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ADAPTATION OF *SCLEROTIUM ROLFSII* TO POLYOXIN-D

POLYOXIN-D is an antifungal antibiotic originally isolated in Japan[1] from *Streptomyces caccoi* var. *asoensis*. It is an inhibitor of chitin synthetase in fungi and is known to inhibit the growth by competitive inhibition of uridine diphosphate N-acetyl glucosamine[2,3]. Due to its effect on chitin synthesis, the antibiotic is known to cause morphological alterations in mycelia[3,4]. The antibiotic was developed mainly for the control of black spot of pear and apple caused by *Alternaria kikuchiana* and *A. mali*, but polyoxin resistance in these fungi soon led to failure of disease control[5,6]. However, very little work has been done on the effect of polyoxin-D on Indian fungi and no knowledge exists regarding adaptation of *Sclerotium rolfsii* to this new antibiotic. Since the fungus survives for long periods in soil as sclerotia and since the fungus is known to adapt to other fungicides[8,9], it was planned to study the adaptation of the fungus to this inhibitor.

Polyoxin Z W.P. (2·2% Polyoxin-D) from a sterilized 1 mg/ml stock solution was impregnated in potato dextrose agar medium to get concentrations ranging from 50 to 400 µg/ml for linear growth and sclerotial germination experiments. The fungus *Sclerotium rolfsii* Sacc. was a local isolate. For linear growth experiments, inoculum discs of 5 mm diameter cut from an actively growing plate culture were inverted onto the centre of treated plates in 4 replicates. The cultures were incubated at 30° C and the linear growth measured daily. From preliminary platings, the ED_{50} value of the antibiotic for the fungus was 75 µg/ml. The mycelia in treated plates, on microscopic examination, showed irregular swellings characteristic of polyoxin response reported elsewhere[4]. From plates containing 400 µg/ml antibiotic, the fungus was reisolated (isolate designated: Exposed) and was transferred along with the original isolate (Unexposed) to media containing 0, 100, 200 and 400 µg/ml antibiotic, and the response of the two were found to be different (Fig. 1).

