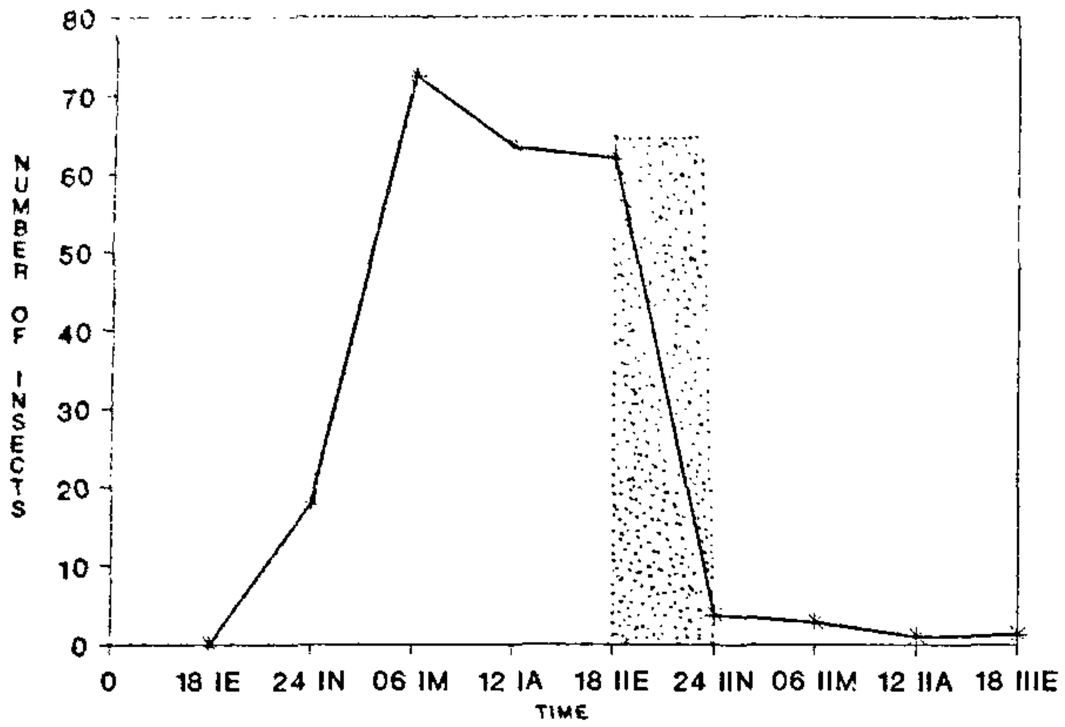

Figure 2. Diel movement of density of insects in the blossom: stippled area reveals the period of pollen rain (E = evening, N = night, M = morning, A = afternoon).

and nocturnal in pollination. In this character it resembles the beetle pollinated *P. margaritiferum*. Since the blossom trapped the blood-sucking female midges, its floral biology is also interesting from the angle of population studies.

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Photoautotrophic shoot culture: An economical alternative for the production of total alkaloid from *Catharanthus roseus* (L.) G. Don.

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An attempt was made to device an economical alternative for the production of medicinally important indole alkaloids in tissue culture of *Catharanthus roseus* (L.) G. Don. Photoautotrophic shoot cultures were established in liquid medium with cotton fibre as a supporting agent in an indigenously designed culture vessel. Autotrophic cultures, which have the potential of a cost-effective system, produce 10% more total alkaloid as compared to mixotrophic cultures.

CULTURED plant tissues usually grow in a mixotrophic mode which use both CO_2 from air and organic carbon source (mostly sucrose) from the medium. In principle, photoautotrophic shoot culture does not require any sugar in the medium, and uses CO_2 as the sole carbon source. Carbon metabolism is essential to all cells and the nature of carbon source (sugar or CO_2) may affect secondary metabolism and the production of useful compounds[1].

Photoautotrophic shoot cultures of *Catharanthus roseus* (L.) G. Don. have not previously been reported. Interest in photoautotrophy stems from the study of high value indole alkaloids in shoot cultures of periwinkle. The dimeric alkaloids, vincristine and vinblastine could be extracted from mixotrophic shoot cultures, but the yield is very low, possibly because of improper development of chloroplasts due to altered carbon metabolism. Autotrophic shoot cultures contain well-developed chloroplasts. The possibility that would stimulate normal metabolism in leaves and synthesize and accumulate these compounds in higher amounts[2] was apparent.

