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## Effect of Spacing and Nitrogen on Growth and Yield of Manioc, *Manihot esculenta* Crantz Grown as an Intercrop under Coconut

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(Paper accepted : 14 January 1980)

**Abstract :** An experiment was conducted at the Coconut Research Institute of Sri Lanka, Lunuwila, to study the effect of spacing and nitrogen application on the growth and yield of manioc when grown as an intercrop under coconut. Level of nitrogen had no effect on tuber fresh weight yield or dry matter accumulation. Spacing had a marked effect on tuber fresh weight yield and  $0.91 \times 0.61$  m spacing was significantly superior to the wider spacings tested. The reduction in leaf, stem and tuber dry matter yields and leaf area index (L) after 8 months of planting indicated that variety MU 72 reaches maturity and is ready for harvesting at this stage. Total and tuber dry matter yields were linearly correlated with leaf area duration (D) showing that leaf area is the major determinant of crop yield.

### 1. Introduction

Owing to the wide range of economic and agronomic advantages of intercropping coconut lands, it is being increasingly recognized in almost all the coconut growing countries as against the outmoded practices of monoculture. Of the many crops that could be cultivated as intercrops under coconut, manioc appears to be one of the most promising crops. It is a popular root crop among many farmers. This popularity is on account of its tuber yield which could be obtained with comparatively low capital expenditure, minimum possible management practices needed by the crops, the longer period for which the crops could be left in the soil with little or no loss of weight, etc. The crop is also remarkable in its adaptation and when established could withstand periods of severe drought which would have adverse effects on most of the crops. It is able to make use of the carbohydrates in the roots and make a rapid start when the moisture is available. It could also be grown on a wide range of soil types and could be filled into poor soils which gives it a comparative advantage over most of the other food crops. There are no serious problems in its culture and it is relatively free of pest and disease incidences. The demand for manioc is predominant as a food crop and most of the manioc produced locally is utilized for human consumption. Manioc could be used as a raw material for a variety of products such as animal feed, starch for laundry work, paper and textile sizing and painting, production of power alcohol, adhesives, beer and liquid glucose. Thus, it could form the basis for a number of agro-based industries and these could create new avenues of employment particularly in the villages where unemployment is a serious problem.

Although intercropping coconut with manioc has been an age old practice, it had been done without any scientific foundation particularly with reference to agronomic practices when this crop is grown in association with coconut. Due to poor culture, the yield of manioc has been very low. The trial reported in this paper is a preliminary attempt to investigate into the effect of plant spacing and nitrogen application on the growth and yield of manioc, and these cultural practices are regarded as being the most important in increasing the yield of manioc when grown under coconut.

## 2. Materials and Methods

The experiment was carried out during May, 1973 to March, 1974 under a mature stand of coconut of about 60 years planted on the square system at a spacing of  $7.92 \times 7.92$  m at Bandirippuwa Estate of the Coconut Research Institute of Sri Lanka, Lunuwila.

The soil was a deep, well drained, brownish to yellow, to yellowish sandy clay loam, with lateritic gravel occurring at an average depth of 50.8 cm. Soil is classified under the great soil group of Red Yellow Podsolc soils.

Climatic data is given in Table 1. In general, weather conditions were satisfactory for plant growth until end of December, 1973. Thereafter, from beginning of January to mid February the crop experienced a period of severe drought during which the rainfall receipts amounted to only 0.05 cm.

TABLE 1. Climatic data, May 1973 — March 1974.

Period	Rainfall, cm	Temperature, °C.	
		Maximum	Minimum
01 — 15	8.2	31.8	25.6
16 — 31	8.7	31.0	25.2
01 — 15	18.6	30.2	25.2
16 — 30	5.6	30.5	25.6
01 — 15	9.7	30.2	24.4
16 — 31	2.1	30.9	25.1
01 — 15	0.8	29.6	24.7
16 — 31	5.8	30.0	25.1
01 — 15	0.5	30.6	23.7
16 — 30	1.6	30.8	25.1
01 — 15	3.8	31.0	24.4
16 — 31	20.8	30.2	23.3
01 — 15	38.4	29.9	22.3
16 — 30	9.1	31.1	22.2
01 — 15	6.9	30.7	21.9
16 — 31	13.6	28.8	21.2
01 — 15	—	30.8	19.8
16 — 31	—	31.7	20.0
01 — 15	0.05	33.2	20.4
16 — 28	1.9	32.9	21.4
01 — 15	1.5	32.6	21.8
16 — 31	6.3	32.3	23.2

The treatments consisted of 4 plant spacings ( $0.91 \times 0.61$  m,  $0.91 \times 0.91$  m,  $0.91 \times 1.21$  m and  $0.91 \times 1.52$  m) and 3 levels of nitrogen application (44.8, 67.2 and 89.6 kg N/ha). All treatment combinations were arranged in randomized blocks replicated three times. Each plot measured an area of  $15.8 \times 15.8$  m, which is equivalent to 4 coconut squares. Half of the total dressing of N as urea (46% N) was applied at the time of planting while the remainder was top dressed 60 days after planting. All plots received a basal application of 44.8 kg  $P_2O_5$ /ha as concentrated super phosphate (42%  $P_2O_5$ ) and 112 kg  $K_2O$ /ha as muriate of potash (60%  $K_2O$ ). 15 cm long cuttings of variety MU 72 containing 3 to 5 buds were planted at 2 cuttings per hill on 7th May, 1973.

The plants were sequentially sampled at monthly intervals, commencing 2 months after planting. At each sampling, a hill selected at random from each plot was sampled, except at the final harvest when 4 hills were sampled per plot. For each sample, tuber fresh weight and the dry weights of tubers, stems and leaves were determined. The leaf area was estimated by the disk method.<sup>6</sup>

### 3. Results and Discussion

**Tuber Data.** The main effects of treatments on tuber fresh weight yield are given in Table 2. Although the differences were non-significant throughout the entire period of growth there was a marked response to levels of applied nitrogen. The lower level of nitrogen reached maximum tuber fresh weight yield 6 months after planting while the intermediate and the higher levels of nitrogen recorded maximum tuber fresh weight yields 7 and 8 months after planting, respectively. Each increment of nitrogen increased tuber fresh weight yield; the higher level of nitrogen recorded the highest yield of tubers which was an increase of 14% and 27% respectively, compared with the intermediate and lower levels of nitrogen.

TABLE 2. Main effect of treatments on tuber fresh weight yield kg/ha.

Level of N	Months after planting									
	2	3	4	5	6	7	8	9	10	
44.8 kgN/ha	209	4223	7658	16992	30332	28722	32112	27958	24896	
67.2 kgN/ha	487	5038	9711	19057	30589	33994	32339	28389	25594	
89.6 kgN/ha	329	4179	11698	16489	26823	37316	38586	28905	24791	
LSD (P = 0.05)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	
Spacing										
$0.91 \times 0.61$ m	323	7656	17761	23504	47847	46191	44709	36891	40264	
$0.91 \times 0.91$ m	385	4370	9611	20981	34375	38845	45937	33541	25123	
$0.91 \times 1.21$ m	382	4009	7000	16456	23635	32470	28239	29830	23789	
$0.91 \times 1.52$ m	272	1851	4304	9109	11137	15871	18495	13405	11197	
LSD (P = 0.05)	n.s	2726.5	7537	5821	11302	11780	11897	12950	12357	

Significant differences in tuber fresh weight yields between spacing treatments were recorded throughout the entire growth except at 2 months after planting. An increase in spacing decreased tuber fresh weight yield and the highest tuber yield (47,847 kg/ha or 19 tons/acre) was recorded\* for the closer spacing (0.91 × 0.61 m). The increase in tuber fresh weight between the closest and the widest spacing (0.91 × 1.52 m) was as high as 150% ( $P = 0.01$ ) while the increase over the recommended spacing of 0.91 × 0.91 m for crops grown in monoculture was 4% ( $P = 0.05$ ). The data clearly indicated that a higher yield of tubers could be obtained by growing manioc at a closer spacing of 0.91 × 0.61 m when grown as an intercrop under coconut.

The tuber fresh weight yield decreased after 8 months of planting for all treatments. At this stage of growth leaf area index (Table 8) also decreased indicating that the variety MU 72 could be harvested around 8 months after planting.\*\* The reduction in tuber bulking and in tuber yield after the maximum has been reached may be associated with dry matter losses in respiration.

The mean tuber bulking rates were calculated over a period of 6 months from the commencement of the tuber growth by using a linear regression equation  $Y = a + bx$ , where  $Y$  = yield of tubers,  $a$  = a constant,  $b$  = slope of the regression and  $x$  = time. The lowest bulking rate was recorded for 0.91 × 1.52 m, followed by 0.91 × 0.61 m spacing, while the highest bulking rate was recorded for 0.91 × 1.21 m spacing. (Appendix, Table 3). The tuber bulking rates were linear ( $R^2 = 67.5\%$ ) however, unlike in the potato tuber, bulking rates were not correlated with the final tuber yield.<sup>2,3</sup> This may be due to the relatively long period of tuber bulking in manioc when compared with potato where the period of tuber bulking is comparatively short. However, Enyi<sup>1</sup> reported a close relationship between tuber bulking rate and tuber yield in coco yams.

TABLE 3. Main effect of treatments on tuber bulking rate.

a. Nitrogen	kg/ha/week	R <sup>2</sup> (%)
44.8 kgN/ha	956.0	73.0
67.2 kgN/ha	662.4	70.0
89.6 kgN/ha	917.3	67.5
b. Spacing		
0.91 × 0.61 m	485.3	69.5
0.91 × 0.91 m	1003.2	68.1
0.91 × 1.21 m	1133.7	76.6
0.91 × 1.52 m	458.7	68.6
LSD ( $P = 0.05$ )	N.S	

\*Such a high yield estimate perhaps is due to the small sample size.

\*\*Whether different times of planting would give similar observations as recorded here need to be investigated.

## Dry matter accumulation

Main effect of treatments on total dry matter yield is given in Table 4. There were no interactions between treatments. Nitrogen increased total dry matter yield upto 8 months after planting and then declined. Each increment of nitrogen increased total dry matter yield but the difference between levels of nitrogen were not significant. Higher level of nitrogen recorded the highest dry matter yield which was 8% and 9% more than the intermediate and lower levels of nitrogen, respectively.

TABLE 4. Main effect of treatments on total dry matter yield kg/ha.

a. Nitrogen	Months after planting									
	2	3	4	5	6	7	8	9	10	
Levels of N										
44.8 kgN/ha	982	2279	4926	8416	14343	13256	15765	12177	11774	
67.2 kgN/ha	847	2671	5185	8355	13825	14559	15862	12888	12791	
89.6 kgN/ha	952	2572	4752	8290	13184	15983	17134	13754	13206	
LSD (P = 0.05)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	
b. Spacing										
0.91 × 0.61 m	1352	3854	7338	12057	22656	20621	21534	18334	19643	
0.91 × 0.91 m	1052	2693	5618	9699	15324	16853	13559	14352	13432	
0.91 × 1.21 m	728	2125	3907	6985	11165	13318	13560	12575	11730	
0.91 × 1.52 m	604	1358	2955	4675	5992	6565	9114	6493	5557	
LSD (P = 0.05)	452	1025	1919	2659	5946	4350	5127	4036	5663	

Significant differences in total dry matter yield between spacing treatments were recorded at all sample harvests and an increase in spacing decreased total dry matter yield. 0.91 × 0.61 m spacing reached the highest dry matter yield 6 months after planting while other spacings recorded highest total dry matter yields in the 7th and 8th months after planting. The difference in total dry matter yield between the closest and the widest spacing was 148%.

Leaf dry matter yield increased upto 6 months after planting and declined for all nitrogen treatments (Table 5). Each increment of nitrogen increased leaf dry matter yield but the differences between treatments were not significant at any stage of growth. The higher level of nitrogen increased leaf dry matter yield by 10% and 12% respectively, when compared with the intermediate and the lower levels of nitrogen at 6 months after planting.