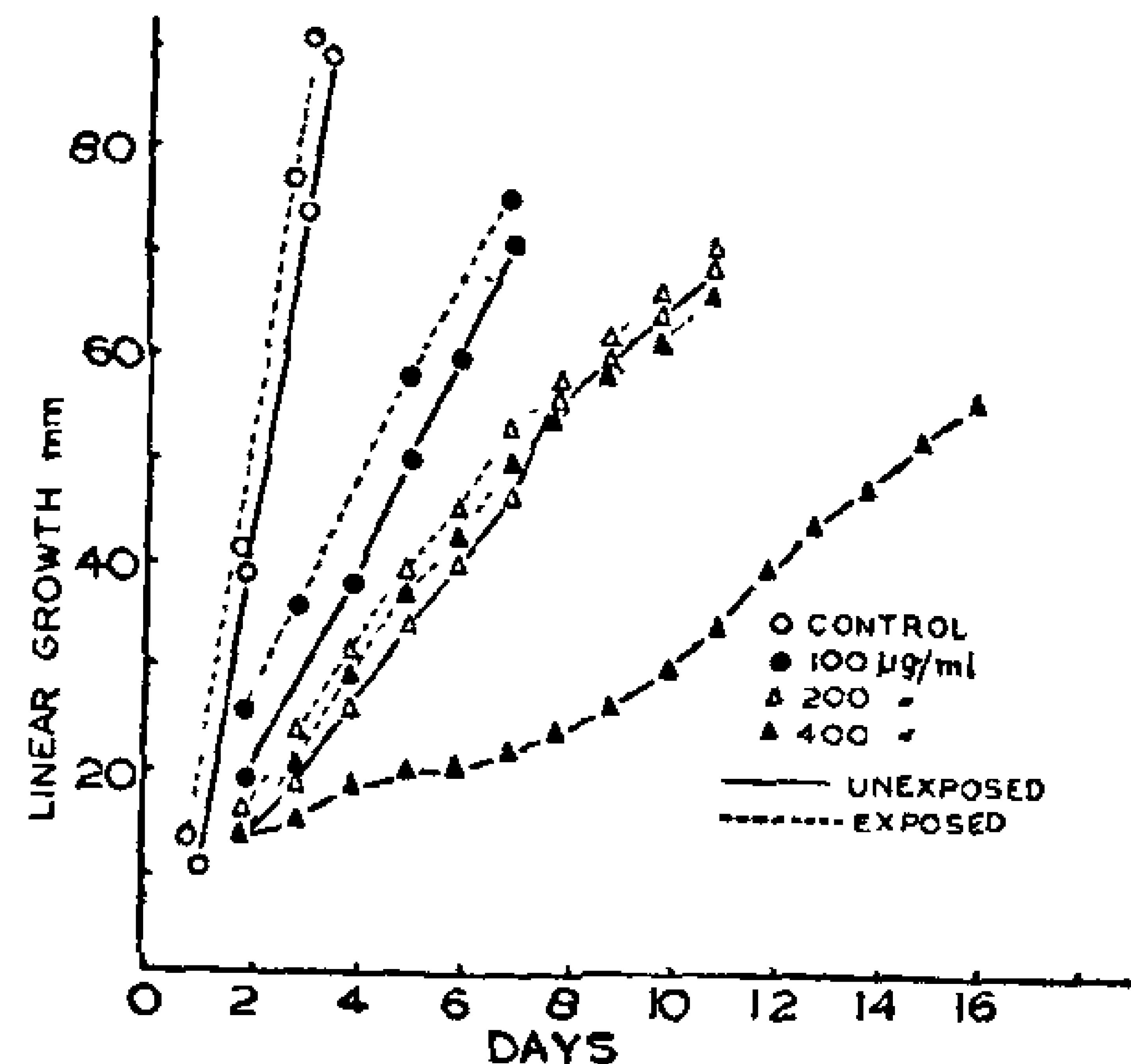

FIG. 1. Effect of polyoxin-D on linear growth of *Sclerotium rolfsii*.

The unexposed fungus showed increasing lag phase with an increase in the concentration of the antibiotic. It reached 65 mm colony diameter in 6 days at 100 µg/ml but it took 18 days to reach the same width at 400 µg/ml. The exposed culture at 400 µg/ml required only 11 days to reach 65 mm diameter. This shows that a single prior exposure to the antibiotic has resulted in a certain level of tolerance in the fungus. Transferring the fungus after a second exposure to 400 µg/ml, to antibiotic-free media successively 7 times and testing the retention of the acquired resistance after each transfer showed that there was neither increase nor decrease in sensitivity. The adaptation, obviously, was retained through the successive generations.

Sclerotia, obtained from the unexposed fungus, were tested for germination in different concentrations of the antibiotic at 30° C. The sclerotia gave 92% germination in two days and reached 100% on the third day at 100 µg/ml. At 400 µg/ml, they took 5 days to reach 97% germination. On further incubation, there was no further increase in germination. The sclerotia remaining ungerminated, at this concentration, were considered sensitive (S) and those germinated as resistant (R). One germinated sclerotium along with the

minute colony and one ungerminated sclerotium were transferred separately to PDA and labelled R and S respectively. The strains were further tested for linear growth and sclerotial germination in different concentrations of the antibiotic. In linear growth experiments (Table I), the strain S, which was sensitive in

TABLE I

Linear growth (in mm) of sensitive (S) and resistant (R) strains of Sclerotium rolfsii *in polyoxin-D*

