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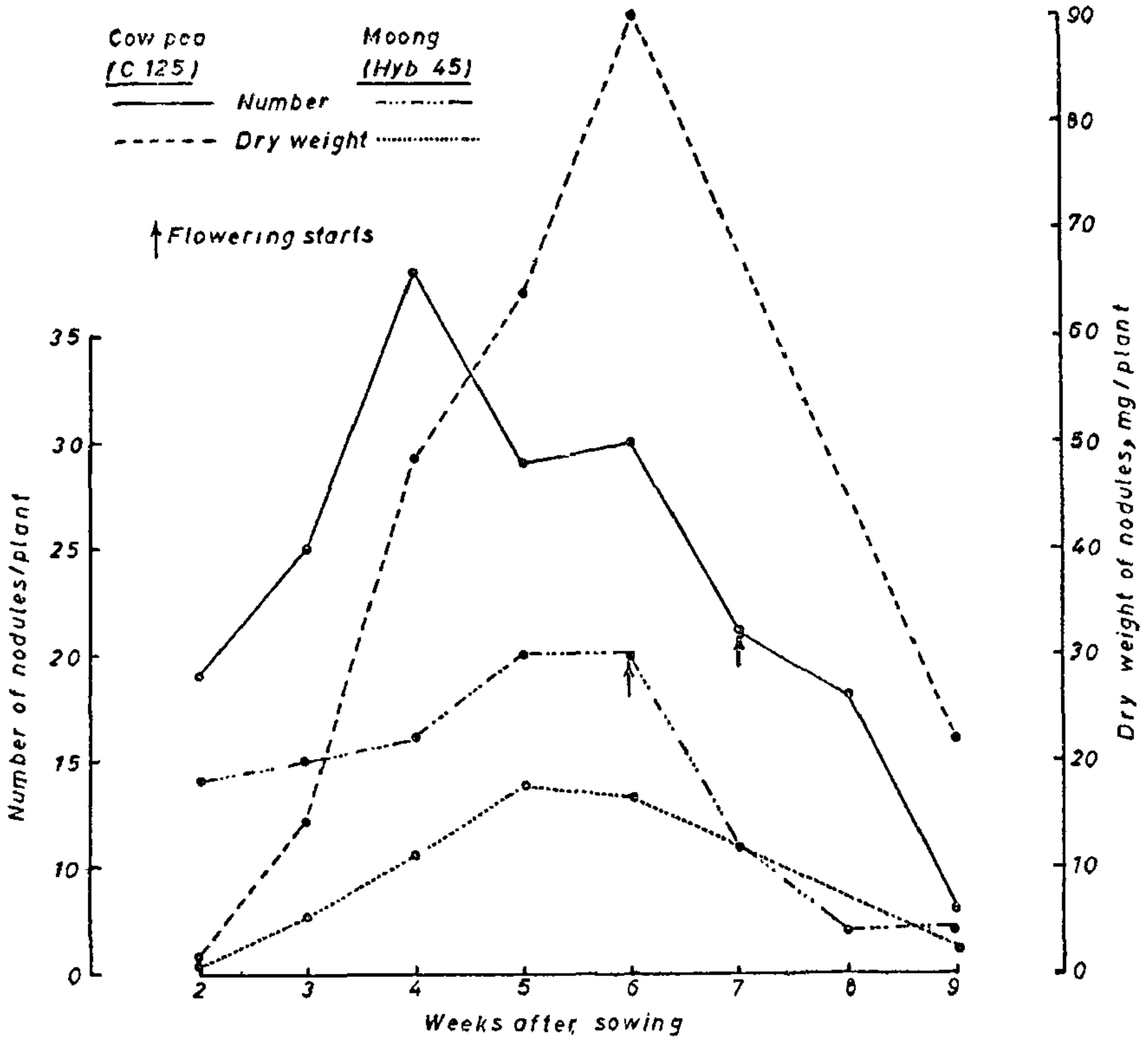

FIG. 2. Nodule number and weight during growth in cowpea and mung.

