

Figure 3b. UV spectrum of toluene utilization by eight strains of hydrocarbonoclastic bacteria (see text). 2 is the spectrum of toluene in MSM (1% v/v).

eventual oil spillage. Further studies in this direction are in progress.

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ACKNOWLEDGEMENTS. We are grateful to the reviewer/s for critical comments and improvement of the manuscript.

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## A low-cost culture vessel for *in vitro* culture of plants

The use of tissue culture in crop improvement, afforestation and micro-propagation in the ornamental horticulture industry is well documented<sup>1</sup>. One of the major difficulties in expanding propagation techniques to other crop areas is the production cost. Indeed, many tissue culture results are interesting from an academic standpoint, but they are not of much benefit to industries because of the high economic cost. Non-availability of glassware restricts the research progress in developing countries. Mass production of photoautotrophic organisms was successfully carried out by using poly-bag bottles<sup>2</sup>. We report here the proposed design of poly-bag bottle aimed at cutting the cost of *in vitro* propagation of plants.

Polypropylene bag of dimensions (26.0 × 16.5 cm) was brought from the local market. A collar (dia 3.0 cm height 2.5 cm) was made by cutting a piece from a cylindrical tube of polyvinyl chloride. The polypropylene bag was inserted into the collar. Then the cotton

plug was inserted into the mouth of the poly-bag bottle after inoculation of media. For convenience, the mouth of the poly-bag bottle can be widened by changing the diameter of the collar.

Young tobacco (*Nicotiana tabacum* L.) leaves were cut into small pieces (0.4 cm<sup>2</sup>), surface sterilized with 0.1% sodium hypochloride and 50% alcohol, washed in sterile water three times and used as explants for callus initiation. The explants were transferred to poly-bag bottle and conical flasks containing 30-40 ml of MS medium<sup>3</sup> supplemented with 2% sucrose, 1 mg/l 2,4-D, 0.2 mg/l BAP and solidified with 0.7% agar. Callus was grown 5 days in dark at 25°C, RH 50-60% and then with 12 h photo-periods. Light intensity was 37 mE m<sup>2</sup> s<sup>-1</sup>.

Dissected embryos (half) of green gram (*Phaseolus radiates* L.), corn, (*Sorghum vulgare* L.) and citrus (*Citrus indica* L.) were sterilized with 0.1% hypo chloride and 50% alcohol for a few seconds and washed three times in sterile water. Poly-

bag bottles were steam sterilized with 30-40 ml of culture medium containing MS salts for 20 min. Sterile embryos were placed on the medium and incubated at 25 ± 1°C under fluorescent light with 12 h photo periods. Callus and seedlings were also raised in 500 ml conical flasks for comparison.

Plant tissue culture is used to achieve many different objectives which require in common the growth of microbe-free plant material in an aseptic environment. Callus culture of tobacco was successfully carried out with poly-bag bottle (Figure 1a). Several manipulated skills may be applied to get expected results in various aspects of tissue culture with poly-bag bottle and the latter can be used for mass propagation in plant tissue culture industry with low labour cost input. As shown in the photograph (Figure 1b), seedlings can grow without any fungal/bacterial contamination in poly-bag bottles. The advantages of poly-bag bottles over glassware in *in vitro* culture of plants are listed in Table 1.