A two-tier vessel was constructed indigenously with two 250 ml conical flasks according to Husemann and Barz[3] with minor modifications (Figure 1). The upper compartment functions as culture vessel, where the *in vitro* raised shoots[4] were kept on cotton support suspended in 60 ml of sugar-free Murashige and Skoog's (MS)[5] liquid medium supplemented with 6-benzyl aminopurine (0.2 mg/l) and naphtheleneacetic acid (0.1 mg/l). Three multiple shoots, each containing three shoots per clump were used as inoculum.

The lower compartment contained 50 ml of a 2 M $KHCO_3$–K_2CO_3 buffer mixture for enriching CO_2 (2% v/v) in the gaseous atmosphere of the entire culture vessel as suggested by Johnson *et al.*[6]. All openings of the culture vessel were tightly closed with cotton plugs followed by sealing with aluminium foil and finally with cling film to check gas exchange. The culture vessels containing photoautotrophic cultures were incubated in 16 h photoperiod under the light intensity of 42–45 $\mu mol\ m^{-2}\ s^{-1}$ and temperature of $25 \pm 2°C$. The

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Figure 1. Autotrophic shoot cultures of *Catharanthus roseus* growing in a two-tier culture vessel.

buffer system was renewed and the plants were subcultured after every three to five weeks.

- The total alkaloid from the biomass was extracted with 95% ethanol[7] and estimated spectrophotometrically as reported by Tu and Li[8].

It was observed that the cost of cotton support is nearly one fourth as compared to agar used as a supporting agent. Nutrient diffusion through cotton support (using liquid media) is reportedly better than that on agar[9]. The contaminants present in the agar, when released, can affect the culture of plant tissues[10].

Photoautotrophic cultures are considered to be costly because of the additional expenses in CO_2 enrichment and increased lighting. However, in practice the cost of CO_2 enrichment is reported to be not very significant[11].

In mixotrophic culture, plants need to be subcultured after every 18 days. In autotrophic cultures plants withstand up to five weeks in unchanged environment. This eliminates the cost of manpower for subculturing. A reduction in the number of vessels for autotrophic culture (half of the number as compared with mixotrophic culture) could be the added advantage.

In our experiment, a 16-hour photoperiod enhanced the growth of plantlets compared to continuous illumination. This could reduce the cost of lighting in conformity with the earlier reports[11]. Photoautotrophic cultures produced 10% more total alkaloid compared to mixotrophic cultures (Table 1).

Contaminations were practically none in photoautotrophic culture system as has been reported earlier[12]. Cultures contaminated with fungi when used as the source of explants, showed good growth in autotrophic

Table 1. Growth and total alkaloid content in mixotrophic and photoautotrophic shoot cultures

Parameter	Mixotrophic	Photoautotrophic
Shoot length (cm)	6.0 ± 1.5	6.0 ± 1.2
Number of leaf/shoot	12 ± 2.5	11 ± 1.5
Number of multiple shoots/inoculum	3 ± 1.5	6 ± 1.5
Fresh wt/inoculum (mg)	650 ± 0.003	600 ± 0.003
Dry wt/inoculum (mg)	85.2 ± 2.3	76.2 ± 2.8
Total alkaloid (µg fresh weight)	446.73 ± 0.55	500.25 ± 1.2

Data recorded after five weeks in case of photoautotrophic culture and three weeks for mixotrophic culture. Each value (mean ± SD) is an average of five replicates. Fresh and dry weights are represented per shoot basis.

mode of nutrition. This finding has much potential for cost reduction in plant tissue cultures.

The cost of autotrophic culture vessels was more than that of culture vessels used for mixotrophic shoot culture. This could be reduced to a large extent if polypropylene material were used for making these culture vessels instead of conical flasks (Borosil) used in the present study. This could also minimize the cost of repairing or replacing damaged culture vessels.

Shoot culture systems are attractive alternatives for the production of high value alkaloids[13]. Callus or cell suspension cultures were not promising approaches. For the production of leaf alkaloids[14], organ differentiation is a prerequisite for the formation of alkaloids of leaf origin[14]. Autotrophic mode of nutrition might possibly play a role in the formation of these alkaloids. In this context, photoautotrophic organ cultures could be a promising and cost-effective approach towards the production of these high-value low-volume alkaloids.

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