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SEASONAL VARIATION OF STRYCHNINE AND BRUCINE IN VEGETATIVE PARTS OF *STRYCHNOS NUX-VOMICA*

J. Bandopadhyay and B. De*

Pharmacognosy Research Laboratory, Department of Botany, University of Calcutta,
35, Ballygunge Circular Road, Calcutta 700 019, India

ABSTRACT

Seasonal variations of strychnine and brucine in different vegetative parts of Strychnos nux-vomica *were determined by HPLC. Root bark and stem bark are rich in these two alkaloids. Root wood, stem wood, and twigs are poor sources of strychnine and brucine. In the stem bark, stem wood, and leaves, the alkaloids gradually decrease in amount up to December (winter). In the root bark, there is a tendency towards a gradual increase in these alkaloids up to December.*

INTRODUCTION

Strychnine and brucine, two important indole alkaloids, are used as a central nervous system stimulant and as an alcohol denaturant respectively. Strychnine is a valuable tool in physiologic and neuroanatomic research (Tyler *et al.*, 1988). Out of about 185–190 species of the genus *Strychnos* (Loganiaceae) (Bisset *et al.*, 1973), strychnine and brucine have only been found in six species. These are *S. nux-vomica* L., *S. wallichiana* Steudel ex DC., *S. ignatii* Berg., *S. lucida* R. Br. in Asia, *S. icaja* Bail in Africa and *S. panamensis* Seem in Central America (Marini-Bettolo *et al.*, 1972).

S. nux-vomica is one of the commercial sources of these two alkaloids (Tyler *et al.*, 1988). The plant also contains a number of other minor alkaloids (Evans, 1994).

Baser and Bisset (1982) investigated variations in content and composition of tertiary alkaloid mixtures on the basis of 12 consecutive monthly collections in different parts of Sri Lanka (Gampaha) and Indonesian (Bogar) *S. nux-vomica* by visual assessment of the relative sizes and intensities of the spots on Si gel plates. In the present paper we report for the first time seasonal variation of strychnine and brucine in different vegetative parts of *S. nux-vomica*. The investigation was carried out with plant parts collected during the period from February to December, 1989.

Keywords: Brucine, indole alkaloid, HPLC, seasonal variation, strychnine, *Strychnos nux-vomica.*

* Author to whom correspondence should be addressed.

MATERIALS AND METHODS

Plant materials were collected between February and December 1989 from Chinsurah (District Hooghly), India. Voucher specimens are kept in the Department of Botany, University of Calcutta.

Extraction of Alkaloids

Alkaloids were extracted following the general method of alkaloid extraction. 1 g powered material was moistened with 25% NH_4OH and extracted in a Soxhlet apparatus with $CHCl_3$ for 2 h. The $CHCl_3$ was extracted with 2% aqueous sulphuric acid (× 3).

The combined acid extracts were alkalized with 25% NH_4OH solution to pH 10 and then extracted with $CHCl_3$ (× 3). The combined organic layers were dried over anhydrous sodium sulphate to dryness and then analysed by HPLC.

HPLC Analysis

The HPLC system consisted of a Milipore-Waters (Milford, MA, USA) Model 510 pumping system, a Waters Model 440 injector and a Waters Model 440 absorbance detector. A Waters C_{18} Bondapak Column (30 cm × 3.9 mm I.D.) was used. The mobile phase was MeOH:2M NH_4OH:M NH_4NO_3 :: 28:1:0.5 (mod-

Table 1. Average maximum and minimum temperature, total rainfall and mean relative humidity in Baghati (Dist. Hooghly, India) during January – December 1989*.

	Average temperature °C		Total rainfall (mm)	Mean Relative Humidity %	
Month	Maximum	Minimum		3.00 A.M.	12.00
January	26.06	10.90	0.0	80.06	36.06
February	28.70	14.90	0.0	77.70	63.60
March	33.40	19.60	0.0	81.00	79.08
April	37.30	24.20	0.0	87.90	67.40
May	37.20	27.90	87.9	88.80	72.80
June	32.50	25.40	166.2	87.60	81.70
July	32.20	24.20	273.2	85.03	84.40
August	33.60	25.90	142.1	88.00	86.30
September	31.05	25.70	191.4	91.10	83.90
October	30.90	24.30	186.3	95.40	85.20
November	30.06	18.20	0.0	88.80	74.70
December	25.90	14.05	12.0	86.90	64.06

*Data provided by Regional Meteorology Centre, Calcutta.

ified solvent system of Dennis, 1984). The flow rate was 2 ml/min. The eluate was monitored at 254 nm.

