indicating thereby that the browning is due to the direct effect of temperature on enzyme activity.

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1. Burton, K. S., *Mushroom J.*, 1986, **158**, 68.
2. Rai, R. D. and Saxena, S., *Curr. Sci.*, 1988, **57**, 434.
3. Saxena, S. and Rai, R. D., *Mushroom J. Tropics*, 1988, **8**, 15.
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
5. Singh, M., Singh, S. S. and Sanwal, G. G., *Indian J. Exp. Biol.*, 1978, **16**, 712.
6. Moore, S. and Stein, W. H., *J. Biol. Chem.*, 1948, **176**, 367.
7. Spiro, R. G., *Methods Enzymol.*, 1966, **8**, 3.
8. Davis, N. C. and Smith, E. L., *Methods Enzymol.*, 1955, **2**, 215.
9. Hammond, J. B. W. and Nichols, R., *Ann. Appl. Biol.*, 1977, **85**, 162.
10. Eby, D. L., McCardle, F. J. and Beelman, R. B., *J. Fd. Sci.*, 1977, **42**, 22.
11. Murr, D. P. and Morris, L. L., *J. Am. Soc. Hortic. Sci.*, 1975, **100**, 16.
12. Goodenough, P. W. and Ricketts, V., *Ann. Appl. Biol.* 1977, **85**, 447.

ALKALOID PRODUCTION BY THE IMMOBILIZED CELLS OF *SOLANUM XANTHOCARPUM*

J. SUBRAMANI, P. N. BHATT and A. R. MEHTA
Department of Botany, The M S. University of Baroda, Baroda 390 002, India.

HIGHER plants produce a variety of secondary metabolites, some of which are an indispensable source of important pharmaceuticals. However, due to the uncontrolled exploitation of natural habitat and the fluctuations in the supply of raw materials the production of secondary compounds by plant cell cultures *in vitro* may prove a viable alternative[1]. It is also possible to increase the secondary metabolite production in cell cultures by various methods. However, when scaling-up of production technology from culture vessels to industrial fermentor is attempted, plant cells pose a series of problems, some of which are: instability of production, susceptibility to shearing force, lack of secretion of active principle in the medium and contamination[2]. These problems could be overcome by immobilizing the cells by entrapping them in an inert matrix and suspending them in the medium. Due to these advantages, we decided to use the immobilized cells of *Solanum xanthocarpum* to produce steroidal alkaloid called solasodine which is a good substitute for diosgenin from *Dioscorea* tubers for the synthesis of corticosteroidal drugs[3]. In this communication we present data on viability and the solasodine production by immobilized cells from the suspension culture of *S. xanthocarpum*.

A batch culture of cell suspension of *S. xanthocarpum* was generated from the leaf callus, developed on Murashige and Skoog's (MS) medium[4] containing 3% w/v sucrose, 0.025% w/v casein hydrolysate, 0.5 ppm, 2,4-D and 0.1 ppm Kn.

About 12–14 month old cell suspension cultures maintained through regular subculturing (once in twelve days) were used for analysis. Five milliliter cell suspension (250–280 mg) was pippetted into 30 ml of fresh culture medium and the cells were harvested at three-day-intervals for fresh weight and dry weight determination. Three replicates were used for each observation and the experiment was repeated (figure 1B).

Our initial experience on the use of various matrices to immobilize *S. xanthocarpum* cells indicated that sodium alginate is a better matrix than agar-agar or agarose as it gave sustained production of the alkaloid for 20 days[5]. Therefore in the present experiment only sodium alginate was used for the immobilization experiment using the protocol of Brodelius[6]. Briefly, the cell suspension of lag, exponential and stationary phases was filtered separately through a sterile stainless steel mesh of 0.7 mm^2. At least 2 g cells in 10 ml medium of each growth phase were mixed separately with an equal volume of 2% (w/v) aqueous solution of sodium alginate. This cell suspension-alginate mixture was pippetted through a wide mouth (4 mm dia) 10 ml pippette into sterile 30 mM aqueous calcium chloride solution.

Beads of 4–5 mm in diameter formed instantly at this step and were left in this solution for 15 min for hardening. Finally, the beads were incubated in 250 ml Erlenmayer flask that contained 10 ml of suspension culture medium. Six sets for each type of cells were prepared. Of the six sets, three flasks were used to determine cell viability. From the remaining