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NODULATION OF *TRIFOLIUM ALEXANDRINUM* BY PENICILLIN TREATED *RHIZOBIUM TRIFOLII*

It has been observed by Hamatova (Personal Communication) that the efficiency of rhizobia increased when grown on a medium containing penicillin. It is also known that additions of alkaloids or high amounts of yeast extract to yeast extract mannitol (YEM) broth induced the formation of bacteroid-like cells in *in vitro* cultures of *Rhizobium*[1]. These findings prompted us to study the influence of penicillin on the morphology of *R. trifolii* and the effectiveness of penicillin treated bacterial cells in nodulating Egyptian clover or

berseem (*T. alexandrinum*). In one of the treatments, the normal content of yeast extract in YEM medium (0·1%) was raised to 0·35% and in the other treatment penicillin (as benzyl chloride) was added at 500 I.U./ml to study the effects on morphology of cells (light microscopy), growth (turbidimetry) and viability (plate counts) of *R. trifolii*. Unwashed cells and cells washed repeatedly by centrifugation with normal sterile saline water (to get rid of the antibiotic) were used separately to inoculate berseem seedlings grown on Jensen's nitrate-free agar slopes[2] incubated in an illuminated growth room (22° C ± 1° C; 12 hr photoperiod) under bacteriologically controlled conditions.

The results revealed that penicillin induced the production of bacteroid-like cells reminiscent of the earlier observation with yeast extract[1]. Growth was exponential upto 6 days although less in yeast extract and penicillin added cultures. However, viability of cells was reduced linearly after 24 hr (Fig. 1). Interestingly enough, it was noticed that