TABLE 5. Main effect of treatments on leaf dry matter yield, kg/ha.

a. Nitrogen	Months after planting.									
	2	3	4	5	6	7	8	9	10	
Level of N										
44.8 kgN/ha	226	504	703	635	1730	1354	1452	603	390	
67.2 kgN/ha	245	545	726	664	1697	1467	1660	545	363	
89.6 kgN/ha	235	558	665	678	1838	1543	1296	587	389	
LSD (P = 0.05)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	
b. Spacing										
0.91 × 0.61 m	346	827	1054	984	2834	1905	2040	807	563	
0.91 × 0.91 m	242	562	804	675	1882	1923	1992	631	506	
0.91 × 1.21 m	203	444	527	609	1527	1306	1056	575	298	
0.91 × 1.52 m	151	310	402	367	776	684	689	300	157	
LSD (P = 0.05)	104	187	N.S	208	792	529	496	283	184	

Maximum leaf dry matter for spacing treatments was recorded at 6 months after planting except for the 0.91 × 0.91 m spacing which recorded maximum leaf dry matter two months later. The maximum leaf dry matter recorded for the closest spacing (0.91 × 0.61 m) was 2834 kg/ha, which was an increase of 42%, 86% and 265% respectively when compared with the wider spacings in an increasing order. This was due to the direct effect of plant population, more plants per unit area progressively increasing the leaf dry matter yield. The decrease in leaf dry matter yield 6 months after planting was due to rapid defoliation.

Nitrogen increased stem dry matter yield upto 9 months from planting and then declined (Table 6). Significant differences between treatments were recorded at the 9th harvest taken 10 months after planting when the intermediate and the higher levels of nitrogen were significantly superior to the lower level of nitrogen (P = 0.05). The reduction in stem dry matter yield after 9 months could be regarded as an index of crop maturity as the fresh weight yield of tubers also have declined after this stage of growth.

A decrease in spacing increased stem dry matter yield due to the production of more stems per unit area. Similar to that of the level of nitrogen, the maximum stem dry matter yield for all spacings were recorded at 9 months after planting. The highest stem dry matter yield recorded for the closest spacing (0.91 × 0.61 m) was 6280 kg/ha, this being an increase of 147% compared with the widest spacing (0.91 × 1.52 m).

TABLE 6. Main effect of treatments on stem dry matter yield, kg/ha.

a. Nitrogen	Months after planting								
	2	3	4	5	6	7	8	9	10
Level of N									
44.8 kgN/ha	718	1067	2356	2944	4928	2965	3355	4002	2966
67.2 kgN/ha	533	1271	1987	3218	5176	2903	3553	4036	4296
89.6 kgN/ha	667	1275	2019	3024	4872	2844	3602	4166	4054
LSD (P = 0.05)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	744
b. Spacing									
0.91 × 0.61 m	953	1895	3094	4329	8332	4522	5536	6280	5576
0.91 × 0.91 m	720	1267	2375	3933	5594	3037	3571	4290	4508
0.91 × 1.21 m	471	904	1571	2332	3742	2246	2758	3159	2920
0.91 × 1.52 m	414	754	1442	1623	2300	1812	2149	3543	2078
LSD (P = 0.05)	355	509	737	1190	815	230	374	905	859

Nitrogen had no significant effect on tuber dry matter yield (Table 7). Highest tuber dry matter yield for all nitrogen levels were recorded at 8 months after planting. The maximum tuber dry matter yield was recorded for the highest level of nitrogen (12,592 kg/ha) was only 2% more when compared with other levels of nitrogen.

TABLE 7. Main effect of treatments on tuber dry weight, kg/ha.

a. Nitrogen	Months after planting								
	2	3	4	5	6	7	8	9	10
Level of N									
44.8 kgN/ha	38	706	1868	4838	7680	8905	11048	7542	8418
67.2 kgN/ha	69	855	2471	4474	6952	10189	9947	9307	8135
89.6 kgN/ha	50	679	2068	4574	6474	11595	12252	9002	8768
LSD (P = 0.05)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
b. Spacing									
0.91 × 0.61 m	54	1050	3190	6743	11490	14494	13958	11208	13504
0.91 × 0.91 m	62	864	2434	5061	7487	11932	14434	9433	8417
0.91 × 1.21 m	54	777	1809	4023	5896	9724	9768	8845	8513
0.91 × 1.52 m	39	294	1110	2686	2909	5069	6171	3650	3329
LSD (P = 0.05)	N.S	503	1156	1745	3713	4266	1572	3841	5492

The tuber dry matter yield increased progressively and reached a peak value between the 7th and 8th months after planting and then declined. An increase in spacing decreased tuber dry matter yield. The highest tuber dry matter yield was recorded for  $0.91 \times 0.61$  m at 7 months after planting. There was no significant difference in highest tuber dry matter yield between  $0.91 \times 0.61$  m and  $0.91 \times 0.91$  m, but both these treatments were superior to the other wider spacings. The closest spacing increased tuber dry matter yield by 135% when compared with the widest spacing ( $0.91 \times 1.52$  m).

#### Leaf area index, L.

Leaf area per unit area of land, the leaf area index was increased by nitrogen application (Table 8). The highest level of nitrogen had a maximum L value of 6.57, this being an increase of 18% and 21% when compared with the intermediate and lower levels of nitrogen.

TABLE 8. Main effect of treatments on leaf area index, L.

a. Nitrogen	Months after planting									
	2	3	4	5	6	7	8	9	10	
Level of N										
44.8 kgN/ha	0.78	1.91	2.29	2.04	4.68	5.17	5.42	1.98	1.00	
67.2 kgN/ha	0.90	2.22	2.26	2.27	4.75	5.52	5.56	2.04	1.02	
89.6 kgN/ha	0.89	2.38	2.30	2.24	5.01	6.57	5.03	2.00	1.15	
LSD (P = 0.05)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	
b. Spacing										
$0.91 \times 0.61$ m	1.28	3.54	3.38	2.96	7.96	8.30	7.67	2.56	1.47	
$0.91 \times 0.91$ m	0.79	2.19	2.72	2.45	5.00	7.49	6.42	2.35	1.28	
$0.91 \times 1.21$ m	0.74	1.56	1.64	1.98	3.85	4.96	4.19	1.90	0.84	
$0.91 \times 1.52$ m	0.62	1.38	1.48	1.33	2.44	3.26	3.07	1.22	0.64	
LSD (P = 0.05)	0.40	1.00	0.89	0.69	2.26	2.25	1.72	N.S	0.54	

The closest spacing ( $0.91 \times 0.61$  m) had the highest L and this value was reached a month in advance of the other spacings. This could be attributed to the increase in shoot number resulting from the higher plant population. The maximum L recorded for the closest spacing ( $0.91 \times 0.61$  m) was 7.96 followed immediately by 7.49, 4.96 and 3.26 for the other wider spacings in an increasing order. After the 8th month of planting, defoliation was rapid and L declined quickly, but the population effects persisted until the final harvest was made 12 months after planting. This would mean that the tuber bulking will be reduced after 8 months of growth due to the reduction in L, leaf being the most important organ in manioc.

Leaf area duration (D), the integral of leaf area index with time was calculated from the first sample harvest taken 2 months after planting to the final sample harvest made 12 months after planting (Table 9). Each increment of nitrogen increased D. The closest spacing (0.91 × 0.61 m) recorded the highest D, followed by a progressive reduction in D as the spacing increased. The total and tuber dry matter yields were linearly correlated with D, and these relationships could be represented by the equations  $Y = -15743.0 + 93.47 D$  ( $P = 0.01$ ) and  $Y = -12746 + 56.73 D$ , ( $P = 0.01$ ), D accounting for 97% and 86% of the variation in total and tuber dry matter yields respectively. Similar relationships between D and total and tuber dry matter yields have been reported for other root crops such as the potato<sup>3</sup> and Coco yams.<sup>1</sup> The results reported supports Watson's<sup>5</sup> contention that leaf area is the chief determinant of crop yield.

TABLE 9. Main effect of treatments on leaf area duration, D.

a. Nitrogen	D, weeks	% increase/decrease with increasing level of nitrogen or spacing
44.8 kgN/ha	97.52	—
67.2 kgN/ha	106.28	9.6
89.6 kgN/ha	108.00	10.7
b. Spacing		
0.91 × 0.61 m	146.66	—
0.91 × 0.91 m	118.42	19.3
0.91 × 1.21 m	83.48	43.1
0.91 × 1.52 m	59.24	59.6

The tubers of a single replicate harvested 8 months after planting were analysed for hydrocyanic acid content. The values ranged between 0.050% to 0.053% in fresh peeled tubers which according to Singha and Nair<sup>4</sup> is innocuous.

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The effect of nitrogen and potassium on the growth and yield of second early potato variety Craig's Royal

Level of N	Increasing level of nitrogen or potassium		Yield (kg/ha)	Tuber yield (kg/ha)	Tuber yield (%)
	1	2			
0	10.1	10.1	108.00	108.00	100.0
100	10.1	10.1	108.00	108.00	100.0
200	10.1	10.1	108.00	108.00	100.0
300	10.1	10.1	108.00	108.00	100.0
400	10.1	10.1	108.00	108.00	100.0
500	10.1	10.1	108.00	108.00	100.0
600	10.1	10.1	108.00	108.00	100.0
700	10.1	10.1	108.00	108.00	100.0
800	10.1	10.1	108.00	108.00	100.0
900	10.1	10.1	108.00	108.00	100.0
1000	10.1	10.1	108.00	108.00	100.0

The effect of a single potassium fertilizer 8 months after planting was analysed for hydrocyanic acid content. The values ranged between 0.020% to 0.033% in fresh tubers which according to Singh and Nair is innocuous.

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## Economically Useful Plants of Sri Lanka. Part III\*. A Phytochemical Screening of One Hundred Medicinal Plant Materials from Ayurvedic Shops

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**Abstract :** Over one hundred medicinal plant materials from Ayurvedic shops have been screened for the occurrence of alkaloids, saponins, steroids/terpenoids, cardiac glycosides and flavonoids. Therapeutic significance of these classes of compounds are outlined and wherever possible the traditional medicinal uses of these plants are discussed with reference to the class(es) of natural product(s) detected in them.

### 1. Introduction

In Sri Lanka, various systems of indigenous medicine utilising medicinal plants are widely practised. This is true especially in rural areas. Even in certain parts where modern health care is readily available, traditional and indigenous systems of medicine have co-existed for many decades. In fact, it has been estimated that the traditional system of medicine in Sri Lanka meets the basic health needs of about 70% of the population.<sup>21</sup>

The therapeutic value of medicinal plants is no doubt due to the presence of biologically active natural products. The goal in investigating plants for biologically active or medicinally useful compounds should be to isolate the one or more constituents responsible for a particular activity. Farnsworth points out<sup>6</sup> that in the phytochemical investigation of a particular plant phytochemical screening techniques can be a valuable aid. It is valuable as reliable methods are available for the detection of some important classes of biologically active phytoconstituents such as alkaloids, saponins, cardiac glycosides, steroids/triterpenoids and flavonoids.<sup>7</sup>

Two systematic phytochemical surveys of Sri Lanka plants for alkaloids have been reported.<sup>15,18</sup> Although several medicinal plants were included in the above studies many were left out. Though alkaloids have been recognised as an important class of pharmacologically active compounds,<sup>9</sup> no systematic survey of Sri Lankan medicinal plants for alkaloids has been conducted hitherto ; neither has it been done for the other biologically active phytoconstituents mentioned earlier.

As a part of our preliminary study of economically<sup>11</sup> and medicinally<sup>10</sup> useful plants we have initiated a phytochemical screening of medicinal plants and herein we report our results obtained on over one hundred medicinal plants commonly employed in Sri Lanka.

\*For Part 2 see A. A. L. Gunatilaka, S. Sotheeswaran and S. Balasubramaniam, *J. Natn. Sci. Coun. Sri Lanka*, 1978, 6, p. 121-128.

## 2. Experimental

All plant materials were purchased from Ayurvedic shops in Kandy. Wherever possible the part of the plant was identified and was then powdered in a mill.

### 2.1 Extraction of plant constituents

The powdered plant part (100g.) was extracted with about 300 ml of rectified spirit in a conical flask. A filter funnel was placed into the neck of the flask which acted as a condenser to prevent evaporation of solvent. After about one hour of refluxing on a steam bath, the flask was cooled to room temperature. The mixture was filtered and the plant material was washed with *ca* 50 ml of fresh rectified spirit. The total volume of the extract was measured and portions were used in the screening tests.