Calibration

Aliquots of 5, 10, 15, 20 and 25 µl of standard stock solution of strychnine and brucine prepared by dissolving 5 mg of the alkaloids in 25 ml $CHCl_3$ were injected. Regression equations for strychnine and brucine were obtained from peak height (y) and concentration (x). Concentrations of strychnine and brucine were calculated from regression equations prepared from peak height (y) and concentrations (x) of authentic alkaloids:

Strychnine: regression equation $y = 18.3x + 0.1$, correlation coefficient 0.991; brucine: regression equation $y = 5.46x + 1.27$, correlation co-efficient 0.998.

Data presented are means of analyses from duplicate extractions ± standard deviation.

RESULTS AND DISCUSSION

Monthly variations of average maximum and minimum temperature, average humidity and total rainfall are shown in Table 1.

Strychnine and brucine showed seasonal variation in all the vegetative plant parts examined. Root bark and stem bark are rich in strychnine and brucine (Figs. 1, 2). Root wood, stem wood and twig are poor sources of these two alkaloids (Figs. 3, 4, 5). Highest strychnine content detected was 5.4 mg/g dry weight of root bark collected in the month of December and highest brucine content detected is 10.12 mg/g dry weight of stem bark collected in the month of February. The amount of strychnine in the vegetative plant parts is lower than that recommended in the Pharmacopoeias (Pharmacopoeia of India, 1966; British Pharma-

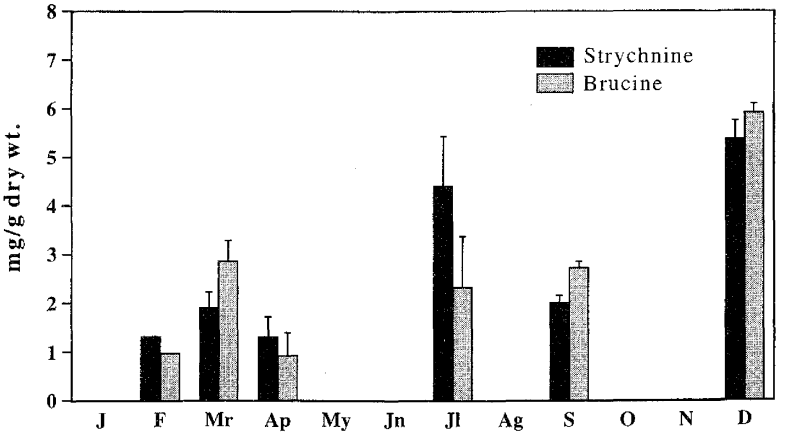

Fig. 1. Variations of strychnine and brucine contents in root bark of *S. nux-vomica*. J = January, F = February, Mr = March, Ap = April, My = May, Jn = June, Jl = July, Ag = August, S = September, O = October, N = November, D = December.

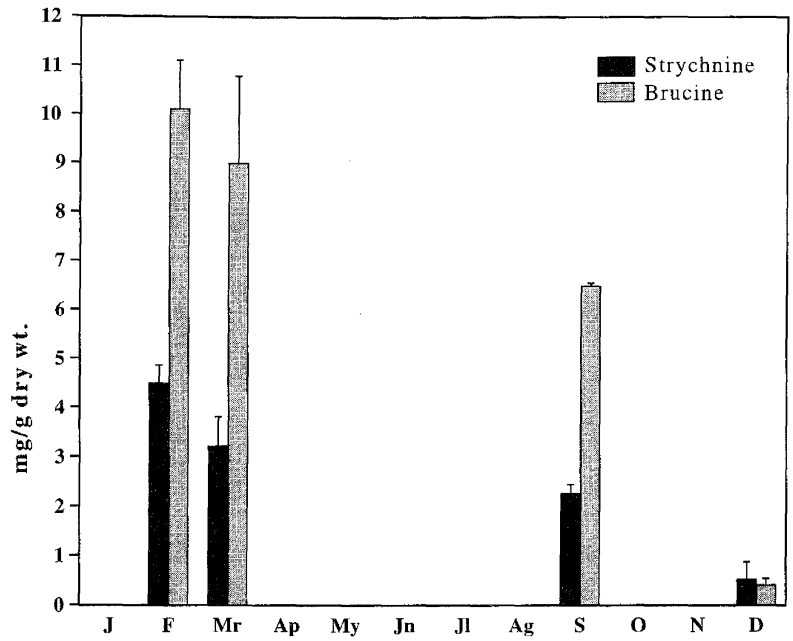

Fig. 2. Variations of strychnine and brucine contents in stem bark of *S. nux-vomica*.