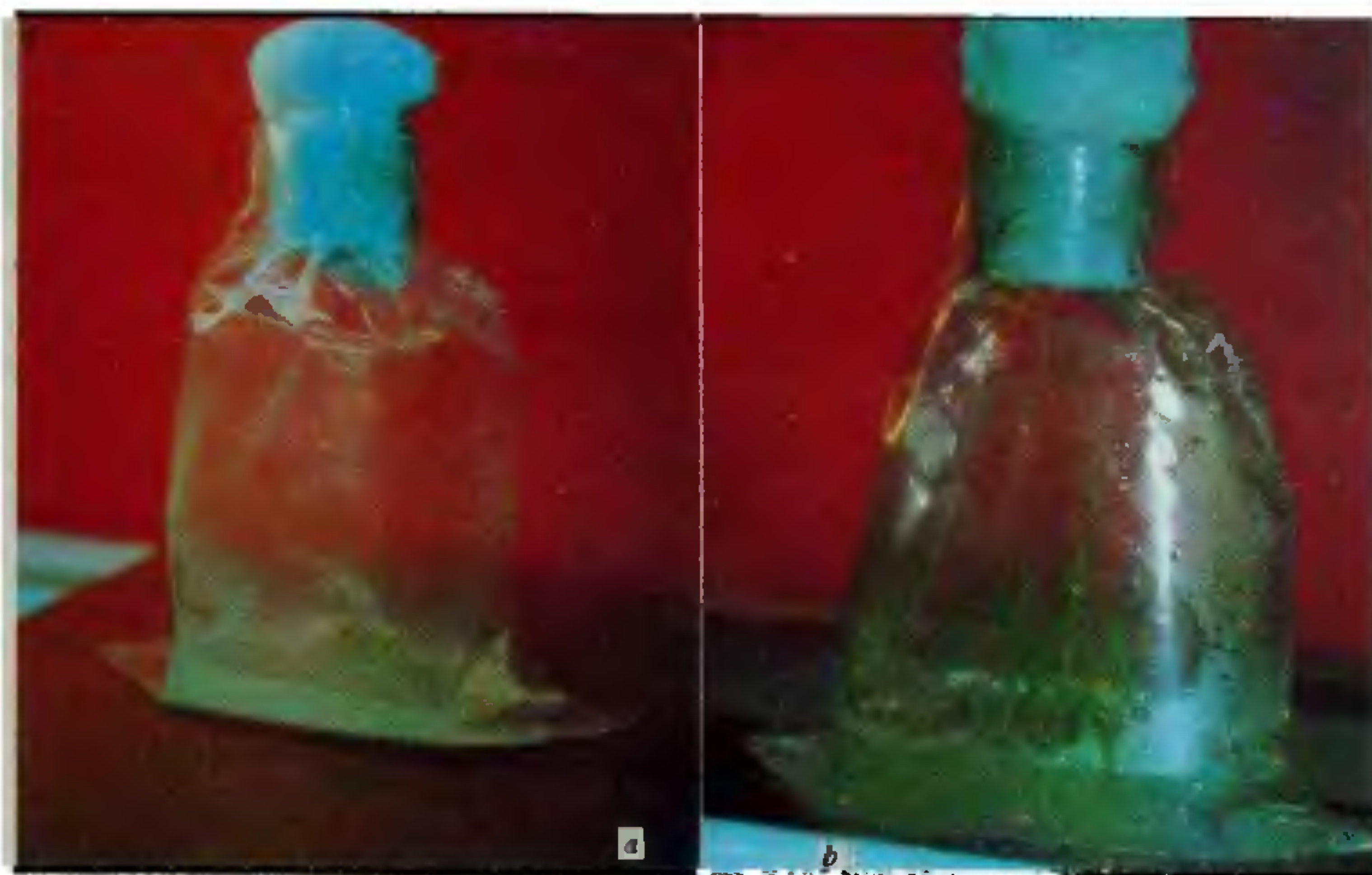


Figure 1. *a*, Tobacco callus growing in the poly-bag bottle. *b*, mass production of aseptic (corn) seedlings for *in vitro* culture studies.

Table 1. Advantages of poly-bag bottles in tissue culture

Poly-bag bottle	Glassware
Low-cost	High-cost
Unbreakable	Breakable
Flexible (more number of bags can be accommodated per unit area)	Not flexible
Sterilizable (120°C)	Yes
No additional labour cost for cleaning as the bags are used new	Additional labour cost is necessary for cleaning for reuse
To protect the environment, the used poly-bag bottle (if contaminated or after use) with medium can be sealed and disposed	Not possible

Following this work, attempts will be made to realize the use of poly-bag bottles in various aspects such as (i) post-tissue culture aspects such as shoot multiplication, rooting and acclimation, (ii) micro-propagation of florist flower crops and (iii) storage of valuable germplasm. The used poly-bag bottle with medium (if contaminated/after use) can be sealed, autoclaved and disposed, without causing any hazard to the environment.

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## Comments on 'A critical appraisal of the type locality of a rare palm from Kumaon Himalaya, India'

In the article referred to above, Rana *et al.* (*Curr. Sci.*, 1995, 68, 590–592) have raised queries about the existence of *Trachycarpus takil* Becc. in wild state in Indian flora, its type locality and its affinity. As a part of the DST-sponsored project on the status of endemic and endangered palms of India, we had an opportunity to track this palm in 1993 in Kumaon Himalayas in collaboration with Botanical Survey of India, Northern Circle,

Dehra Dun. A brief resume of our observations is given below to clarify the queries raised by Rana *et al.* for the benefit of naturalists interested in this endemic endangered taxon of Himalayan flora.

The widely growing population of this palm is found in Badkot forest (about 2000 m) between Pandavkuli and Badkot of Almora district. The palms grow along the slopes of the limestone mountain under the shades of *Quercus dialata*

associated with *Rhododendron arboreum*, *Ilex* sp., *Acer* sp., *Berberis aristata*, *Paeonia emodi*, etc. They are 5–6 m high; their stems are tightly covered with a network of persistent leaf-sheath fibres up to the base; a 6–12 leaved crown lies above the cover of persistent reflexed green older leaves.

The palms are distantly distributed. Seeds or seedlings were very infrequent on the forest floor. The palms are locally