### 2.2 Screening for alkaloids

A volume equivalent of 50g of plant material was evaporated to dryness. 2N-HCl (10.0 ml.) was added and the mixture was heated on a steam bath for 5 min, with stirring. This was filtered and was divided into four equal parts in separate test tubes.

- (a) A few drops of Mayer's reagent were added to one of the test tubes and was observed for slight turbidity (+), definite turbidity (++) or heavy precipitate (+++).
- (b) A few drops of Wagner's reagent were added to the second test tube and observations were recorded as in (a).

If no precipitate or turbidity was observed in either of the two test tubes, then alkaloids were assumed to be absent from the plant material being examined.

If positive results for Mayer's or Wagner's tests were noted then the two remaining fractions were combined, basified with concentrated ammonia ( $pH = 9$ ) and the solution was extracted with chloroform. The combined chloroform extracts were dried ( $MgSO_4$ ) and the concentrated solution was subjected to a thin layer chromatographic (TLC) examination. The developer solvent was either chloroform-methanol (9 : 1) or chloroform-methanol (19 : 1). The TLC plates were sprayed with iodoplatinate or Dragendorff reagents.

### 2.3 Screening for Saponins

**Froth Test :** The plant material (100 mg) was taken in a test tube and water (10 ml) was added. The mixture was shaken vigorously. If a froth formed remained 3 cm above the water level for more than 1/2 hr, the test was considered positive.

**Haemolysis Test :** Only froth positive plant materials were subjected to this test. A medium double layered plate of 5% bovine red-cell suspension in isotonic phosphate buffer of pH 7.4 in 1.5% agar-buffer was overlaid on 1.5% agar-buffer. A disc of filter paper was saturated with the plant extract dissolved in phosphate buffer, and was placed on the surface of the well dried agar plate. The disc was left for 24 hr, and was observed for haemolysed zone, if any.

A plant extract which answered the froth, haemolysis and the Liebermann-Burchardt test (see below) was considered to contain saponins.

#### 2.4 Screening of Steroids/Triterpenoids

**Salkowski Test :** A volume equivalent to 10 g of plant material was evaporated to dryness. The residue was stirred with light petroleum (10 ml) and the organic layer was discarded. The residue was dissolved in chloroform (10 ml) and was divided into three test tubes. One test tube was held at an angle of 45° and 2 ml of conc.  $H_2SO_4$  was allowed to run down the inside of the test tube. Sulphuric acid was gently mixed and a cherry red colour if formed was considered to be indicative of the presence of unsaturated steroids. The solution in the second test tube was used as a reference solution.

**Liebermann-Burchardt Test :** To the third test tube about three drops of acetic anhydride was added. After mixing, one drop of conc.  $H_2SO_4$  was added and after further mixing the colour changes were observed immediately and over a period of one hr. The presence of a bluish-green colour was considered to indicate the presence of steroids whilst a red, pink or violet colour was considered to indicate the presence of triterpenoids. A pale yellow colour was considered to indicate saturated sterols/triterpenes.

#### 2.5 Screening for Cardiac glycosides

If Salkowski and Liebermann-Burchardt tests gave positive results, then the Kedde Test (for the presence of unsaturated lactones) and Keller-Killiani (for the presence of 2-deoxy sugars) Test were performed. If the latter two tests were positive then the plant extract was considered to have cardiac glycosides.

##### **Kedde Test :**

On the centre of a piece of filter paper 0.2 ml of the rectified spirit extract of the plant material was placed and a circular chromatography was performed with chloroform as the developer. The paper was dried and was sprayed with the Kodde reagent (2% methanolic solutions of 3, 5-dinitrobenzoic acid mixed in equal volumes with 2N KOH). The paper was air dried and the formation of a purple spot was considered to give a positive result.

**Keller-Killiani Test :**

The original rectified spirit extract (10 ml) was evaporated to dryness. The dried extract was defatted with light petroleum. The residue was treated with 3 ml of the  $\text{FeCl}_3$  reagent (0.3 ml of 10%  $\text{FeCl}_3$  in 50 ml of glacial acetic acid). The test tube was held at an angle of  $45^\circ$  and 2 ml conc.  $\text{H}_2\text{SO}_4$  was added carefully along the wall of the test tube. If a purple ring was observed at the interphase, this was considered to be a positive response to the Keller-Killiani test.

**2.6 Screening for flavonoids**

A volume equivalent to 3g of the plant material was evaporated to dryness. The residue was de-fatted with light petroleum and was dissolved in 2 ml rectified spirit. The solution was divided into two test tubes. To one conc.  $\text{HCl}$  (0.5 ml) and *ca* three magnesium turnings were added and if an exothermic reaction occurred the test tube was cooled and was shaken with octanol. The colour of the solution was observed and was compared with the colour of the solution in the second test tube. A colour change to orange, red, crimson within ten minutes indicated the presence of flavonoids in the plant extract.

**3. Results and Discussions**

All plant materials were purchased using their Sinhala names. Their probable botanical names, synonyms and Tamil names were obtained from published works (see Table), which give these names as against the Sinhala names. However, for the following plant materials sold no botanical names were available : agil, heen-aratha, kurasaani, maha-aratha, morawessa, thubarala, walangassaal and wal-anoda. In some instances an accurate assignment of the botanical names was not possible, as a number of species belonging to a single genus bear the same Sinhala name (e.g. all *Sida* species are called *bevilas*).

In each case the plant material was extracted with hot rectified spirit and the extract was subjected to a phytochemical screening for alkaloids, saponins, steroids/triterpenoids, cardiac glycosides and flavonoids by the method of Farnsworth and co-workers.<sup>7</sup> The results of the screening of one hundred and four medicinal plants are presented in the Table which gives also the medicinal uses of these plants.

Out of the 104 samples tested, 68 (*ca* 65%) were shown to be alkaloid positive. The probable number of alkaloids present in each extract was obtained from a TLC analysis and is indicated in the Table 1. The incidence here is very much higher than the estimated distribution of alkaloids in vascular plants (i.e. *ca* 15% to 20%).<sup>13</sup> It is possible that alkaloids may be responsible for the pharmacological action of the majority of plants included in this study. The negative alkaloid reaction obtained for *Sida rhombifolia* (Malvaceae) is surprising as this species has been reported to contain alkaloids.<sup>12</sup> It is possible that either the alkaloid content was below the detectable level or that this is a case of mistaken identity.

TABLE I.

Family Botanical Names (Sinhala, Tamil names)	Reported Medicinal Uses	Plant Part(s) Used <sup>b</sup>	Screening results <sup>c</sup>				
			Alkaloids (No. of alkaloids by t.l.c.) <sup>d</sup>	Saponins	Steroids/tri- terpenoids	Cardiac Glycosides	Flavonoids
<b>ACANTHACEAE</b>							
<i>Barleria mysorensis</i> Roth. (= <i>Barleria prionitis</i> L.) (S: Katukarandu ; T: Vavamula)	Antiseptic, <sup>4</sup> Catarrhal affections of children <sup>1</sup>	Br	++ (1)	—	+	—	—
<i>Hyprophylla spinosa</i> (S: Ikiya)		Wp	+++ —	—	+	—	—
<b>APOCYNACEAE</b>							
<i>Holarrhena antidysenterica</i> (L) Br. (S: Kelindahaal)			+++ (2)*	—	—	—	—
<i>Rauwolfia serpentina</i> (L.) Benth ex Kurz (S: Ekaweriya ; T: Covanna milipori)	Antidote to reptile venom, dysentery, emmenagogue <sup>1</sup>	Rt	+++ (4)	—	+	—	—
<i>Rejovia dichotoma</i> (Roxb.) Gamble (S: Divi-kaduru ; T: Nanthiyavata)	Cooling agent for boils		+	—	—	—	—
<b>ARACEAE</b>							
<i>Acorus calamus</i> L. (S: Wadakaha ; T: Vashambu)	Rheumatism <sup>1</sup>	Rh	++	—	—	—	—

Family Botanical Names <sup>a</sup> (Sinhala, Tamil names)	Reported Medicinal Uses	Plant Part(s) Used <sup>b</sup>	Screening results <sup>c</sup>				
			Alkaloids (No. of alkaloids by t. l. c.) <sup>d</sup>	Saponins	Steroids/tri- terpenoids	Cardiac Glycosides	Flavonoids
<b>ARISTOLOCHIACEAE</b> <i>Aristolochia indica</i> L. (S : Sapsanda ; T : Perularundu)	Fever, attenuant, emmenagogue <sup>1</sup>		+ (2)	—	—	—	—
<b>ASCLEPIADACEAE</b> <i>Hemidesmus indicus</i> (L.) Ait. f. (S : Eramasu ; T : Nanari)	Skin diseases <sup>4</sup>	Bk	++ (1)	—	+	—	+
<b>BIGNONIACEAE</b> <i>Oroxylum indicum</i> (L.) Vent (S : Totila ; T : Vanga adanthay)	Dasamula decoc- tion, dysentery		+ (2)	+	+	—	—
<i>Stereospermum suaveolens</i> (Roxb.) DC (S : Palol ; T : Padri)	Dasamula decoction <sup>1</sup>		+++ (3)	—	+	—	—
<b>CAPPARIDACEAE</b> <i>Cratogeomys religiosa</i> Forst. f. (S : Lunuwarana ; T : Marilankai)	Lithonriptic action <sup>3</sup>	Bk	+ (5)*	—	—	—	—
<b>COMBRETACEAE</b> <i>Terminalia arjuna</i> (Roxb) Wight Arn (S : Kumbuk ; T : Vellai maruthu)	Astringent, cardiac stimulant, dysentery, fever, heart diseases <sup>3</sup>	Bk	—	+	—	—	+
<i>Terminalia bellerica</i> (Gaertn) Roxb. (S : Bulu ; T : Tantri)	Astringent, laxative <sup>3</sup>	Fr	+++ (2)	+	+	—	—

<i>Terminalia chebula</i> Retz. (S : Aralu ; T : Kadukkay)	Laxative gum <sup>4</sup>	Fr	++(2)*	+	+	-	-
<b>COMPOSITAE</b>							
<i>Anacyclus pyrethrum</i> (S : Akkarappata ; T : Akki-rakaram)	Alleviation of bronchitis, stimula- tion of salivary glands <sup>4</sup>	TW	++(3)	-	+	-	-
<i>Vernonia anthelmintica</i> (L.) Willd. <sup>2</sup> (S : Sanninayan ; T : Kattushugam)	Leucoderma <sup>1</sup>	TW	+	-	-	+	-
<i>Vernonia anthelmintica</i> (L.) Willd. <sup>2</sup> (S : Bodhi ; T : Karpokarishi) ( <i>Psoralea corylifolia</i> LEGUMINOSAE) <sup>3</sup>	Leprosy, Leucoderma <sup>1,3</sup>	Sd	-	-	+	+	-
<b>CONVOLVULACEAE</b>							
<i>Evolvulus alsinoides</i> (L.) L. (S : Vishukranti)	Bronchitis, asthma, antidycentric <sup>4</sup> antiseptic	Wp	++(2)	-	+	-	-
<i>Ipomoea mauritiana</i> Jacq. (S : Kiribadu ; T : Nellikumbalu)	Aphrodisiac <sup>1</sup>	Fr	-	-	-	-	-
<i>Operculina terpenium</i> (L.) Manso. <sup>2</sup> (S : Trastavallu ; T : Sivada)	Purgative <sup>1</sup>	Fr	++(4)	-	-	-	-
<b>CRUCIFERAE</b>							
<i>Brassica juncea</i> (L.) Coss. (S : Aba ; T : Kaduku)	Rheumatism <sup>1</sup>	Sd	+++	-	-	-	+