Incubation period (in days)	Conc. of Polyoxin-D					
	100 μg/ml		200 μg/ml		400 μg/ml	
	S	R	S	R	S	R
2	21	18	14	14	..	..
3	38	36	20	16	13	11
4	48	44	23	19	14	12
5	57	52	27	22	16	12
6	67	63	31	25	17	14
7	75	67	34	28	18	15
8	..	..	36	33	20	16
9	..	..	44	36	21	16
10	..	..	45	42	24	17
11	..	..	51	46	29	20
12	..	..	54	52	33	25
13	..	..	59	56	37	30
14	..	..	63	59	41	34
15	..	..	64	60	44	39
16	..	..	69	64	47	42

the first exposure, seemed to show greater resistance than the strain R which had initial resistance. The same pattern was found in the germination of the sclerotia of the two strains (Fig. 2). Both the prior exposed strains (R and S) were more resistant than the sclerotia obtained from unexposed culture. But among the exposed strains, R and S, strain S had become more resistant than R. This may be due to the fact that in strain R, the sclerotium having germinated and formed mycelia, the hyphal segments on transfer to antibiotic-free medium lost a little of their resistance. In strain S, since no germination occurred at 400 μg/ml, the sclerotium had greater time to imbibe more antibiotic and slowly get adapted. Mycelia produced from this sclerotium had, therefore, gained resistance. Polyoxin resistance has been shown to be a membrane phenomenon, involving decreased permeability, in *A. kikuchiana*[4]. No genetic basis for resistance is known and in this case also, the adaptation does not seem to be a genetic phenomenon as there was fluctuation in the level of resistance on serial transfer to drug