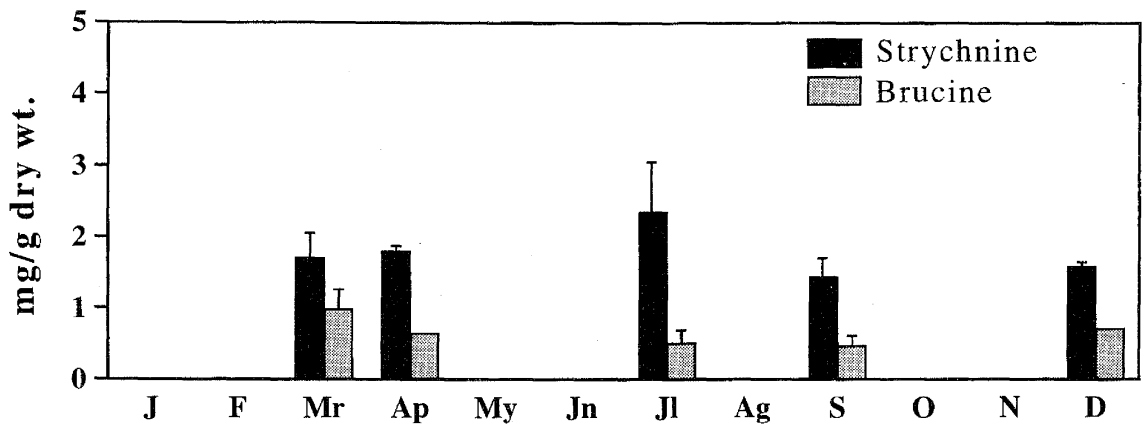

Fig. 3. Variations of strychnine and brucine contents in the root wood of *S. nux-vomica*.

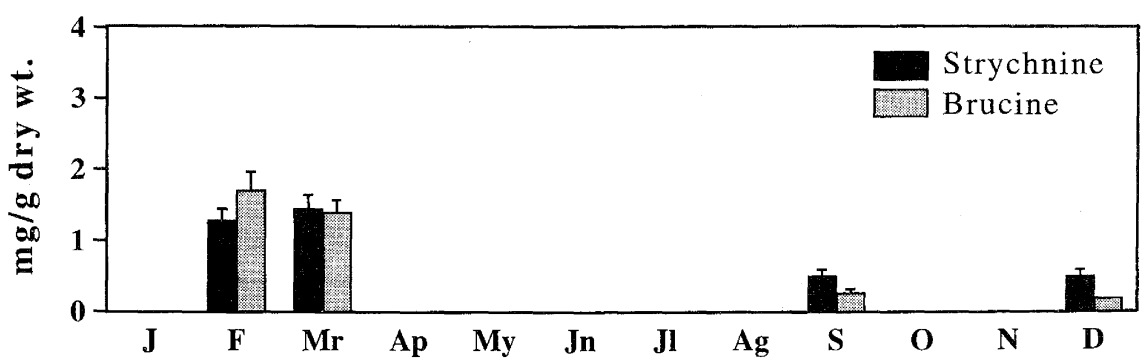

Fig. 4. Variations of strychnine and brucine contents in the stem wood of *S. nux-vomica*.

