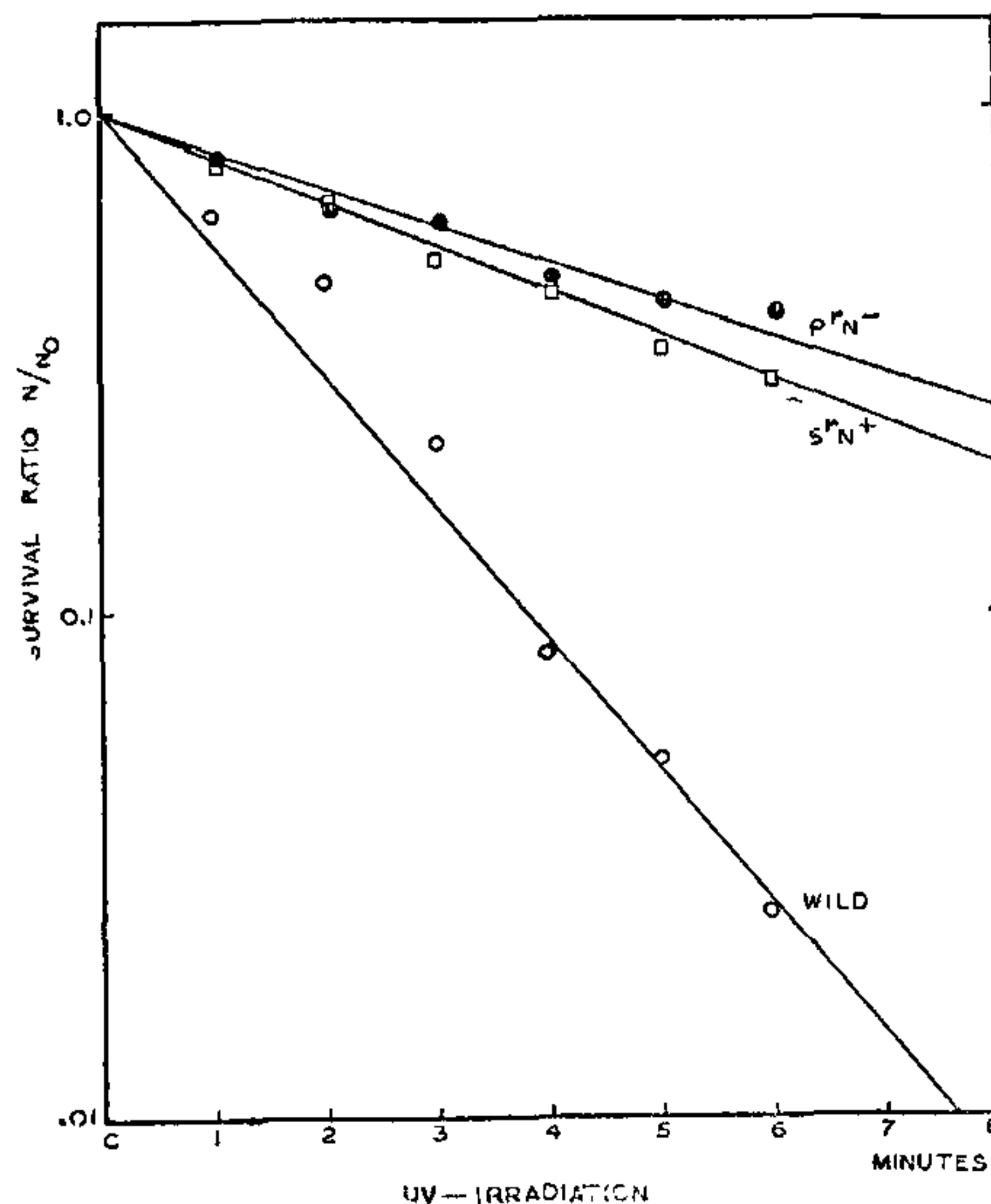


FIG. 2. UV survival curves of W, P^+N^- and S^+N^+ strains of *Nostoc linckia*.

and S'N⁺ strains, it was about 4 min. Drug-resistant strains are known among blue-green algae.⁶⁻⁷⁻⁹ Of particular interest is the loss of nitrogen-fixing capacity in the streptomycin-resistant S'N⁺ strain, which proved to be radio-resistant also. The use of variants with such biochemical markers in studies on the mechanism of nitrogen fixation has been shown in the case of *Azotobacter*.⁴⁻⁵ The relationship between drug resistance and the loss of a biochemical activity needs further examination.