Family	Botanical Names <sup>a</sup> (Sinhala Tamil names)	Reported Medicinal Uses	Plant Part(s) Used <sup>b</sup>	Screening results <sup>c</sup>				
				Alkaloids (No. of alkaloids by t. l. c.) <sup>d</sup>	Saponins	Steroids/tri- terpenoids	Cardiac Glycosides	Flavonoids
<b>CUCURBITACEAE</b>								
	<i>Cucumis melo</i> (S: Pitakiri ; T: Velapalam)	Cooling, diuretic <sup>3</sup>	Tw & Lf	++(2)	-	+	-	+
<b>ERYTHROXYLACEAE</b>								
	<i>Erythroxylum monogynum</i> DC. (S: Devadara)		St	-	-	+	-	-
<b>EUPHORBACEAE</b>								
	<i>Apurosa cardiosperma</i> (Gaertn) Merr. (S: Pathpadagam)	Nervous depres- sions, chronic malaria <sup>4</sup> and febrifuge	Wp	++	-	+	-	+
	<i>Phyllanthus embilica</i> L. (S: Nelli ; T: Nelli)	Haemorrhage, diarrhoea, dysentry, scurvy, <sup>4</sup> diuretic, laxative, anaemia	Fr	++(1)	-	+	-	+
	<i>Croton tiglium</i> L. (S: Jayapala ; T: Neervalam)	Purgative <sup>1</sup>	Sd	+++(4)*	-	-	-	-
<b>GRAMINEAE</b>								
	<i>Panicum antidotale</i> Retz. (S: Krimisastru)	Vermifuge <sup>1</sup>	Ls	++(3)	-	+	-	+
	<i>Vetiveria zizanioides</i> (L.) Nash (S: Savandara)	No medicinal uses, used as a perfume <sup>4</sup>	Rt	++(3)	-	+	-	-
<b>HIPPOCRATEACEAE</b>								
	<i>Salacia reticulata</i> Wight (S: Kotala-himbutu-wel)	To allay thirst in cases of diabetes	Ls	-	+	+	-	+



Family	Botanical Names <sup>a</sup> (Sinhala, Tamil names)	Reported Medicinal Uses	Plant Part(s) Used <sup>b</sup>	Screening results <sup>c</sup>				
				Saponins	Steroids/tri- terpenoids	Cardiac Glycosides	Flavonoids	Alkaloids (No. of alkaloids by t. l. c.) <sup>d</sup>
<i>Pterocarpus santalinus</i> L.f. (S : Rat handun ; T : Santhanum)		Cooling, astringent, headache <sup>3</sup>	St	-	+	+	+	+
	<i>Pongamia pinnata</i> (L.) Pierre (S : Karanda)	Dysentery and skin diseases <sup>1</sup>	St	+	+	-	+	+
		Asthma, cough, blood purification, bleeding piles, gonorrhoea, dyspepsia <sup>3, 4</sup>	Rt	-	+	-	+	+
<i>Trigonella foenumgraecum</i> L. (S : Ulhal ; T : Venbhayam)		Diarrhoea <sup>e</sup>		+	+	-	-	-
<b>LILIACEAE</b>								
<i>Asperagus racemosus</i> Willd. (S : Hatawariya ; T : Sathkawari)		Hair oil, chronic rheumatism <sup>1</sup>	Rh	-	-	-	-	+
<b>LOGANIACEAE</b>								
<i>Strychnos potatorum</i> L.f. (S : Igni ; T : Teirankottai)		Diuretic <sup>1</sup>	Sd	-	+	-	-	-
<b>LYTHRACEAE</b>								
<i>Woodfordia fruticosa</i> (L.) Kurz. (S : Malitha)		Stimulant, astringent dysentery, irritant, haemorrhage <sup>3</sup>	Fj	-	-	+	+	+

MAGNOLIACEAE

*Michelia champaca* L.  
(S : Sapu ; T : Sanpakam)

Astringent, diuretic, dyspepsia, nausea, fevers<sup>3</sup> FI + + + (3) - - + - +

MALVACEAE

*Sida cordifolia* L.  
(S : Wal-bevila ; T : Sevakanpundu)

Fever, urinary complaints<sup>1</sup> Wp + - - - - +

*Sida rhombifolia* L.  
(S : Koti-kan bevila ; T : Ariwal-manaippundu)

Fever, urinary complaints<sup>1</sup> Wp - - - - - +

MELIACEAE

*Azadirachta indica* A. Juss.  
(S : Kohomba ; T : Vembu)

Antiseptic, astringent, fever, skin diseases<sup>3</sup> rheumatism, leprosy<sup>3</sup> Bk - - - - - +

*Azadirachta indica* A. Juss.  
(S : Kohomba, T : Vembu)

Wp + + + (2) - - - - - +

*Murroneia pumila* Wight  
(S : Bin-kohomba ; T : Nilavembu)

Wp + + + (3) - - - - - +

MENISPERMACEAE

*Cissampelos pareira* L.  
(S : Diyamitta)

Muscle relaxant, relieves pain, snake bite, scorpion stings<sup>4</sup> St + + + (5) - - - - - +

*Coscinium fenestratum* (Gaertn) Colebr.  
(S : Weai-wel ; T : Maramanchal)

Tetanus, asthma, urine trouble, fever, bronchitis, tuberculosis<sup>4</sup> Bk, Tm + + + (6) - - - - - +

Family	Botanical Names <sup>a</sup> (Sinhala, Tamil names)	Reported Medicinal Plant Part(s)		Screening results <sup>c</sup>				
		Uses	Used <sup>b</sup>	Alkaloids (No. of alkaloids by t. l. c.) <sup>d</sup>	Saponins	Steroids/tri- terpenoids	Cardiac Glycosides	Flavonoids
	<i>Tinospora cordifolia</i> (Willd.) Miers ex Hook. f. (S : Rasakinda ; T : Amuthawalli)	Fever, jaundice, skin diseases, rheumatism. Urinary diseases, stomach irritability <sup>1</sup>	St	+	—	—	—	+
<b>MORACEAE</b>								
	<i>Ficus religiosa</i> L. (S : Bo ; T : Arasu)	Astringent, fever dysentry <sup>1</sup>	Sd	—	—	+	+	—
<b>MYRISTICACEAE</b>								
	<i>Myristica fragrans</i> Houtt (S : Sadikka ; T : Sadikai)	Carminative, digestive expectorant <sup>1</sup>		—	—	—	—	+
<b>NYCTAGINACEAE</b>								
	<i>Boerhavia diffusa</i> L. (S : Pitasudu-pala ; T : Mukkarachi)	Laxative, diuretic, stomachic, pres- cribed in jaundice, dropsy <sup>1</sup>	Rt, Lf	—	—	—	—	+
<b>NYMPHAEACEAE</b>								
	<i>Nelumbo nucifera</i> Gaertn. (S : Nelum ; T : Tamarai)	diuretic <sup>4</sup> for fever as a refrigerant <sup>4</sup>	An	+	—	+	—	—
<b>PEDALIACEAE</b>								
	<i>Pedaliium murex</i> L. (S : Et-nerenchi ; T : Peru nerunji)	Gonorrhoeal rheumatism <sup>1</sup>		—	—	—	—	+

PIPERACEAE

*Piper chaba*  
(S : Siviyu)

*Piper cubeba* L. f.  
(S : Wal-gammiris)

(*Ocimum basilicum* L. LABIATAE)

*Piper longum* L.  
(S : Tippili ; T : Thippili)

*Piper nigrum* L.  
(S : Gammiris ; T : Milaku)

PLUMBAGINACEAE

*Plumbago indica* L.  
(S : Rat-nitul ; T : Chittira)

PUNICACEAE

*Punica granatum* L.  
(S : Delum ; T : Mathulai)

RANUNCULACEAE

*Aconitum heterophyllum*  
(S : Attudayan ;  
T : Attudayan)

RUBIACEAE

*Ixora coccinea* L.  
(S : Rat-mal ; T : Vedsii)

*Paederia foetida* Linn.  
(S : Apasu madu)

*Pavetta indica* L.  
(S : Pavetta ; T : Pavattai)

	+++ (1)	-	+	-	+	-	+
Fr	+	-	+	-	+	-	+
Sd	++ (2)	-	+	-	+	-	+
Sd	+(1)	+	+	-	+	-	+
	-	-	-	-	-	-	+
Fr	+(5)	-	-	-	-	-	+
Rh	-	-	-	-	-	-	-
Fl	-	-	-	-	-	-	+
Lf, Tw	+	-	+	-	+	-	+
Rt	+++ (3)	-	+	-	+	-	+

Family Botanical Names <sup>a</sup> (Sinhala, Tamil names)	Reported Medicinal Plant Part(s) Used <sup>b</sup>	Uses	Alkaloids (No. of alkaloids by t. l. c.) <sup>d</sup>	Screening results <sup>c</sup>			
				Saponins	Steroids/terpenoids	Cardiac Glycosides	Flavonoids
<i>Randia dumetorum</i> Lam. (S : Kukurman ; T : Karai)	Fr	Emetic, diaphoretic, antispasmodic, nut, bruised and thrown into pools, intoxicates fish <sup>3</sup>	—	+	—	+	
<i>Rubia cordifolia</i> L. (S : Welmadata ; T : Manjidi)	Tw, St	Diuretic, emmenagogue, jaundice <sup>3</sup>	+	+	+	—	
<b>RUTACEAE</b> <i>Aegle marmelos</i> (L.) Corr. (S : Noli ; T : Vilvum)	Rt	Chronic bronchitis, asthma, scabies, skin complaints <sup>3,4</sup>	+++ (4)	+	—	—	
<b>SANTALACEAE</b> <i>Santalum album</i> L. (S : Sudu handun ; T : Senthanam)	Tm	Pimples-local application <sup>e</sup>	—	—	—	—	
<b>SAPINDACEAE</b> <i>Cardiospermum halicacabum</i> L. (S : Wel-penela ; T : Mudakattran)	Lf, Tw		+	—	—	+	
<i>Sapindus emarginatus</i> Vahl <sup>2</sup> (S : Penela ; T : Ponnankottai)		Hot and alexipharmic, anthelmintic, diarrhoea and cholera <sup>3</sup>	—	+	+	—	
<i>Sapindus trifoliatus</i> L. <sup>3</sup>			—	+	+	—	
<b>SCROPHULARIACEAE</b> <i>Bacopa monniera</i> (L.) Wettst. (S : Lunuwila ; T : Noerpirami)	Wp	Diuretic, <sup>1</sup> bowels and skin eruptions	—	+	+	—	



Family	Botanical Names <sup>a</sup> (Sinhala, Tamil names)	Reported Medicinal Uses	Plant Part(s) Used <sup>b</sup>	Screening results <sup>c</sup>				
				Alkaloids (No. of alkaloids by t. l. c.) <sup>d</sup>	Saponins	Steroids/tri- terpenoids	Cardiac Glycosides	Flavonoids
	<i>Trachyspermum roxburghianum</i> (DC.) Craib (S: Asanodagam; T: Omum)	Diarrhoea, carminatives	Fr	-	-	-	-	+
<b>URTICACEAE</b>	<i>Fleurya interrupta</i> (L.) Wight (S: Kahambilya)	Not recorded but produces dermatitis	If, St	+	-	+	-	-
<b>VALERIANACEAE</b>	<i>Nardostichys jatamansi</i> (D. Don) (S: Jatamansa, D C T: Jatamanshi)	Nervine tonic, carminative rheumatism <sup>1</sup>	Rt	-	-	+	-	-
<b>VERBENACEAE</b>	<i>Gmelina arborea</i> Roxb. (S: El-demata; T: Kumulamaram)	Rheumatism, indigestion, fever <sup>4</sup>	Tm	+++ (1)	-	+	-	-
	<i>Premna herbacea</i> Roxb. (S: Siritekkui)	Rheumatism <sup>4</sup>	Rt	+(2)	-	+	-	+
	<i>Vitex negundo</i> L. (S: Nika; T: Nirmochi)	Febrifuge, expectorant <sup>1</sup>	Rt	+	-	-	-	+
<b>ZINGIBERACEAE</b>	<i>Zingiber officinale</i> Roscoe (S: Inguru; T: Inji) (S: viyali inguru; T: Sukku)	Nausea, asthma, cough, colic, palpitation of the heart <sup>1</sup>	Rh	++ (2)	-	+	-	-



Only 16 (ca 15%) plants screened were found to contain saponins and 18 (ca 17%) of them showed a positive response for cardiac glycosides. However, 62 (ca 59%) species gave a positive test for triterpenoids/steroids. Since saponins and some cardiac glycosides may answer the Liebermann-Burchardt test employed in the screening for triterpenoids/steroids, it was not possible to find out whether triterpenoids/steroids were present in addition to saponins and cardiac glycosides in samples showing positive response to this test.