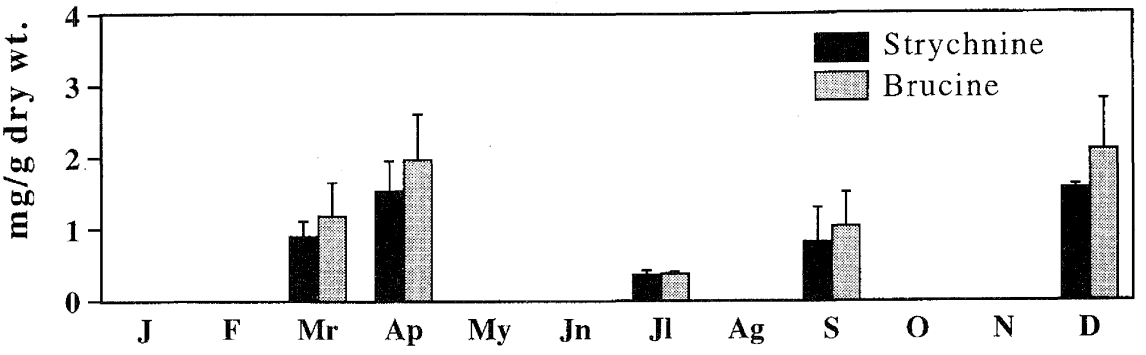

Fig. 5. Variations of strychnine and brucine contents in the twig of *S. nux-vomica*.

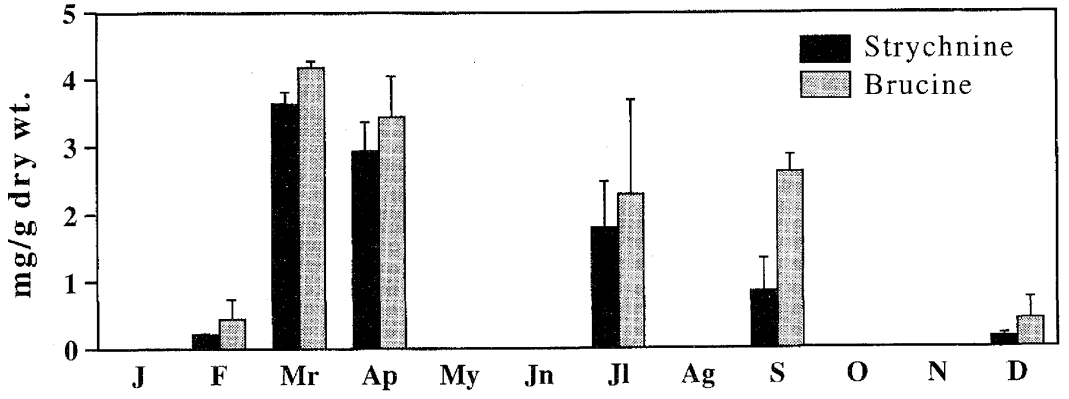

Fig. 6. Variations of strychnine and brucine contents in the leaves of *S. nux-vomica*.

copoeia, 1973) for seeds which are the chief commercial sources of strychnine and brucine (The Wealth of India, 1976).

In the leaves, variations in strychnine and brucine contents show a direct relationship with leaf maturation (Fig. 6). The concentrations of these two alkaloids are high in the month of March (spring) after the new leaves appear on the almost leafless plant after winter. The contents then gradually decrease in amount and are lowest in December when the leaves start falling from the plant with the onset of winter. In the month of February leaves of the previous year show a very low amount of strychnine and brucine. From the limited data it appears that the stem wood and stem bark also show the same patterns of variation of strychnine and brucine which, being high during spring (February/March), gradually decrease in quantity approaching winter (December) (Figs. 2, 4). Throughout the year brucine is present in higher quantity than strychnine in the stem bark, twigs, and leaves.

We have observed a reverse pattern of seasonal variation of strychnine and brucine in the root bark. Although the root bark shows ups and downs in alkaloid content in different seasons, there is a tendency towards a gradual increase up to December (Fig. 1). In the root bark and root wood strychnine is present in comparatively high quantity in July (rainy season). The twig, however, shows a different picture (Fig. 5). Here strychnine and brucine are present in lowest quantity in the month of July (rainy season), gradually increasing up to December and decreasing in March (flowering time) and then rising in April. Further work is required to ascertain whether these results are typical for all years.

It has been suggested for the Asian *Strychnos* species (Bisset and Phillipson, 1976) that on transportation from the root, which is the main site of alkaloid synthesis, to leaves, the alkaloids of the normal series become converted via the *N* oxides to the corresponding bases of the pseudo- and then *N*-methyl-*sec.*-pseudo series. Seasonal

variation of minor indole alkaloids of *S. nux-vomica* are under study. Seasonal conversion of strychnine and brucine (alkaloids of normal series) to the corresponding bases of pseudo- and *N*-methyl-*sec.*-pseudo series is yet to be proved in this plant.

ACKNOWLEDGEMENT

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