

liquor-2, Na<sub>2</sub>HPO<sub>4</sub>-3, KH<sub>2</sub>PO<sub>4</sub>-0.9, pH-7) for 24 hr in a rotary shaker at 28°C. The substrate cholesterol acetate 200 µg/ml (dissolved in alcohol) was added to the growing cells and was allowed to incubate for another 24 hr. One control which received no substrate was also maintained. The culture filtrate was extracted with methylene chloride (3 × 100 ml). Methylene-chloride extracts were washed with aqueous sodium bicarbonate (5%, w/v) followed by washing with distilled water, and drying over anhydrous sodium sulphate and finally the solvent was evaporated. A semi-solid mass was obtained only from the experimental flask while control flasks yielded only an oily residue. The semi-solid mass was then purified by column chromatography and crystallised (solvents: benzene 30%, pet-ether 70%). The bioconverted mass was also acetylated by pyridine and acetic anhydride and purified.

The homogeneity of the bioconverted material was tested on silica gel G plates which showed single spot having *R<sub>f</sub>* of 0.64 different from that of the starting material which had an *R<sub>f</sub>* of 0.75 and the control furnished practically nothing. The purified bioconverted material showed m.p.-148°C, quite similar to cholesterol, hence the bioconverted material was acetylated. The purified acetylated material showed single spot on TLC having *R<sub>f</sub>* of 0.75, identical to that of authentic cholesterol acetate. Hence to ascertain the identity of the bioconverted product the m.p. of acetylated product was determined on H<sub>2</sub>SO<sub>4</sub> bath, which was 115°C, exactly identical to that of the authentic acetylated product. On the basis of these observations the product was identified as cholesterol thus establishing the fact that cholesterol acetate was completely deacetylated to cholesterol by a bacterial strain belonging to *Bacillus* group. Such deesterification performed by bacterial enzymes seems to be quite interesting and promising.

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## EFFECT OF RELATIVE HUMIDITY ON SURVIVAL OF SUSPENDED POLLENS OF *TYPHA ANGUSTATA*

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POLLENS of anemophilus plants are subjected to various stress conditions during their transit through atmosphere. Very little information is available on the effect of various factors on suspended pollens<sup>1,2</sup>. In this communication, the effect of humidity on survival of suspended pollens of *Typha angustata* is presented.

Fresh *T. angustata* male inflorescences, at dehiscing stage were used as source of pollens. A sealed glass and wood-stirred settling chamber of 60 × 30 × 68 cm was employed for suspension of pollens<sup>3</sup>. This chamber was adjusted to 100% RH by exposing it to water trays overnight and to 0% RH by exposing it to anhydrous CaCl<sub>2</sub> trays (BDH, Glaxo Lab., Bombay) overnight. Pollen samples (25 mg) are introduced from the port situated at the top of chamber, and stirred for 2 min by the fan, fitted to the chamber. Samples of settling pollens are collected by exposing sticky microscope slides through the sampling port.

Pollen viability is determined by using 1% 2,3,5-triphenyl tetrazolium chloride<sup>4</sup> reaction (TTC). Pollens collected on the slides are washed with distilled water, concentrated by low speed centrifuge (1000 rpm) for 5 min and treated with 1% TTC for 1 hr, recentrifuged and observed under microscope. Stained (viable) and unstained (non-viable) pollens are counted. The results are tabulated in table 1 and figure 1.

**Table 1** Survival of pollen (% viability) under atmospheric humidity (62% RH), 100% & 0% RH

Time of sampling (hr)	% viability (average of 3 trials)		
	RH 0%	Atmospheric	100% RH
1	96.2	89.7	87.5
2	92.6	74.3	66.9
3	89.9	66.8	47.8
4	85.0	54.2	35.7
5	80.7	39.8	22.4

As can be seen from data presented *T. angustata*, pollen survival is directly affected by RH changes. Possible explanation can be that at 0% RH values, metabolic activities of pollens are suppressed due to loss of moisture and thus increasing the survival rate.