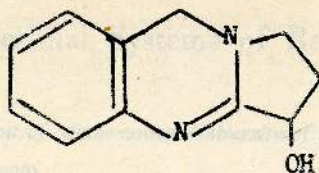
Although the value of saponins as medicinal agents is open to question, the economic importance of steroidal sapogenins, mainly because of their facile conversion to the medicinally useful steroid hormones is well documented<sup>5,11</sup> and recognised. However, by the procedure employed in the present study the steroidal saponins could not be differentiated from the terpenoid saponins.

The importance of cardiac glycosides as medicinal agents requires no elaboration. However, there has not been much effort to seek newer and better agents of this type in the plant kingdom by means of phytochemical screening programmes. Nevertheless a few surveys have been conducted in which plant extracts were evaluated biologically for cardiotoxic activity.<sup>16,19</sup> In the case of cardiac glycosides, the minimum structural features required for this biological activity are so well defined that the chemical methods of detection are acceptable.<sup>6</sup> There are two groups of cardiac glycosides viz. cardenolides and bufadienolides. Of these, the cardenolides are the most commonly encountered and most useful as therapeutic agents.

Out of the 104 samples screened, 42 (ca 40%) showed the presence of flavonoids. More than thirty three different biological activities are reported for some 30 of 137 known natural flavonoids.<sup>20</sup> These, as well as the more recent claims about the antiviral,<sup>17</sup> anti-inflammatory,<sup>6</sup> and cytotoxic properties<sup>14</sup> of flavonoids have made it worthwhile carrying out surveys for this class of compounds. In our screening, some of the medicinal plants with alleged astringent properties showed a positive reaction to the test for flavonoids. Though tannins are known to be responsible for this property in plants, it is possible that the presence of flavonoids may also account for this property.

Five medicinal plant materials included in this survey viz. kotikan-bevila (*Sida rhombifolia*), atiudayan (*Aconitum heterophyllum*), sudu-handun (*Santalum album*), maha-aratha and walangasaal, showed negative responses for all the five classes of natural products. It is possible that either these classes of natural products are present in small concentrations or that their medicinal properties are due to some other classes of natural product(s).

It was stated above that a confusion exists about the botanical identification of medicinal plants sold at the drug stores. Pavetta (*Pavetta indica*) sold by Ayurvedic drug dealers was found chemically to be *Adathoda* (*Adathoda vasica*). To check this, a pharmacognostic investigation of the sample sold as pavetta was undertaken. The major alkaloid isolated from this was found to be vasicine (I), which compared well with the major alkaloid of *Adathoda vasica*.<sup>12</sup> *Pavetta indica* collected by us did not contain any vasicine and further work is in progress to isolate and identify the major alkaloids of *Pavetta indica*. Such nomenclatural confusion may exist with other medicinal plants as well. For instance, the plants sold as nerenchu and gokatu in Ayurvedic shops in one botanical assignment,<sup>2</sup> are given the same species name viz. *Tribulus terrestris*. A pharmacognostic study of all the Ayurvedic drugs is therefore timely.



(1)

Figure 1. VASICINE

### Acknowledgements

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# Conditionally Infinitesimal Systems of Random Variables

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**Abstract :** Infinitesimal systems of random variables, defined for a sequence of independent random variables are an important class of random variables in Probability Theory. In this paper, conditionally infinitesimal systems are defined for a sequence of dependent random variables and some properties are obtained.

## 1. Introduction

Let  $\{ \{ X_{n,k} \} \} k = 1, 2, \dots, k_n; n = 1, 2, \dots$  be a double sequence of random variables which are row-wise independent. That is for  $n = 1, 2, \dots; k_n \rightarrow \infty$  as  $n \rightarrow \infty$  and for every  $n$  (which denotes the row) the random variables  $X_{n,1}, X_{n,2}, \dots, X_{n,k_n}$  are independent.

### Definition 1.1

A sequence of independent random variables  $\{ \{ X_{n,k} \} \}$  is said to be infinitesimal if for every sequence of integers  $k$  which satisfy  $1 \leq k \leq k_n$  for all  $n$  we have,  $X_{n,k} \rightarrow 0$  in probability as  $n \rightarrow \infty$ .

*Notation.*

$X_{n,k} \rightarrow 0$  in probability as  $n \rightarrow \infty$  will be written as  $X_{n,k} \xrightarrow[n \rightarrow \infty]{P} 0$ .

Consider a double sequence  $\{ \{ X_{n,k} \} \} k = 1, 2, \dots, k_n; n = 1, 2, \dots$  random variables. For every  $k$  ( $1 \leq k \leq k_n$ ) and  $n = 1, 2, \dots$  we have an increasing sequence of  $\sigma$ -fields  $F_{n,0} \subset F_{n,1} \subset \dots \subset F_{n,k_n}$  such that every  $X_{n,k}$  is  $F_{n,k}$  measurable.

We shall extend the definition 1.1 to a sequence of dependent random variables and define "conditionally infinitesimal" as follows.

### Definition 1.2

A sequence of random variables  $\{ \{ X_{n,k} \} \}$  is said to be conditionally infinitesimal if

$$\max_{1 \leq k \leq k_n} P(|X_{n,k}| > \epsilon | F_{n,k-1}) \xrightarrow[n \rightarrow \infty]{P} 0 \quad (1)$$

for every  $\epsilon > 0$ .

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If the  $\{\{X_{n,k}\}\}$  are independent then it can be shown that the above definition is equivalent to definition 1.1 and in the general case implies it. [3]

## 2. Equivalent Definition and Properties.

### Lemma 2.1

$\{\{X_{n,k}\}\}$  is conditionally infinitesimal if and only if

$$\max_{1 \leq k \leq k_n} E \left( \frac{X_{n,k}^2}{1 + X_{n,k}^2} \mid F_{n,k-1} \right) \xrightarrow[n \rightarrow \infty]{P} 0 \quad (2)$$

**Proof :**

Let  $F(x \mid F_{n,k-1})$  be a regular conditional distribution for  $X_{n,k}$  given  $F_{n,k-1}$ . [1]

$$\begin{aligned} E \left( \frac{X_{n,k}^2}{1 + X_{n,k}^2} \mid F_{n,k-1} \right) &= \int_{-\infty}^{\infty} \frac{x^2}{1 + x^2} F(dx \mid F_{n,k-1}) \\ &\leq \int_{|x| \leq \epsilon} x^2 F(dx \mid F_{n,k-1}) + \int_{|x| > \epsilon} F(dx \mid F_{n,k-1}) \\ &\leq \epsilon^2 + P(|X_{n,k}| > \epsilon \mid F_{n,k-1}) \end{aligned} \quad (3)$$

Taking  $\max_{1 \leq k \leq k_n}$  and first letting  $n \rightarrow \infty$  and then  $\epsilon \rightarrow 0$  in (3) we get (2) if (1) is true.

$$\begin{aligned} \text{Also } E \left( \frac{X_{n,k}^2}{1 + X_{n,k}^2} \mid F_{n,k-1} \right) &\geq \int_{|x| > \epsilon} \frac{x^2}{1 + x^2} F(dx \mid F_{n,k-1}) \\ &\geq \frac{\epsilon^2}{1 + \epsilon^2} \int_{|x| > \epsilon} F(dx \mid F_{n,k-1}) \\ &= \frac{\epsilon^2}{1 + \epsilon^2} P(|X_{n,k}| > \epsilon \mid F_{n,k-1}) \end{aligned}$$

Hence

$$P(|X_{n,k}| > \epsilon \mid F_{n,k-1}) \leq \frac{1 + \epsilon^2}{\epsilon^2} \cdot E \left( \frac{X_{n,k}^2}{1 + X_{n,k}^2} \mid F_{n,k-1} \right)$$

which implies (1) if (2) is true.

**Note :** If the  $\{\{X_{n,k}\}\}$  are independent then lemma 2.1 reduces to lemma 1,2

**Theorem 2.2**

If the double sequence  $\{\{X_{n,k}\}\}$  of non-negative random variables is conditionally infinitesimal then for every  $t > 0$ .

$$\max_{1 \leq k \leq k_n} E(1 - e^{-tX_{n,k}} | F_{n,k-1}) \xrightarrow[n \rightarrow \infty]{P} 0$$

**Proof :**

$$\begin{aligned} E(1 - e^{-tX_{n,k}} | F_{n,k-1}) &= \int_0^{\infty} (1 - e^{-tx}) F(dx | F_{n,k-1}) \\ &= \int_0^{\epsilon} (1 - e^{-tx}) F(dx | F_{n,k-1}) + \int_{\epsilon}^{\infty} (1 - e^{-2tx}) F(dx | F_{n,k-1}) \\ &\leq t\epsilon + P(|X_{n,k}| > \epsilon | F_{n,k-1}) \end{aligned} \tag{4}$$

Theorem 2.2 is proved by taking  $\max_{1 \leq k \leq k_n}$  and first letting  $n \rightarrow \infty$  and then  $\epsilon \rightarrow 0$  in (4).

**Theorem 2.3**

If the double sequence  $\{\{X_{n,k}\}\}$  is conditionally infinitesimal then

$$\max_{1 \leq k \leq k_n} |E(e^{itX_{n,k}} - 1 | F_{n,k-1})| \xrightarrow[n \rightarrow \infty]{P} 0.$$

**Proof :**

$$\begin{aligned} |E(e^{itX_{n,k}} - 1 | F_{n,k-1})| &= \left| \int_{-\infty}^{\infty} (e^{itx} - 1) F(dx | F_{n,k-1}) \right| \\ &\leq \int_{|x| \leq \epsilon} |e^{itx} - 1| F(dx | F_{n,k-1}) + \int_{|x| > \epsilon} |e^{itx} - 1| F(dx | F_{n,k-1}) \\ &\leq \epsilon |t| + 2.P(|X_{n,k}| > \epsilon | F_{n,k-1}) \end{aligned} \tag{5}$$

The proof of the theorem is complete by taking  $\max_{1 \leq k \leq k_n}$  first letting  $n \rightarrow \infty$  and then  $\epsilon \rightarrow 0$  in (5)

**Note :** If the  $\{\{X_{n,k}\}\}$  are independent theorem 2.3 reduces to Theorem 1.2

### 3. Generalization of results in Section 2 to $d$ — dimension ( $d > 1$ ).

Let  $\{\{X_{n,k}\}\}$  be a double array of  $R^d$  valued random vectors.

#### Definition 3.1

A sequence of random vectors  $\{\{X_{n,k}\}\}$  is said to be conditionally infinitesimal if and only if

$$\max_{1 \leq k \leq k_n} P(\|X_{n,k}\| > \epsilon \mid F_{n,k-1}) \xrightarrow[n \rightarrow \infty]{P} 0 \quad (6)$$

for every  $\epsilon > 0$ , where  $\|x\| = (x_1^2 + x_2^2 + \dots + x_d^2)^{1/2}$  for  $x = (x_1, x_2, \dots, x_d) \in R^d$ .

#### Lemma 3.2

$\{\{X_{n,k}\}\}$  is conditionally infinitesimal if and only if

$$\max_{1 \leq k \leq k_n} E\left(\frac{\|X_{n,k}\|^2}{1 + \|X_{n,k}\|^2} \mid F_{n,k-1}\right) \xrightarrow[n \rightarrow \infty]{P} 0.$$

#### Theorem 3.3

If the double array  $\{\{X_{n,k}\}\} \in R + d$  is conditionally infinitesimal then for every  $t \in R + d$

$$\max_{1 \leq k \leq k_n} E(1 - e^{-(t \cdot X_{n,k})} \mid F_{n,k-1}) \xrightarrow[n \rightarrow \infty]{P} 0.$$

Lemma 3.2 and theorem 3.3 are multidimensional versions of lemma 2.1 and theorem 2.2 respectively and the proofs are similar to the proofs in one-dimensional case.