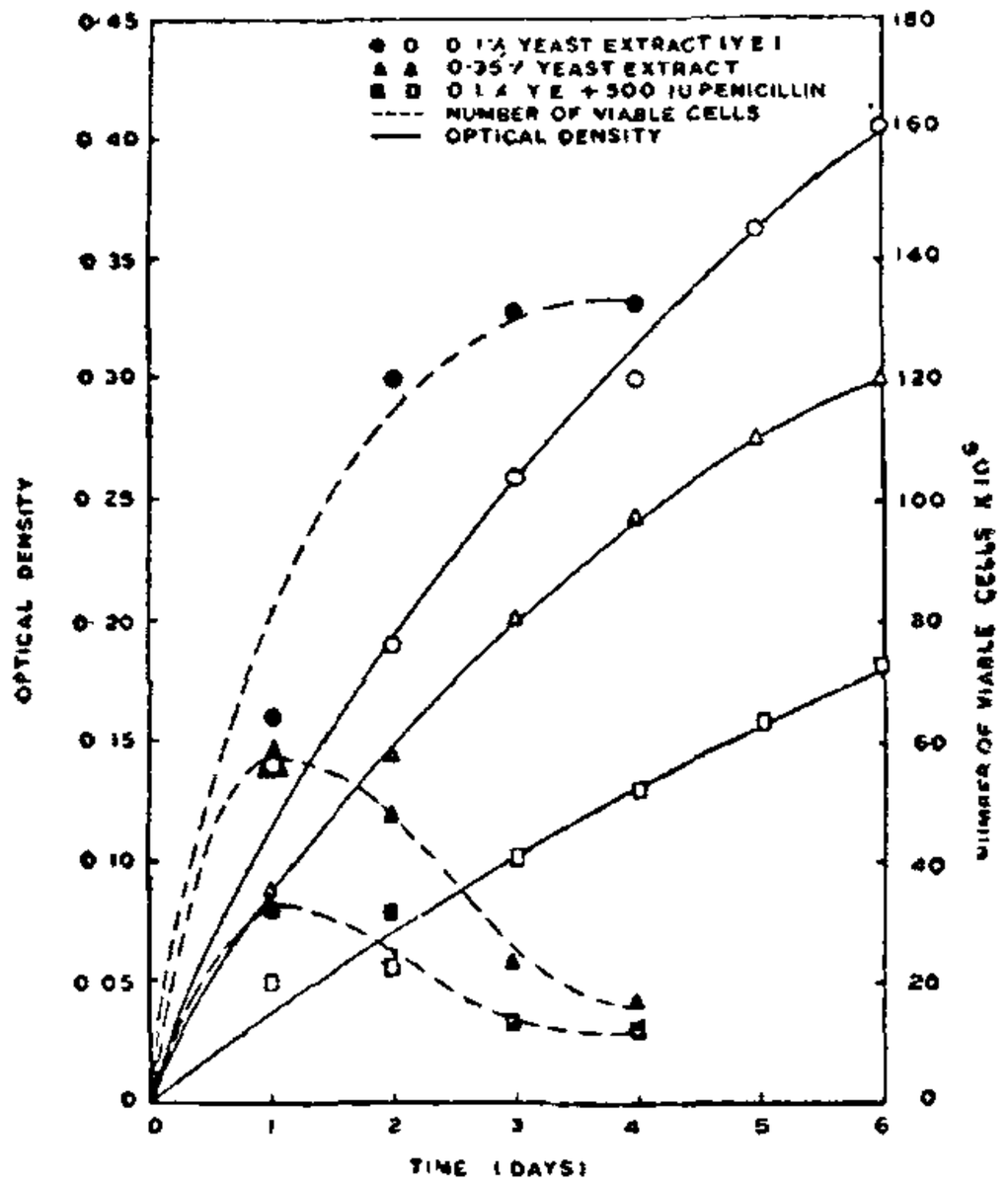

FIG. 1. Growth and viability of *R. trifolii* in the presence of yeast extract and penicillin.

penicillin-treated cells produced more number of nodules resulting in better seedling growth than the untreated bacteria. There was, however, no difference between washed and unwashed cells in their nodulation effects indicating that the residual penicillin had no additional influence (Table I) In our studies, penicillin-treated *Rhizobium* cells (at 500 I.U./ml) grew well after subculturing on penicillin-free medium thereby precluding the

TABLE I

Effect of pretreatment of R. trifolii *(for 72 hrs) with penicillin on growth and nodulation of* T. alexandrinum *(mean of 12 replicates) Data taken at the end of 30 days of plant growth*

Penicillin (I.U.)	Nodule number		Plant length (cm)		Plant fresh weight (mg)	
	A	B	A	B	A	B
0	4·2	5·3	24·0	19·8	120·0	101·0
100	5·2	C	26·0	C	139·0	C
300	6·4*	9·8*	23·2	21·7	114·0	112·0
500	7·4*	8·8*	25·8	28·0*	154·0*	154·0*
* C.D. at 5%	2·0	3·5	4·0	4·4	30·0	42·0

A—in seedlings inoculated with washed bacterial cells; B—in seedlings inoculated with unwashed bacterial cells; C—not tried.

mutagenic effects of the antibiotic on the bacterium. On the other hand, penicillin is known to inhibit specific steps involved in cell wall synthesis in bacteria[3]. Certain physico-chemical changes in the cell wall of root hairs mediated by bacterial activity at the locus of infection have been postulated earlier[4] and it is likely that loosening of the cell walls of *Rhizobium* by penicillin treatment might aid in successful entry of bacteria at many loci on the root system leading to better nodulation.

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OCCURRENCE OF *NUMMULITES PENGARONENSIS* VERBEEK, AN INDO-PACIFIC LARGER FORAMINIFERA, IN THE MIDDLE EOCENE FULRA LIMESTONE OF CUTCH, GUJARAT

TERTIARY rocks ranging in age from Palaeocene to Pliocene are well exposed in the coastal strip of the mainland of Cutch, Gujarat[1]. Here, the Middle Eocene part of the succession is highly fossiliferous and contains abundant larger and smaller foraminifers. Larger foraminifers which constitute the dominant element in the fauna are represented by several stratigraphically significant genera including the most common Lower Tertiary genus *Nummulites* Lamarck. Since Sowerby's[2] publication on the Tertiary fossils from Cutch, several species of *Nummulites* have been reported by workers[3-5]