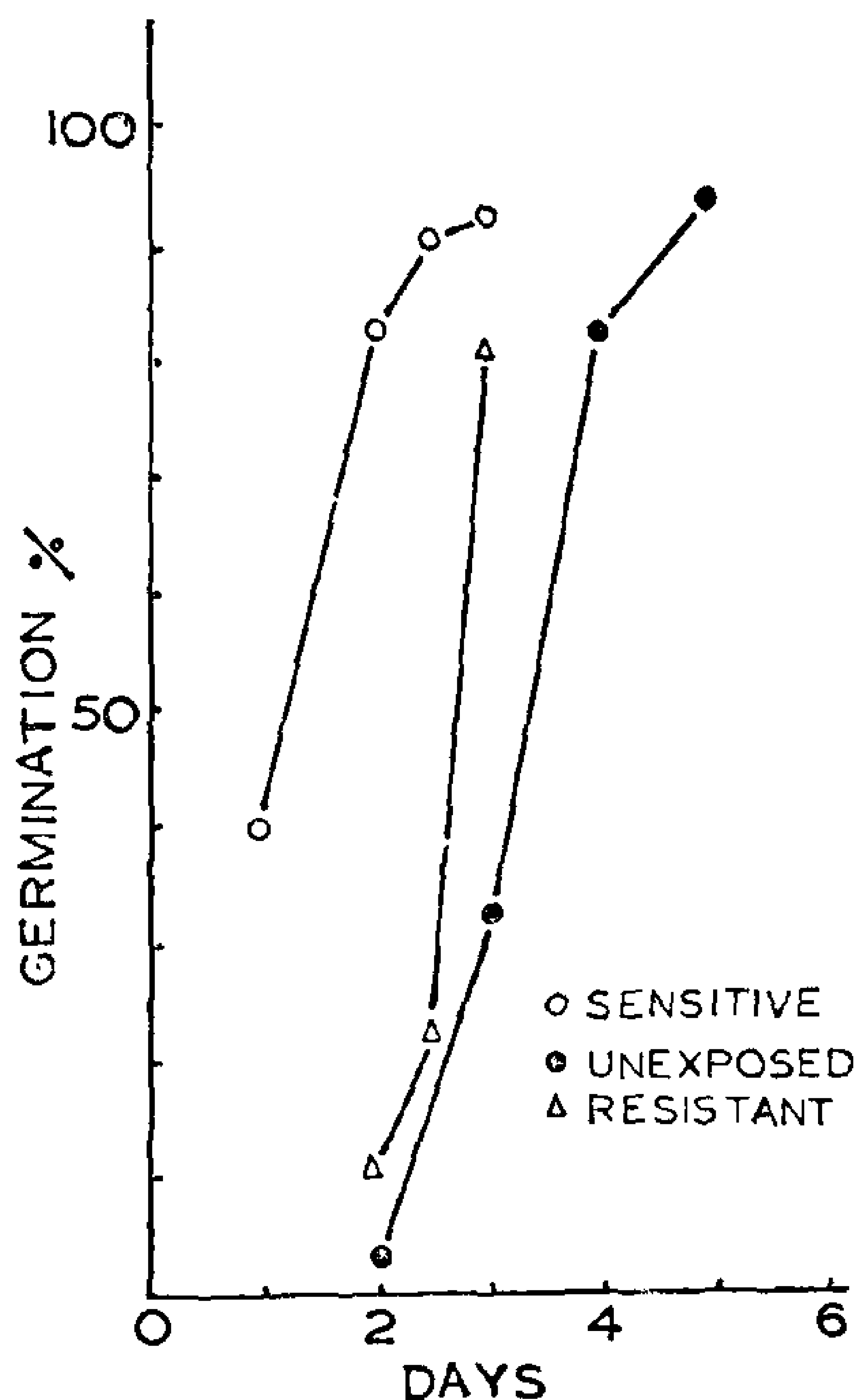

FIG. 2. Germination of sclerotia of *Sclerotium rolfsii* in 400 μg/ml of polyoxin-D.

free medium. Sclerotial adaptation seems to be more durable than mycelial adaptation. Though the sclerotia are also morphologically compressed hyphae, the thickness of the wall and the level of chitin content may play a part in the adaptation of sclerotia. Since the biosynthesis of chitin is a process going on only in actively growing mycelia, the adaptation of sclerotia may be assumed to be a surface phenomenon.

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CONIDIAL PRODUCTION IN *HELMINTHOSPORIUM GRAMINEUM* RABH. IN CULTURE

SPORULATION of *H. gramineum* in artificial culture has long been an intriguing problem. Majority of the attempts to induce sporulation in artificial culture have been unsuccessful[1-3]. However, Houston and Oswald[4] recorded sporulation of the fungus by exposing cultures in Petriplates to the diurnal variations of the outdoor conditions. Similar efforts by us yielded negative results. Using a modified technique we succeeded in inducing spore production of *H. gramineum* in artificial culture. It comprised of inoculating ten days old culture of the fungus on sterilized potato dextrose agar in Petriplates, the surface being covered with a sterilized cellophane disc. The culture was incubated at room temperature (18–25° C) for seven days and then exposed to the outdoor conditions. Care was taken to protect them from direct sunlight. The experiment was conducted in February 1979 when the outside temperature varied between 10–20° C. After one week of exposure to the external environment, sporulation was noticed. Sporulation was, however, sparse. Short secondary conidiophores formed from apical cells produced bicelled secondary conidia.