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## Studies on *Mucuna* Species of Sri Lanka

### I. The L-DOPA Content of Seeds

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**Abstract :** Several local selections of *Mucuna* were investigated for L-DOPA content. The most promising type was a selection of *Mucuna atterrima* which contained about 4.5% L-DOPA in its seed. The L-DOPA could be recovered at 80% efficiency provided the seeds are fresh and used immediately after powdering. Climatic factors appear to have little or no effect on L-DOPA content and succeeding generations appear not to vary significantly from the parent plant with respect to this parameter.

#### 1. Introduction

3-(3, 4-dihydroxyphenyl)-L-alanine (L-DOPA) is a drug, used for some years in the treatment of Parkinson's disease.<sup>3</sup> More recently this compound has found application in the treatment of mental disorders.<sup>8</sup> As a result of its importance as a drug considerable attention has been paid to its occurrence in plant material.

L-DOPA was first isolated from *Vicia faba* as early as 1913.<sup>5</sup> In 1971, Daxenbichler *et al.*,<sup>3</sup> screened 724 species (from 447 genera and 135 families) and found L-DOPA only in 4 legumes species (*Mucuna*, *Dolichos*, *Baptisia* and *Vicia*). Previously L-DOPA had been reported in *Lupinus*,<sup>3</sup> *Euphorbia*,<sup>7</sup> *Robinia*,<sup>9</sup> and *Sarothamnus*<sup>6</sup> species. However, all studies including that of Bell and Janzen<sup>2</sup>, showed that only *Mucuna* had sufficient levels for commercial exploitation. The studies of Bell and Janzen<sup>2</sup> showed that 6 species of *Mucuna* had L-DOPA contents of more than 5%. Further, Daxenbichler<sup>3</sup> screening plants from 5 different countries (3 continents) reported that *M. atterrima* selections had L-DOPA levels of 4% to 5%.

In this study, local *Mucuna* species have been investigated for the first time for L-DOPA content. The primary aim being to collect information on the factors affecting L-DOPA content of *Mucuna* seeds in order to study the feasibility of setting up an industry in Sri Lanka, for the commercial extraction of L-DOPA.

#### 2. Experimental

##### 2.1. Plant Material

The original seed material was collected from different parts of the country either during field surveys or through intermediate sources. All types containing viable seeds were grown in experimental plots located at the CISIR. The plants were grown under controlled conditions using supports for the climber to ascend.

## 2.2. Identification of Plant Material.

A major problem of this study was the lack of authoritative identification of the plant material. Very little documentation on the taxonomy of *Mucuna* is available, both in Sri Lanka and abroad. However with the limited quantity of available data, 4 of the collected varieties were identified. They were (1) CISIR code No. A (see results)—a *M. atterrima* selection (2) CISIR code No. L—a *M. utilis* selection, (3) CISIR code No. R—a *M. deringian* selection. (4) CISIR code No. B. *M. nivia*.

— For this reason, all varieties are described here with code numbers. A detailed description of the plants have been communicated elsewhere.<sup>1</sup> All plant specimens have been sent to Kew, Royal Botanic Gardens, who due to controversy in classification have advised the authors to retain CISIR classification number and herbarium specimens. Experimental plants are also being maintained at the CISIR.

## 2.3. Extraction of L-DOPA.

L-DOPA was extracted using 0.25% acetic acid as described previously.<sup>9</sup>

## 2.4. Assay of L-DOPA.

L-DOPA was assayed by the method of Daxenbichler<sup>4</sup> as modified by Pieris.

## 2.5. Recovery of L-DOPA.

L-DOPA was recovered after deproteinising, concentrating under vacuo, decolourising and further concentration. This concentrate yields crude L-DOPA on cooling. The purity of the recovered L-DOPA was of the order of 90% (determined by UV absorption<sup>4</sup>).

## 2.6. Purification of L-DOPA.

L-DOPA was purified by recrystallisation from a water-ethanol mixture. The purity of the product was at least 98%. The UV and IR absorption spectra were identical with that of authentic L-DOPA (obtained by courtesy of Dr. N. R. Farnsworth, Academy of Sciences, USA). Thin layer chromatography (cellulose) with ethyl acetate : acetic acid : water (10 : 3 : 6) as solvent gave a purple spot with ninhydrin reagent with a R<sub>f</sub> value identical to authentic L-DOPA. The melting point of the L-DOPA (isolated) was 282°C (lit. 283°C to 286°C).

## 3. Results

### 3.1. Selection

Seeds of 19 different *Mucuna* plants from varying parts of the country were collected and analysed for L-DOPA content, by the UV Absorption method.<sup>3</sup> These selections fell into at least seven different morphological types. Results, shown in Table 1, clearly illustrate the wide variation in L-DOPA content of the selections.

TABLE 1. L-DOPA Content of Different Selections of Seeds.

Mucuna CISIR Code No.	Colour	Average seed Weight (g)	Percentage L-DOPA (on dry weight)
A ( <i>M. atterrima</i> )	Black	1.20	4.2
B ( <i>M. nivea</i> )	Grey	1.00	2.5
B.1	Greyish brown	1.10	1.6
B.2	Black	0.90	2.3
C	Black	0.85	2.8
D	Mottled brown on grey	0.75	2.2
E	Mottled brown on grey	0.70	3.5
F	Black	0.80	2.2
G	Black	N.D.	4.4
H	Black	N.D.	3.7
I	Black	0.85	3.8
J	Black	0.65	3.4
L ( <i>M. utilis</i> )	Black	1.00	4.1
N	Mottled brown on grey	1.15	4.2
O	off-white	0.90	2.8
P	Black	0.95	4.5
Q	Brown-black	0.70	2.3
R ( <i>M. deringiana</i> )	Mottled black on grey	0.80	2.7
T	Black	0.80	4.1

N.D. — Not determined.

### 3.2. Recovery of L-DOPA.

Efficiency of recovery of L-DOPA varied to a great extent and appeared to depend on a number of factors mainly,

(a) age of seeds

(b) L-DOPA content of the seeds.

When the seeds were old, low recoveries were obtained and in all these cases the cotyledons were discoloured and extracts therefore highly coloured. Both factors are illustrated by results shown in Table 2.

TABLE 2. Factors affecting L-DOPA Recovery

Batch Code	L-DOPA content (%)	Recovery (%)
Fresh seeds		
A	4.2	81
J	3.4	76
Q	4.3	80
B.1	1.6	26
B	2.5	17
C	2.8	25
*Old Seeds		
G	4.4	No Recovery
H	3.7	17
N	4.2	29

\*Old seeds refer to the seeds that are discoloured and at least six months old. The fresh seed was obtained directly from the tree.

Another factor that affects both L-DOPA content and recovery is the time that elapses between powdering of seeds, and their extraction with acetic acid. Losses of the order of 15% (one week) and 30% (3 to 4 weeks) have been observed.

The maturity of the seeds used and their mode of drying (sun drying or oven drying) had no effect on both L-DOPA content and recovery.

In an industrial process, materials of construction are of considerable importance. Metals were found to have a profound adverse effect on L-DOPA recovery; use of Cu, Zn, Fe, Ni, Sn in fine powdered form all caused oxidation of L-DOPA and therefore low recoveries. The only metal used which did not effect recovery adversely was Al.

### 3.3. Effect of Propagating Seeds of known- L-DOPA content under different Conditions.

The variation in climatic conditions appeared to have little effect on the average L-DOPA content. Plants of the new generation planted at CISIR had approximately the same L-DOPA content as the respective parent plant. Studies showed that L-DOPA content does not vary much in the first two generations. When subjected to detailed study *M. atterima* showed the following. In the first generation when the individual plants were grown under different conditions which resulted in different habits and L-DOPA content ranged from 3.0% to 4.8%. It is interesting to note that the plants grown on supports gave values of 4.5, 4.2 and 4.8 while the plant allowed

to trail along the ground (without a support) gave an L-DOPA content of only 3.4%. In the next generation 21 plants were studied and only 3 of them showed abnormal L-DOPA contents. Two were low (3.4% and 3.6%) while one plant gave seeds with a high L-DOPA content of 6.1%. Mean L-DOPA content of the seeds of separate plants was  $4.4 \pm 0.5$ .

#### 4. Discussion

Although the presence of L-DOPA in *Mucuna* has evoked some interest due to its possible role as a chemical barrier to insect attack<sup>10</sup> the main investigations on this subject has been nearly confined to the extraction of this compound for use as a drug.

While considerable data on the L-DOPA levels of several tropical varieties of the species is available, the local *Mucuna* varieties have not been investigated previously. This is indeed surprising as the seeds of many local varieties (notably varieties similar to B and R) have been used for some time in Ayurvedic medicine in Sri Lanka.

The primary aim of this study was to determine the L-DOPA content of local varieties. Here, L-DOPA content of the seeds of the varieties collected (excluding seed coat) was determined by the UV absorption method. It should be noted that the L-DOPA value given includes the tetra-hydro isoquinolines reported by Daxenbichler<sup>4</sup> (which are generally present at levels of less than 0.3%).

Only 6 selections contained more than 4% L-DOPA. Of these, the high seed yielding type A was selected for further study. This plant was found to produce 0.5 kg to 1 kg seed per plant when planted 2 meters apart on supports. It had been identified as a variety of *M. atterrima*.<sup>1</sup>

Studies showed that L-DOPA content did not vary significantly with both (i) the location of cultivation and (ii) from generation to generation. The other factor that appeared to affect L-DOPA levels was the habit of the plant where it was found that supports are necessary for both high yields of seed and high L-DOPA content. The only other feature worthy of note was that one plant produced seeds with a rather high L-DOPA content (6%). Maturity of the seed (unlike previously reported) had no effect on L-DOPA levels.

The optimum conditions of extraction (including acetic acid strength and volume and time of extraction) have been reported previously.<sup>9</sup> It is now reported that L-DOPA can be extracted at 80% efficiency using this method, provided the seeds are fresh and are used soon after powdering. It is very likely that L-DOPA is lost through oxidation on keeping.

Conclusions reached from this study include the point that the local requirement of L-DOPA (approx. 100 kg) can be produced by the cultivation of a mere 10 acres of this plant, the only major problem encountered so far being the susceptibility of most *Mucuna* varieties to a mosaic virus.

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## A composite medium for the Standardised Measurement of Aflatoxin accumulating capacity of *in-vitro* culture systems

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**Abstract :** Conventional methods which have used single incubation periods for the study of aflatoxin production in solid substrates, have the disadvantages of not taking into account, significant variations of toxin content that occur with time. A single step method, using a double layered composite medium is described here for the determination of the maximum quantity of aflatoxin accumulated in experimental culture. This method is based upon the capacity of silica gel to adsorb aflatoxin and remove it from degradative mechanisms which operate in the substrate, and which are probably responsible for the phasic variations of toxin content which occur in the adsorbent free medium. This method permits of standardisation in terms of adsorbent, substrate, inoculum, incubation conditions and extraction procedure.

### 1. Introduction

The measurement of aflatoxin production has been the basis of several important aspects of research on these biologically and economically important mycotoxins. These aspects include (1) the capacity of substrates to support toxin production, upon which data, agronomically useful substrates which show resistance to aflatoxin accumulation can be selected for propagation ; (2) the capacity of fungal strains to produce aflatoxin. This data could be used to select high yielding strains for the laboratory production of aflatoxin for experimental purposes, for the differentiation of genera, species and strains of fungi and in field studies on the accumulation of aflatoxin under natural conditions ; (3) the effects of cultural conditions, inhibitors and promoters on toxin production ; (4) the biosynthesis and metabolism of aflatoxins.

Most studies done *in vitro* have hitherto used cultures of aspergilli, incubated for arbitrarily selected single incubation periods, which have generally been between the fifth and tenth day. Some studies have used determinations over a few days while only a few have been made serially over three to four weeks.

We have reported earlier<sup>3</sup> that cultures of *Aspergillus flavus* and *A. parasiticus* on grated coconut showed multiple peaks of aflatoxin content when replicate cultures were assayed serially for two to three weeks. The patterns shown by different culture systems may differ in respect of :—

- the time relations of the peaks of toxin content
- the occurrence of monophasic or multiphasic patterns
- the patterns shown by the individual aflatoxin components
- shifts produced by modifications of the substrate, the inoculum or the conditions of incubation.