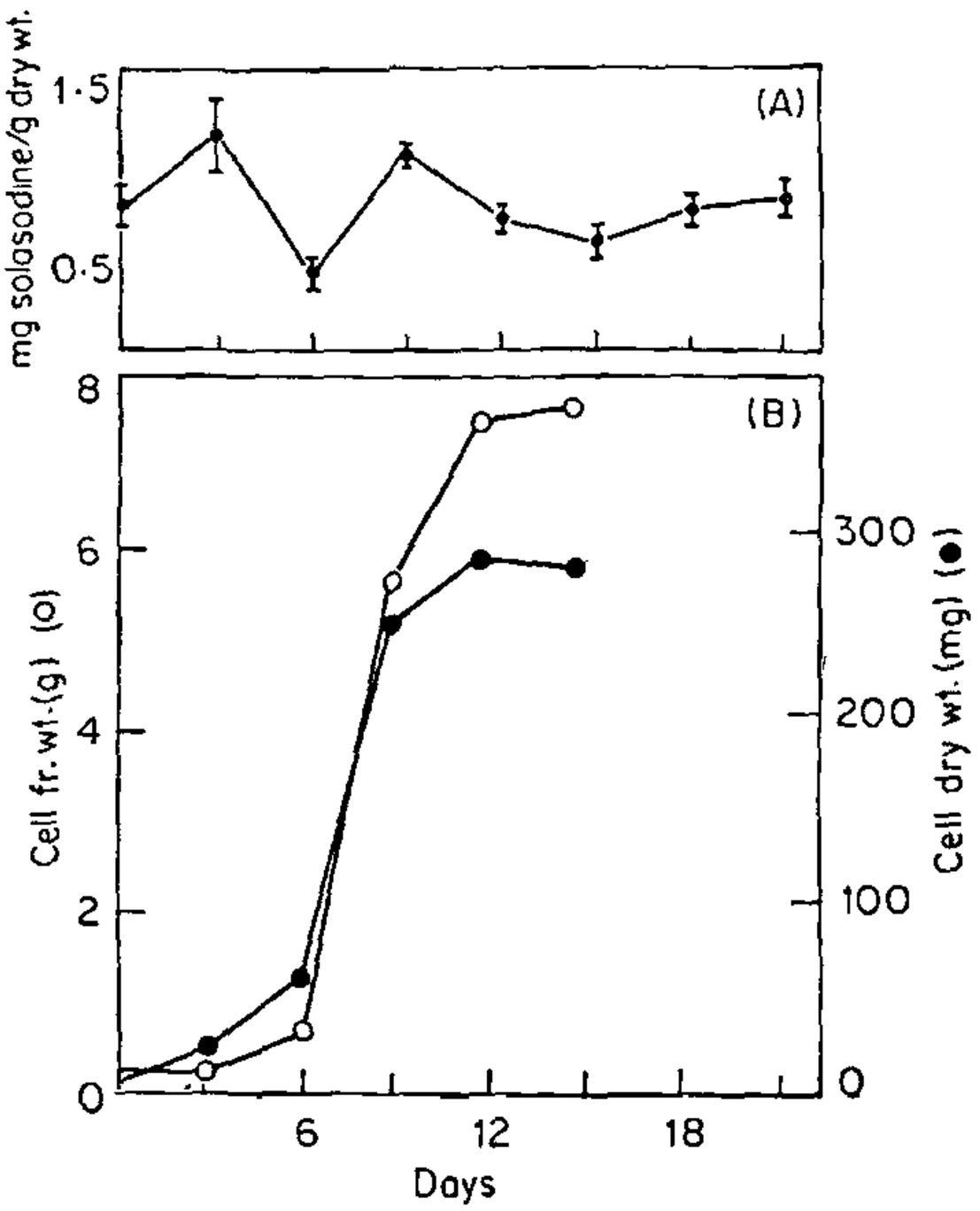

Figure 1A, B. **A.** Changes in content of steroidal alkaloid in cell suspension cultures of *Solanum xanthocarpum*, and **B.** Growth kinetics of cell suspension culture.

three flasks, culture medium drawn every six days for the solasodine analysis was replaced by 10 ml of fresh MS medium of the same composition. The viability and the alkaloid content were analysed till the cell viability dropped to 30% (on day 30) in immobilized stationary phase cell culture and till the disintegration of the alginate beads (on day 60) in immobilized lag and exponential phase cultures.

About 10 beads from each flask were removed at six-day intervals and treated with 5 ml of 0.2 M potassium phosphate buffer pH 7.5 for 5–7 min to release the cells. Viability of the cells was determined using fluorescein-diacetate method of Widholm[7].

The spent medium (10 ml) of the immobilized cells was removed at six-day intervals and the amount of solasodine was determined spectrophotometrically using the procedure of Birner[8]. The presence of solasodine was confirmed on silica gel TLC plate by chromatography with an authentic standard using the three different solvent systems as described by Bhatt *et al*[9].

Immature berries of pale yellow colour (1–1.5 cm diameter) and the cells of suspension culture were dried at 80°C to a constant weight and analysed for solasodine content as described above.

Among all plant organs of *S. xanthocarpum* maximum accumulation of solasodine was reported in the immature berries[10]. Therefore only immature berries were used to determine solasodine. About 3.66 mg solasodine/g dry weight of berries was measured. Changes in solasodine content in batch cultures are shown in figure 1A. Maximum solasodine content of 1.25 mg per g dry weight of cells was detected in 3-day-old and 9-day-old cultures.