Dept. of Microbiol., H. AHMAD.
I.A.R.I., New Delhi-12, G. S. VENKATARAMAN.
October 12, 1970.

1. Bryson, V. and Demerec, M., *Ann. N.Y. acad. Sci.*, 1950, **53**, 283.
2. Demerec, M., *J. bacteriol.*, 1948, **56**, 63.
3. Eagle, H., Fleischman, R. and Levy, M., *Ibid.*, 1952, **63**, 623.
4. Fischer, A. G. and Winston, J. B., *Biochem. biophys. Acta*, 1959, **184**, 99.
5. Green, M., Alexander, M. and Wilson, P. W., *J. bacteriol.*, 1953, **66**, 623.
6. Kumar, H. K., *J. exptl. bot.*, 1964, **15**, 232.
7. —, *Plant & Cell Physiol.*, 1964, **5**, 465.
8. Lederberg, J. and Lederberg, E. M., *J. bacteriol.*, 1952, **63**, 399.
9. Luria, S. E. and Delbrück, M., *Genetics*, 1943, **28**, 491.
10. Singh, R. N., Singh, H. N. and Sinha, R., *Indian J. Genetics*, 1966, **26A**, 405.

ROOT-KNOT NEMATODES AND BACTERIAL NODULATION IN SOYBEAN

THE interrelationships between nitrogen-fixing bacteria (*Rhizobium* spp.), plant-parasitic nematodes and their leguminous hosts have not been adequately studied. The possible role of nematodes in modifying the nodulation of legume roots needs to be understood. Hence, a study was undertaken to determine whether three species of root-knot nematodes, viz., *Meloidogyne incognita* (Kofoid and White, 1919; Chitwood, 1949), *M. javanica* (Treub, 1885) Chitwood, 1949, and *M. hapla*, Chitwood, 1949, could cause reduction of bacterial nodulation in soybean and to find out the effect of different inoculum levels of *M. javanica* on the nodulation, indexed 35 days after inoculation.

The contents of 7 ounce bag of Legume Aid *R. japonicum* (Kirchner, 1895), Buchanan, 1926, were thoroughly mixed with the soil to fill 4-inch pots. Five seeds of soybean variety Adams were sown. Pure cultures of *Meloidogyne* spp. started from single egg mass were obtained from the green house where they

were maintained in tomato plants. The egg masses of *M. incognita*, *M. hapla*, *M. javanica* were teased out from the roots of tomato plants and the second stage larvae were obtained, following the method of Dropkin *et al.*¹ One thousand larvae were inoculated per pot, ten days after germination. Five replications were maintained in each case. Five pots without nematodes served as control. Thirty-five days after inoculation, the plants were removed, the root system washed free of soil and indexed to the degree of nodulation from 0 to 5 (0—no nodulation; 5—heavy nodulation). The root-knot index was based according to Smith and Taylor.² To determine the inoculum levels of *M. javanica*, 0, 10, 100, 1,000 second-stage larvae were inoculated on soybean plants in pots with *R. japonicum* containing soil, as described earlier and root-knot and nodulation were indexed.

All the three species of root-knot nematodes used in this study (*M. javanica*, *M. hapla* and *M. incognita*) caused reduction in bacterial nodulation (Table I). The root nodule index

TABLE I
The effect of 1,000 larvae of *Meloidogyne javanica*, *M. hapla* and *M. incognita* on soybean bacterial nodulation

Treatment	Nodule index*	Root-knot index†
<i>M. javanica</i>	0.1	3.7
<i>M. hapla</i>	1.2	3.3
<i>M. incognita</i>	2.0	3.0
Control (no nematodes)	3.8	0.0

* Mean of 25 plants; index based on 0—no nodules; 5—heavy nodulation (indexed 35 days after inoculation), † After Smith and Taylor, 1947.

0—No roots galled
1—1-25% roots galled
2—26-50% "
3—51-75% "
4—76-100% "

varied from 0.1 to 3.8. The plants inoculated with *M. javanica* suffered a severe reduction of root nodulation and showed a higher root-knot index. The nodules of the control plants were bigger and softer than in nematode infected plants where the nodules were smaller and harder. In general, root-hair production was less in the plants inoculated with the nematodes. Reduction of bacterial nodulation was marked in the plants inoculated with 100 and 1,000 larvae. There was no difference in nodulation indices between the plants which received 100 or 1,000 larvae per plant even though there were differences in root-knot