The results of van Walbeek *et al*<sup>8</sup> also showed that a similar phenomenon was occurring in one of their experiments at the time of termination of their observations ; they however did not comment on this phenomenon. Lafont and Lafont<sup>6</sup> described similar phasic variations of aflatoxin content in a synthetic liquid medium and the phenomenon was also reported by Applegate and Chipley.<sup>1</sup>

It would therefore appear that an assay of a conventional culture at a given incubation period without data on the time course of the variation of toxin content, may provide erroneous conclusions regarding the identity or concentration of the components of aflatoxin which a culture system can produce. Examples of possible errors were pointed out earlier. The assay of several replicate cultures will therefore have to be made before the patterns characteristic of a given culture system are defined and for obtaining valid conclusions on the capacity of the system to accumulate toxin. This approach however entails the cumbersome use of replicate cultures over prolonged periods.

The technique of de Vogel *et al*<sup>4</sup>, for the screening of fungi for aflatoxin production, seemed to provide a basis of an abbreviated method. Their technique used hyflo-superpel as an adsorbent with an overlying culture medium on which the strain under investigation was inoculated. These authors considered that the removal of the toxin into the underlying adsorbent would have differentiated the greenish or greyish fluorescence of the invading mycelium in the agar medium, from the fluorescence of the aflatoxin in the adsorbent, when the adsorbent side of the plate was viewed under ultraviolet light. Their cultures were examined on the third or fourth day after inoculation.

We used the techniques of de Vogel *et al*<sup>4</sup> to screen wild strains of aspergilli for aflatoxin production and to study the time course of aflatoxin accumulation which was determined semiquantitatively with reference to standard solutions of quinine sulphate, over prolonged periods. These plots showed that strains which produced phasic variations of toxin content with time on conventional culture, gave patterns which consisted of a rise of the toxin content to a maximum within a few days ; this maximum was maintained for two or three weeks as a plateau and then declined gradually. Apparently the toxin was removed from the culture by the adsorbent which thus prevented the degradation or inactivation of the toxin and hence the multiple peaks which would have occurred in the adsorbent free medium.

This paper reports a technique based upon the use of silica gel as an adsorbent, which eliminated the phasic variations of toxin content and which produced a plateau which represented the maximum amount of toxin which had accumulated over two or three weeks, during which period the plateau was maintained. During this period, a single assay provided an estimate of this maximum.

We prefer to use the term aflatoxin 'accumulation' rather than aflatoxin 'production' since the aflatoxin content of a culture at a given period of incubation, is the resultant of production and inactivation or loss and because the method which we have described here, measures the maximum content of toxin which was accumulated upto the time of assay.

## 2. Experimental

**Test strains and inocula.** *A. parasiticus* NRRL 2999 was used in all the experiments in establishing the method. *A. flavus* F209 and F218 in our collection, isolated from mouldy copra, were used in addition, to test the system.

Spores from 3 week old cultures on potato dextrose agar ('Difco') slopes were suspended in sterile 0.1% Tween 80 in distilled water (autoclaved at 115°C/10 min.) and the spore count was made in a blood cell counting chamber. The inoculum used was 0.2 ml of suspensions containing 20,800 spores/c.mm. **Culture medium.** Agar-media containing peanut or other substrates, homogenised as in the method of de Vogel *et al*<sup>4</sup> were replaced by the substrate alone in as finely divided a state as possible. Agar media had the disadvantage of producing relatively low levels of toxin with only 10% of the homogenised substrate as used in the original method. On the other hand, natural substrates alone had the advantage of yielding data which was more indicative of the levels of toxin contamination under natural conditions in the field.

Pulses and grains were pulverised in a mortar or mill. With these substrates it was possible to obtain particles with a sieve size of BS 22 or even 44. Oilseeds such as peanut and coconut were minced and used as particles of approximately BS 10 mesh size. Preliminary drying at 40°C to 50°C of the oilseeds made it easier to reduce their particle size; oil extrusion which occurred with more intensive grinding was avoided. Substrates in replicate tests were obtained from the same nut or batch of seeds.

### Adsorbent.

Silica gel H ('Merck') was found to produce higher yields of toxin and more consistent results than hyfflosuperpel.

### Preparation of the medium.

Weighing bottles of 3 cm diameter and 6 cm height provided a suitable container for the double layered composite medium. The composite medium was prepared as shown in Figure 1 by packing the silica gel powder into a uniform layer at the bottom of the bottle. The finely divided substrate was then lightly packed over the adsorbent. In early experiments 2 g of the adsorbent and 4 g of the substrate were used but in later experiments the amount of substrate was reduced to 1.5 g.

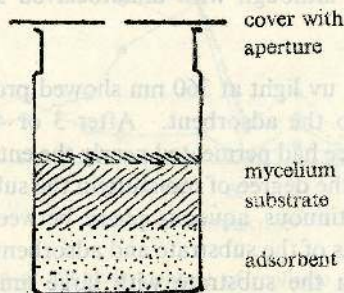


Figure 1. Diagrammatic representation of the weighing bottle containing the double layered composite medium.

With both unautoclaved and autoclaved (115°C/10 min.) composite media, 2 ml of sterile distilled water was added after packing of the components. There was no increase in the moisture content (as determined by weighing) of the composite medium after autoclaving. There was no alteration of the pH of the substrate in the sterilised medium. The bottle was covered with a glass plate which had a central aperture (3 to 4 mm in diameter) which allowed of inoculation and access to air. Duplicates were used for each test. The preparation and inoculation of the composite medium was done as aseptically as possible with sterile solutions and glassware.

#### *Incubation.*

The bottles were incubated in a moist chamber at 24°C in a box which while excluding direct sunlight, permitted intermittent observation of the bottles.

#### *Assay of toxin.*

After incubation, the bottles were steamed for 10 min. The entire composite medium was extracted by the aqueous acetone method of Pons *et al*<sup>7</sup>, substituting three successive homogenisations in a 'MSE' homogeniser with an overhead drive, instead of shaking as in the original procedure. The extracts in chloroform were titrated on TLC plates (Silica Gel G 'Merck') run in two different solvent systems, methanol : chloroform, 3 : 97 v/v and in acetone : chloroform, 1 : 9 v/v respectively, by visual estimation against inocula of standard aflatoxin B1 and G1 solutions in chloroform, of known concentration. The results are expressed as micrograms of aflatoxin per gram of the wet weight of the original substrate.

### 3. Results

In the composite medium, fungal growth was visible on the surface of the substrate after three or four days of incubation. With autoclaved substrates, growth of contaminant fungi did not occur although with unautoclaved substrates, contamination occurred frequently.

Exposure of the bottles to uv light at 360 nm showed progressive diffusion of blue fluorescence downwards into the adsorbent. After 3 or 4 days of incubation and within a week, the fluorescence had permeated nearly the entire depth of the adsorbent and this suggested that with the degree of moisture in the substrate and the adsorbent there appeared to be a continuous aqueous phase between the substrate and the adsorbent. Separate analysis of the substrate and adsorbent after incubation, showed low amounts of aflatoxin in the substrate with large amounts in the adsorbent ; hence both substrate and adsorbent were extracted together for estimation of the total content of toxin in the composite medium.

There was no mycelial growth into the adsorbent, sufficient to bind the adsorbent particles into a matt as with the substrate. At the end of the incubation period, even with appreciable amounts of toxin, the particles of the adsorbent retained their discreteness as when originally packed.

Autoclaved or oven dried substrates showed both B1 and G1 components of aflatoxin with strain 2999 whereas with unsterilised substrates G1 was present in very low concentrations or was absent.

Strain NRRL 2999 was previously reported by us<sup>3</sup> to produce biphasic curves for both aflatoxin B1 and G1 in conventional culture on loose, freshly grated coconut. Figure 2 compares the patterns of the variations of aflatoxin content with time in cultures of this strain on adsorbent-free loose, grated coconut and in the substrate packed as a layer as in the composite medium, but without adsorbent. With both the loose and the packed substrates, biphasic curves with similar time relationships were obtained indicating that packing of the substrate had no effect on the phasic alterations of toxin content. The loose substrate yielded more aflatoxin; this was probably due to greater accessibility of the particles in the loose medium, to mycelial spread.

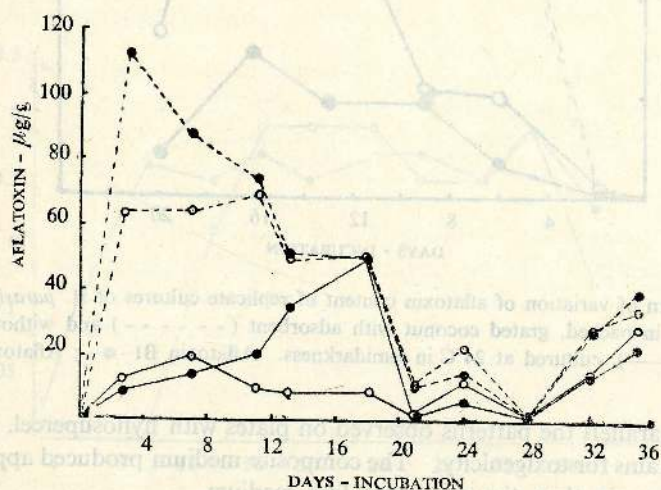


Figure 2. Pattern of variation of aflatoxin content of replicate cultures of *A. parasiticus* NRRL 2999 in grated coconut, in loose form (----), and as a packed layer (—), in the absence of adsorbent; cultured at 24°C in semidarkness. Aflatoxin B1—●—; Aflatoxin G1—○—.

Figure 3 compares the patterns of variation of aflatoxin content, with the packed substrate but without the adsorbent, with that in the composite medium with the adsorbent. The elimination of the variations and their replacement by the plateau in the latter system indicate that it was the adsorbent which was responsible for the modification of the pattern. The packing of the substrate apparently merely served to provide a continuous phase for the rapid diffusion of toxin through the substrate into the adsorbent.

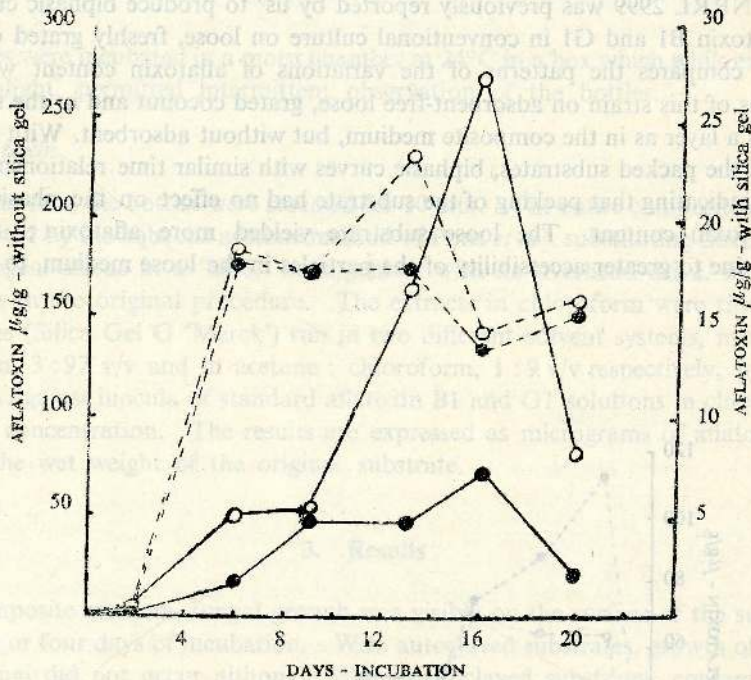


Figure 3. Pattern of variation of aflatoxin content of replicate cultures of *A. parasiticus* NRRL 2999 in packed, grated coconut with adsorbent (-----) and without adsorbent (———), cultured at 24°C in semidarkness. Aflatoxin B1—●—; Aflatoxin G1—○—.

This effect parallels the patterns observed on plates with hyflosupercel, used in the screening of strains for toxigenicity. The composite medium produced approximately ten-fold more toxin than the adsorbent free medium.