Time course data on per cent viable cells in alginate beads and the content of solasodine released in the media by the immobilized cells are shown in table 1. Sustained (up to 60 days) and high degree of viability (as much as 64%) of immobilized cells was noticed when the cells of exponential phase of suspension culture were used. Viability was lowest (only 30% at the end of 24 days) when cells of

Table 1 *Viability and solasodine content of immobilized cells and in the incubated medium*

		Cell viability and alkaloid content on day										
		0	6	12	18	24	30	36	42	48	54	60
Early lag phase (3-days-old)	% Cell viability	90	71	63	48	43	51	52	55	59	61	61
	Solasodine μg/10 ml medium	—	17	8.7	5.75	2.5	0	1.25	9	3.5	0	0
		Total = 47.7 μg/100 ml medium										
Exponential phase (9-days-old)	% Cell viability	92	78	72	57	53	55	61	59	60	64	64
	Solasodine μg/10 ml medium	—	24.6	19.3	29.3	3.5	6.3	4.8	5.3	1.1	1.3	0
		Total = 95.5 μg/100 ml medium										
Stationary phase (15-days-old)	% Cell viability	86	64	51	46	30	---------- dead ----------					
	Solasodine μg/10 ml medium	—	39	24.3	13.6	3.7						
		Total = 80.6 μg/40 ml medium										

stationary phase from the suspension culture were used. Further cell division did occur albeit at a slower rate in the immobilized cells of lag and exponential phase cultures, as was evident by the formation of callus over the surface of alginate beads and the beads started disintegrating after 60 days of incubation. Immobilized stationary phase cells did not show this behaviour indicating very low or no cell division in them during immobilization. Our observation agrees with those of Jones and Veliky[11]

The yield of solasodine in the spent medium used for cell immobilization was higher in stationary phase culture (80.6 μg solasodine in 40 ml of medium in 24 days) compared to that in the media used for immobilization of exponential phase cell suspension where it is 95.5 μg per 100 ml medium over 60 days. Solasodine content within the immobilized cells of all three types, viz. lag, exponential and stationary phase suspension cultures was 2–2.2 fold greater than freely suspended cells of suspension culture of identical stages respectively.

Clearly this explains the increased capacity for alkaloid production in the immobilized condition by the cells compared to their freely suspended state, indicating the potential of this technique for large scale production of secondary metabolites of plants.

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1. Berlin, J., In: *Biotechnology*, (eds) H. J. Rehm and G. Reed, VCH Publishers, 1986, Vol. 4, p. 636.
2. Fowler, M. W., In: *Plant biotechnology*, (eds) S. H. Mantell and H. Smith, Cambridge University Press, London, 1983, p. 8.
3. Mann, J. D., In: *Advances in agronomy*, (ed) N. C. Brady, Academic Press, New York, 1978, p. 207.
4. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473.
5. Subramani, J., Phale, P., Bhatt, P. N. and Mehta, A. R., *Proc. Int. Workshop on Tissue Culture and Biotechnology of Medicinal and Aromatic Plants*, Lucknow, 14–16, December, 1987, (in press).
6. Brodelius, P., Mosbach, K. and Zenk, M. H., *FEBS Lett.*, 1979, **103**, 93.
7. Widholm, J. M., *Stain Technol.*, 1972, **47**, 189.
8. Birner, J., *J. Pharm. Sci*, 1969, **58**, 258.
9. Bhatt, P. N., Bhatt, D. P. and Sussex, I. M., *Physiol. Plant.*, 1983, **57**, 159.
10. Weiler, E. W., Kruger, and Zenk, M. H., *Planta Med.*, 1980, **39**, 112.
11. Jones, A. and Veliky, I. A., *Can. J. Bot.*, 1981, **59**, 2095.

AFLATOXIN CONTAMINATION IN SEEDS OF MEDICINAL VALUE

VINEETA KUMARI, H. K. CHOURASIA and A. K. ROY
Department of Botany, Bhagalpur University, Bhagalpur 812 007, India.

AFLATOXIN contamination in seeds of various agricultural commodities[1-3] has been reported from different parts of India and abroad. However, occurrence of aflatoxin in seeds of medicinal value has not been reported so far from India. Earlier reports[4,5] stated that the traditional methods adopted for the storage of medicinal seed samples led to the association of moulds with them. In the present communication, aflatoxin-producing potential of *Aspergillus flavus* isolates and aflatoxin contamination in seeds of two plants, viz. *Argyreia speciosa* and *Embelia ribes*, which have carminative, alterative, anthelmintic and stimulant activity, have been studied.

Ten different samples of each seed were collected from storage centres of private concerns located in plateau regions of Bihar. One hundred seeds of each sample were plated on moist blotting paper[6] and the growing fungi were isolated and identified under a Nikon stereomicroscope. The aflatoxin-producing potential of *A. flavus* isolates was tested in SMKY liquid medium[7]. All the samples were observed under long-wave UV light for the characteristic fluorescence[8] and the samples that fluoresced were extracted chemically to determine the natural occurrence of aflatoxins[9]. Aflatoxins were qualitatively detected on thin-layer chromatography plates[10] and were quantified by spectrophotometry[11]. The results are presented in table 1. The table also gives (last column) natural occurrence of aflatoxin B_1 in the two seeds. Ten different samples each of *A. speciosa* and *E. ribes* were screened for natural aflatoxin contamination. Six samples of