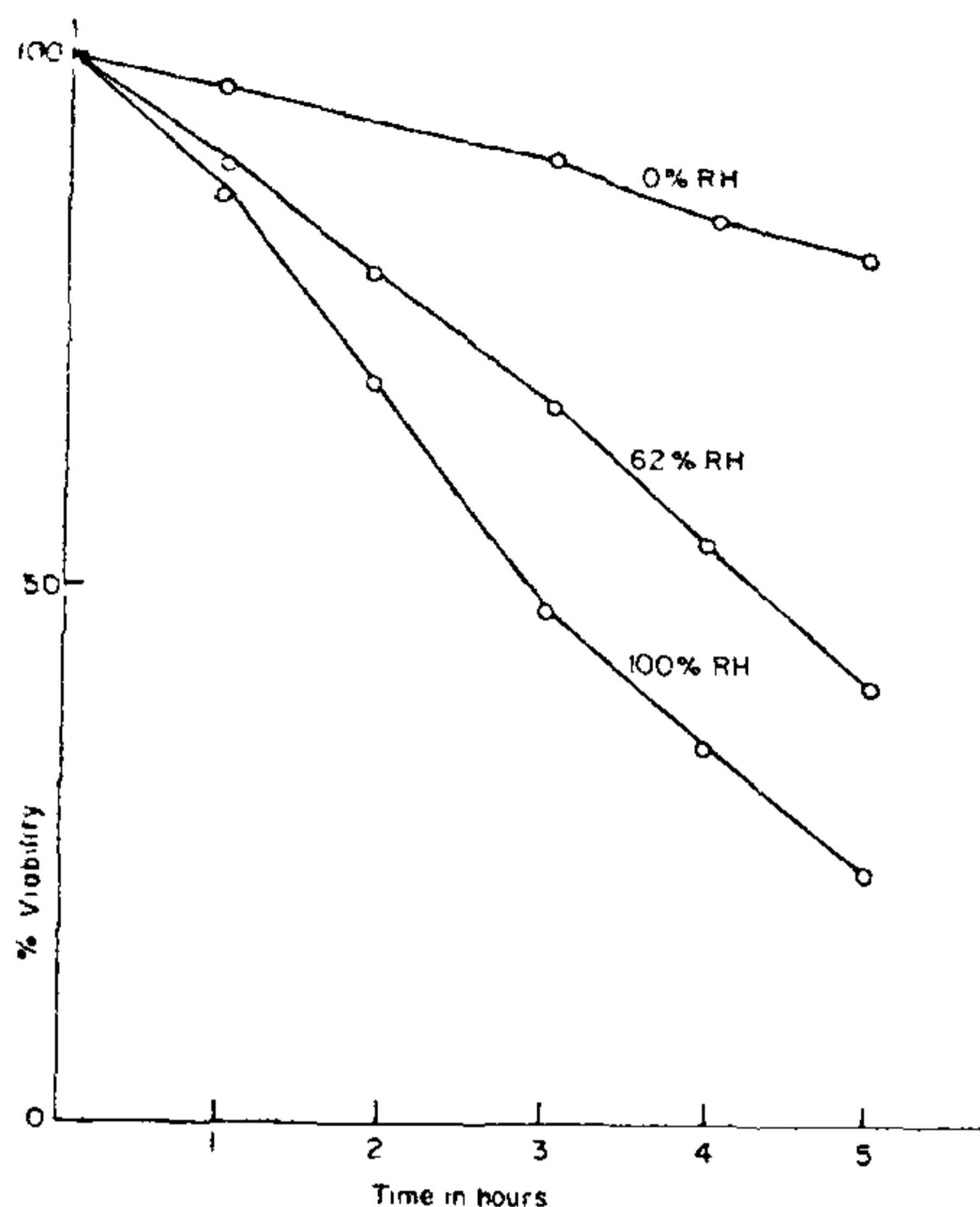


Figure 1. Survival of suspended *Typha* pollen in stirred chamber under controlled humidity.

Implications of this effect are difficult to assess in in-field conditions as humidity fluctuations are difficult to assess.

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## THE NEED FOR *RHIZOBIUM PHASEOLI* INOCULATION TO ESTABLISH AMERICAN BEANS IN INDIA

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ALMOST all the species of *Phaseolus*, indigenous to India, have been included in the genus *Vigna*<sup>1</sup>. This classification has been supported by studies in this laboratory concerning the host-group specificity in legume-*Rhizobium* symbiosis<sup>2</sup>. These studies show that all the species of *Vigna*, placed earlier in the genus *Phaseolus*, are indeed nodulated by rhizobia of the cowpea miscellany group (*Rhizobium* sp.), whereas the true species of *Phaseolus* are nodulated only by rhizobia of the bean group (*R. phaseoli* Dangeard). These species of *Phaseolus* are commonly known as the 'American beans' or 'temperate beans', some of which have been introduced in India and are unsuccessfully cultivated during the cooler part of the year in plains and in the warmer part of the year in the hills.

The foregoing presentation relates to studies carried out to show that rhizobia specific to the introduced temperate beans of the *Phaseolus* group are virtually absent from Indian soils, and inoculation with specific strains of *Rhizobium phaseoli* is necessary to overcome one of the limitations in the establishment of American beans in India.

Thirteen types of soil collected from various locations in India, having diverse agroclimates were amended with superphosphate (100 kg P<sub>2</sub>O<sub>5</sub>/ha), and used for nodulation tests in pots. The pots were seeded with the following beans: *Phaseolus coccineus* Linn. var. scarlet runner, *P. lathyroides*, Linn. var. phasmy bean, *P. lunatus*, var. limabean and *P. vulgaris*, vars., black prince, french bean, giant springles, kentucky wonder, plentiful and tender green.

The results showed that nodulation was virtually absent in plants grown in all the soils except for stray occurrence of ineffective tiny nodules on roots due to the promiscuous nature of indigenous strains of rhizobia. Such ineffective symbiosis has been reported by several workers<sup>2,3</sup>.

A second set of pot trials was done by planting seeds of *P. vulgaris* var. french bean, inoculated with three different strains of bean-rhizobia (*R. phaseoli*) using the slurry inoculation method. The local strain VPKAS, was supplied by Vivekananda Laboratory for Hill Agriculture, Almora (U.P.) and strains CIAT 166 and 255 were obtained from Centro Internacional de