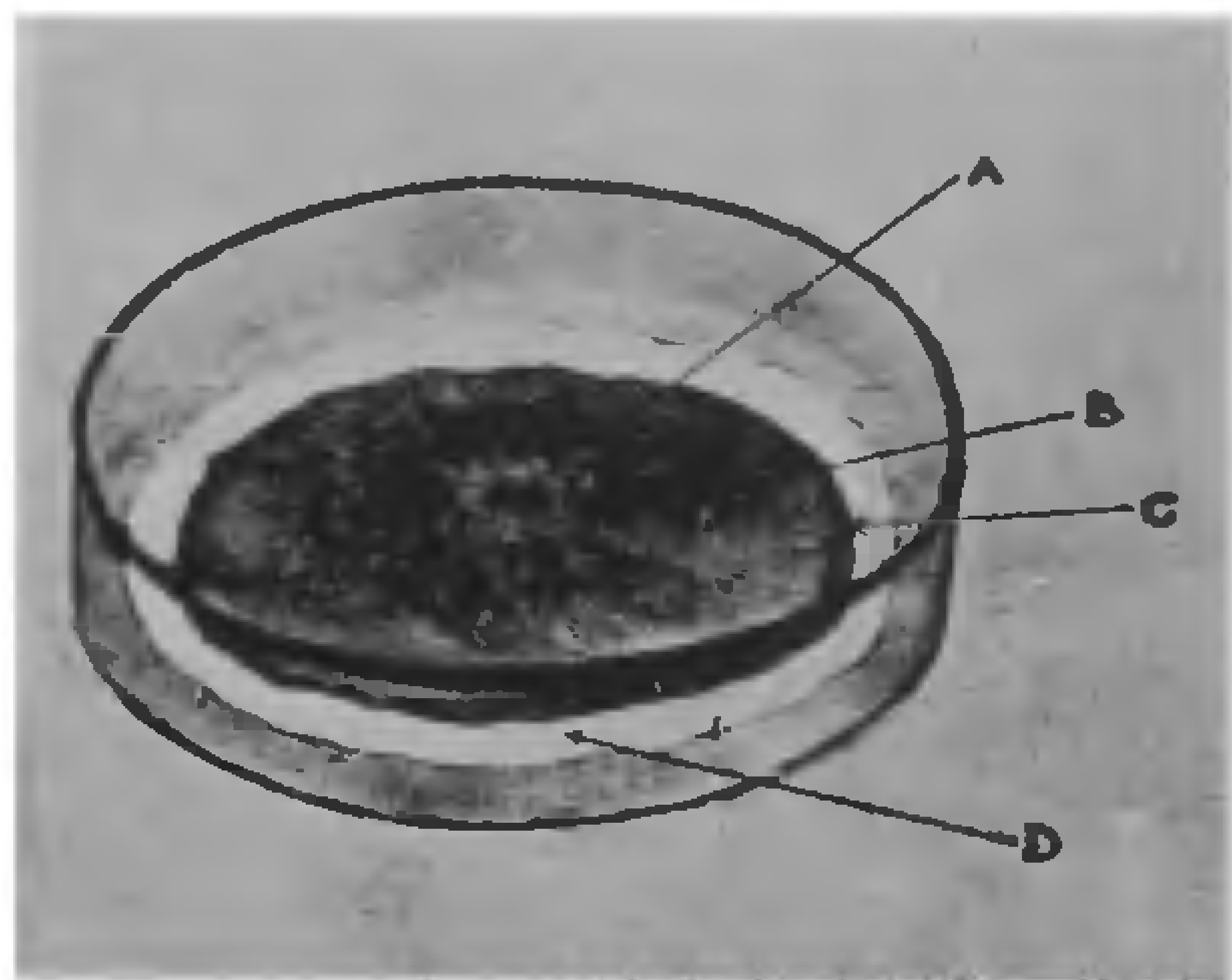

FIG. 1. Diagram of Petriplate culture of *Helminthosporium gramineum* on cellophane disc overlying medium. A, Inoculum; B, Mycelial mat with spores; C, Cellophane disc; D, Potato dextrose agar medium.

It is reported that the normal requirement of light for sporulation of *Ascochyta pisi* and *Phoma* spp. has been substituted by using cellophane or cellulose discs[5,6]. However, in this case, the role of the cellophane appears to provide a contact stimulus in inducing sporulation or even it may be to act as a filter for esesential metabolites required for sporulation of the fungus. The decisive role of temperature in sporulation is confirmed as the culture did not sporulate at 30–40° C, when the experiment was repeated in May-June, 1979. Further studies to obtain abundant sporulation in artificial cultures of *H. gramineum* and on the nutritional requirements for sporulation of the fungus are in progress.

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A NOTE ON THE OCCURRENCE OF *TRICHOTHECIUM ROSEUM* LINK. ON *DOLICHOS LAB-LAB* L. IN INDIA

IN recent years, "Hebbal Avare" a newly introduced variety of field bean (*Dolichos lab-lab*) is extensively grown in the State during Kharif months. During July–August, the authors observed avare pods infected with brownish spots accompanied by rotting in experimental plots at Agriculture College, Dharwar. The characteristic symptom of the disease was small irregular lesions which appeared on pods in initial stages, later lesions enlarged turning to brown colour and extending inside the pods. The affected pods were covered by pink mycelial mat with mouldy appearance. Repeated isolations from infected pods consistently yielded a fungus which was identified as *Trichothecium roseum* Link. The pathogenicity was proved by spraying spore suspension six days old pure culture of *T. roseum* on pods both in field and glass house. Visible symptoms appeared on pods after 2–3 days