heterocyst frequency of 8.9 and 6% respectively, compared with values of 4–5% in the other isolates. This observation further substantiates the importance of heterocysts in nitrogen fixation. Fairly young (3-day) and very old (30-day) cultures showed lower ARA than those in log phase (7-day), suggesting that the enzyme nitrogenase is synthesized significantly more in the log phase. ARA was also altered by changes in medium and culture conditions (data not shown), suggesting that the history of cultivation and age of cultures play an important role in the regulation of ARA (nitrogen fixation) in blue-green algae.

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## TROPANE ALKALOIDS FROM *LYCIUM BARBARUM* LINN., *IN VIVO* AND *IN VITRO*

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TROPANE alkaloids, which are pharmacologically important compounds, have been reported from solanaceous plants such as *Atropa*[1,2], *Hyoscyamus*[3,4], *Datura*[5,6], *Duboisia*[7] and *Scopolia*[8]. However, there is no report on the production of tropane alkaloids from intact plant parts and in tissue culture of *Lycium barbarum* of an Indian arid zone. Therefore the present investigation was undertaken.

Plant materials (roots, shoots and fruits) were freshly collected from Davi Kund Sagar, Bikaner. The voucher specimen of *L. barbarum* was collected from the Department of Botany, University of Jodhpur, Jodhpur and deposited at the same place. Unorganized tissue of *L. barbarum* was established from seedlings on Murashige and Skoog's[9] medium supplemented with 5 ppm of kinetin. The growth index (GI) of each of the tissue samples was calculated from

$$GI = \frac{\text{Final dry weight of tissue} - \text{initial dry weight of tissue}}{\text{Initial dry weight of tissue}}$$

The various plant parts and 6-week-old tissues at maximum GI (7.5) were dried and ground to a fine powder. The powdered material was extracted with chloroform for 24 h. Thin-layer chromatography (silica gel G; chloroform: methanol: ammonia, 30:60:2) gave two spots in all the plant parts and tissue samples tested. The spots were visualised under UV light (254 nm) and developed by spraying with modified Dragendorff's reagent[10] and heating at 100°C until the characteristic colours developed. The spots corresponded with reference atropine ($R_f$ 0.35, dark orange) and hyoscyamine ($R_f$ 0.36, light orange). The alkaloids present in the extracts were obtained by preparative (0.4 to 0.5 mm) TLC on silica gel plates eluted with chloroform. Each isolated compound was crystallized and further analysed for m.p., m.m.p. and IR spectra, and compared with the authentic sample for confirmation. The various extracts were subjected to quantitative estimation by the procedure of Feldman and Robb[11] for atropine and the method of Gaur[4] for hyoscyamine. Five replicates were examined in each case, and mean and 95% confidence limits obtained.

The callus tissue was hairy, compact in texture, and whitish-green in pigmentation. The maximum GI observed was 7.5 in a six-week-old tissue. Tropane alkaloids in each of the plant parts and in 6-week-old tissue at maximum GI were identified as atropine and hyoscyamine. Atropine and hyoscyamine were confirmed by m.p. (atropine, 117–118°C and hyoscyamine, 108°C), m.m.p. (undepressed) and IR spectroscopy.

Total alkaloid was nearly the same in shoots and fruits lower in calli, and still lower in roots (table 1). Fruits had the highest atropine content and shoots the highest hyoscyamine content.

**Table 1** *Tropane alkaloid content (per cent dry weight) of Lycium barbarum in vivo and in vitro*

| | Alkaloid content* (%) | | | |
|---|---|---|---|---|
| Alkaloid | Roots | Shoots | Fruits | Callus |
| Atropine | 0.42 ± 0.07 | 0.93 ± 0.02 | 0.95 ± 0.04 | 0.74 ± 0.02 |
| Hyoscyamine | 0.25 ± 0.02 | 0.33 ± 0.02 | 0.29 ± 0.01 | 0.09 ± 0.05 |
| Total alkaloid | 0.67 ± 0.04 | 1.26 ± 0.02 | 1.24 ± 0.02 | 0.83 ± 0.03 |

*Mean and 95% confidence limits.

Mitra[1] and Khanna *et al.*[2] have estimated the atropine content of roots (0.45%) and seedling callus cultures (0.53%) of *A. belladonna* respectively. Hocking[3] showed the presence of alkaloids in roots (0.08–0.11%), stems (0.01–0.025%), leaves (0.04–0.08%), flower tips (0.07–0.10%), seeds (0.06–0.10%) and whole herbs (0.02–0.08%) of *H. niger*. Gaur[4] showed significant amounts of hyoscine (0.019%) and hyoscyamine (0.57%) in seedling callus cultures of *H. niger*. Tropane alkaloids have also been reported from the seeds (0.32%) of *D. innoxia*[5]. Prabhakar *et al.*[6] have described the commercial production of tropane alkaloids such as hyoscine from the leaves and seeds of *D. innoxia*, *D. metal* and *D. fastuosa*.

The present results show that yields of tropane alkaloids from tissue culture are lower than those from intact plant parts and are dependent on added growth factors in the medium.

The fruits of *L. barbarum* are widely eaten by camels and goats in the Indian arid zone. Studies on the effects of these alkaloids on these animals will be interesting.

*L. barbarum* growing in Indian arid zone is a new source of tropane alkaloid. The presence of high amounts of atropine in the fruits of the intact plant is of great commercial importance and merits the attention of the pharmacological industry.

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## PRELIMINARY REPORT ON CARDIAC DEPRESSANT EFFECT OF *HEMIDISCUS HARDMANNIANUS* (BACILLARIOPHYCEAE)

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IN recent years, investigations of marine plants and animals for useful drugs have become increasingly important. At present no diatom has been shown to be a potential source of drugs other than antibiotics. *Hemidiscus hardmannianus* is a common diatom in the Indian seas. It was tested for possible cardiac activity.

*H. hardmannianus* was collected from the mouth of

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