The Table records the aflatoxin levels obtained in replicate cultures on the composite medium containing 4 g of the substrate, indicating an acceptable degree of inter-replicate variation and reproducibility of the method.

TABLE 1. The aflatoxin content of replicate cultures of *A. parasiticus* NRRL 2999 on grated, pulverised coconut in the composite medium containing 4 g of substrate and 2 g of adsorbent, cultured at 24°C in semidarkness.

Aflatoxin content ug B1/g, in replicate cultures	mean	coefficient of variation
487.5	464.6 ± 18.4	3.9
475		
462.5		
437.5		
450		
475		

The plateau pattern was also produced by four other substrates incorporated in the composite medium, cow-pea and Lanka dhal (Figure 4) and Green-gram and peanut (Figure 5), with strain NRRL 2999. Only aflatoxin B1 was detected in these cultures. These four substrates however produced much lower levels of aflatoxin in comparison with coconut which was earlier shown by us<sup>2</sup> to be an excellent medium for the production of very high levels of toxin. This strain NRRL 2999 was found to produce biphasic curves with crushed peanuts in conventional culture (Arseculeratne, unpublished data).

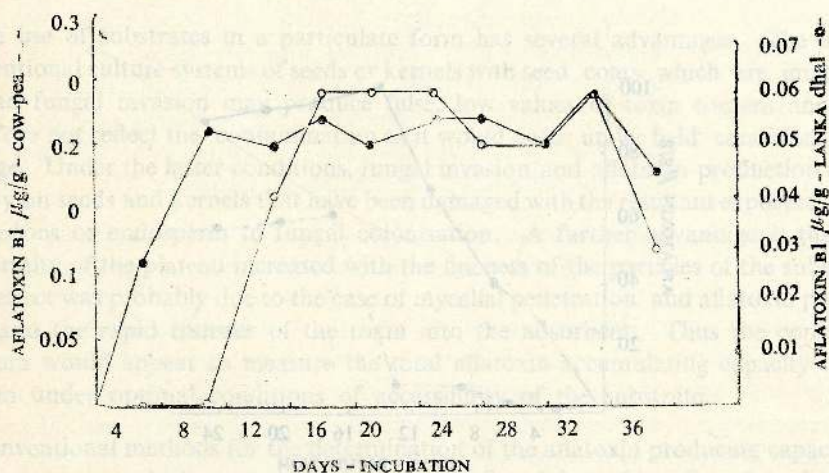


Figure 4. Pattern of variation of Aflatoxin B1 content of replicate cultures of *A. parasiticus* NRRL 2999 on the composite medium containing Lanka dhal -o-; cow pea-g-. Cultured at 24°C in semidarkness.

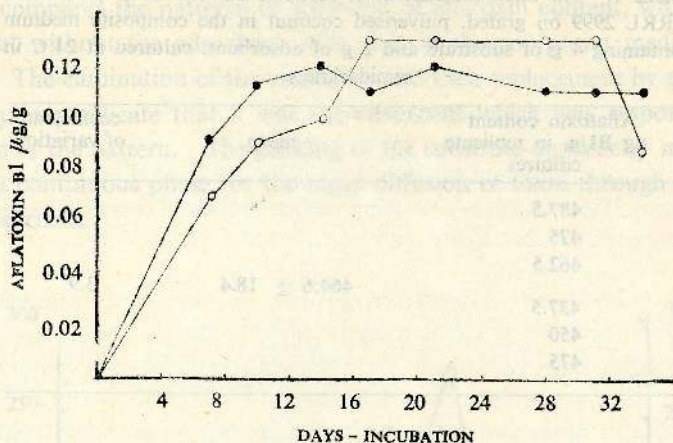


Figure 5. Pattern of variation of Aflatoxin B1 content of replicate cultures of *A. parasiticus* NRRL 2999 on pulverised green gram—●—; peanut—○—; in the composite medium at 24°C in semidarkness.

Figure 6 shows the patterns obtained with the strain F208 and F218 on the composite medium with grated coconut. The plateau of the curve with aflatoxin B1 was maintained for approximately ten days in both instances.

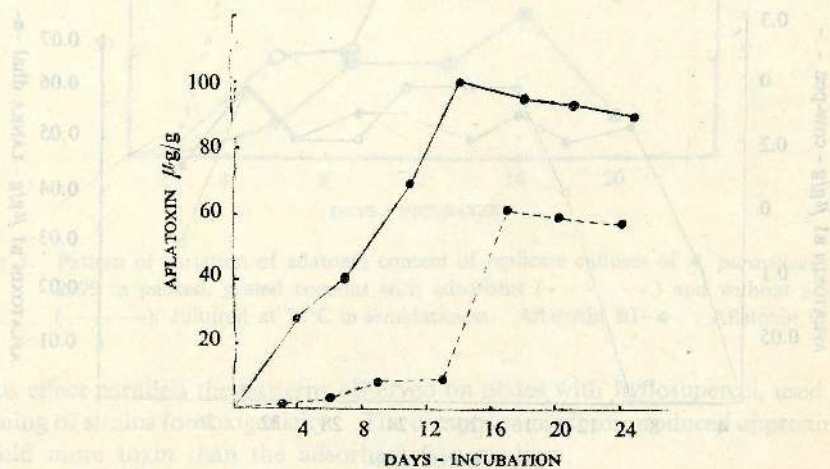


Figure 6. Pattern of variation of Aflatoxin B1 content of replicate cultures of *A. flavus* F208 (—) and F218 (- - -) on grated coconut in the composite medium at 24°C in semidarkness.

#### 4. Discussion

Although the original expectation of de Vogel *et al*<sup>4</sup> in the use of an adsorbent was that it would have prevented interference from the nor-aflatoxin greenish fluorescence of the mycelium in the agar medium with the fluorescence of the aflatoxins, the use of the adsorbent now appears to have a second advantage which is the removal of the aflatoxin from degrading or inactivating mechanisms which probably operate within the substrate. The evidence for this view is as follows : (1) the higher yields of toxin in the composite medium than in the adsorbent-free medium. An alternative explanation could be that substances which were inhibitory to aflatoxin production were removed by the adsorbent ; (2) the occurrence of phasic variations of aflatoxin content in the adsorbent-free medium whereas in the composite medium, these variations were eliminated and a plateau replaced the multiple peaks.

The reasons for our view that the phasic variations were not artefactual due to technical errors but were real variations are :—

- (1) the variations were reproducible in degree and time relations.
- (2) the variations were much larger (50—90%) than the general range of variation of the TLC values due to technical error ( $\pm 20\%$ )
- (3) the same assay procedure was applied to both adsorbent-free and the composite media while the levelling of the curves was seen consistently with the latter medium.

The use of substrates in a particulate form has several advantages. The use in conventional culture systems of seeds or kernels with seed coats which are impermeable to fungal invasion may produce false, low values of toxin content and may therefore not reflect the contamination as it would occur under field conditions or in storage. Under the latter conditions, fungal invasion and aflatoxin production occur mainly on seeds and kernels that have been damaged with the resultant exposure of the cotyledons or endosperm to fungal colonisation. A further advantage is that the uniformity of the plateau increased with the fineness of the particles of the substrate. This effect was probably due to the ease of mycelial penetration and aflatoxin production and the rapid transfer of the toxin into the adsorbent. Thus the composite medium would appear to measure the total aflatoxin accumulating capacity of the system under optimal conditions of accessibility of the substrate.

Conventional methods for the determination of the aflatoxin producing capacity of culture systems which use solid substrates incubated for arbitrarily selected, single incubation periods, have the disadvantage of not taking into account the significant variations of toxin content that occur with time. On the other hand the serial assay of replicate samples is a time consuming and cumbersome procedure. A further

disadvantage of conventional methods is the difficulty of standardisation to permit of valid comparisons between the results obtained in different laboratories or in the same laboratory on different occasions, in respect of both the toxigenic capacity of *Aspergilli* on a standard substrate or of the capacity of a given substrate to support toxin production using a standard strain of *Aspergillus*.

The method described in this paper appears to provide a simple and convenient alternative procedure which would not only eliminate the need for performing serial assays, but would also allow of the standardisation of the determination of aflatoxin production, in respect of the following factors :—

*the container* with cover, of specified dimensions

*the substrate*, of defined particle size, quantity and thickness of layer

*the adsorbent*, obtained from a single manufacturer and of defined quality, quantity and thickness of layer

*the inoculum*, a standard toxigenic strain is grown on a specified medium, cultured under defined conditions, and its spores are suspended in 0.1% Tween 80 to a standard spore count. A specified inoculum is used. To prevent loss of toxigenicity on repeated subculture on laboratory media, the standard strain may be periodically passaged through moist crushed peanuts.\*

**Incubation.**

The composite medium could be incubated under defined conditions of temperature, light, humidity and atmosphere. From our observations on the 5 different substrates tested, it would appear that an interval of three weeks after inoculation would coincide with approximately the midpoint of the plateau. Hence the single assay could be performed after an incubation period of three weeks, after inoculation of the composite medium.

**Extraction and assay procedure**, for aflatoxins could be prescribed in terms of solvents, methods of extraction, purification and titration of extracts.

The use of such standard conditions, and the measurement of the cumulative maximum amount of aflatoxin which the culture system has produced, may provide greater uniformity in the results obtained from studies on aflatoxin production. Hitherto, divergent results which are reported in the literature have been obtained by the use of conventional methods of culture. Examples of such controversial results include the claim that certain varieties of peanut are resistant to aflatoxin accumulation. It is possible that the occurrence of phasic variations of toxin content in conventional culture systems could have contributed to these discrepancies.

### Acknowledgement

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### 1. Introduction

The rubber tree is a tropical pine now grown to the extent of about 32,000 acres in Sri Lanka. Investigations on the tapping and microwave output of the *Phytophthora* species of *Phytophthora* had been reported previously by this group. In this study the terpentine content of the oleoresin when the plant is grown under local conditions as well as investigations aimed at the commercial extraction of the

several methods for isolating the terpenes of Pinus. (i) Tapping of the tree for latex collection. (ii) Steam distillation of wood and chips. (iii) Dry distillation. However, only the first two are widely used for producing terpenes. In other parts of the world the tapping method is still popular, but in addition the next step is necessary, the separation of resin and terpentine by water distillation. Most resin producing countries use large centrifugal machines as stills using steam under pressure. This situation clearly not suitable for small industry steps. An alternative method is the use of a simple fire still, which is popular in the early part of the century. Unfortunately, the critical factors of the operation of a fire still are not well understood.

This work has been carried out as a part of the Ph. D. Thesis of A. Chandrasekara.



## Studies on the *Pinus* Species growing in Sri Lankan Plantations II. Turpentine Content of *Pinus caribaea* Oleoresin and Studies Directed towards the Commercial Extraction of Turpentine

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(Paper accepted : 28 January 1980)

**Abstract :** The average turpentine content of *Pinus caribaea* oleoresin (Erabedde Plantation) is about 18% to 20%. However, individual trees showed marked variations not only from each other but also from season to season and day to day. Turpentine content may be as high as 30% or as low as 10%. The rate and efficiency of recovery of turpentine depends on (1) the quantity of water present and (2) cohobation. Detailed laboratory studies have led to a refinement in operating conditions, so that a simple fire still (50 kg capacity) can be made to yield turpentine quantitatively in a very short distillation time.

### 1. Introduction

*Pinus caribaea* is a tropical pine now grown to the extent of about 32,000 acres in Sri Lanka.<sup>1</sup> Investigations on the tapping and oleoresin output of the *P. caribaea* plantation of Erabedda had been reported previously by this group.<sup>3</sup> In this study we report the turpentine content of the oleoresin when the plant is grown under local conditions as well as investigations aimed at the commercial extraction of the turpentine.

There are several methods for isolating the terpenes of Pine. (1) Tapping of the oleoresin.<sup>4</sup> (2) Solvent extraction.<sup>5</sup> (3) Steam distillation of wood and chips, and (4) Dry distillation.<sup>7</sup> However, only the first two are widely used for producing rosin and turpentine with the solvent extraction process being favoured by most developed countries. In other parts of the world, the tapping method is still popular, and in this situation the next step is necessarily the separation of rosin and turpentine by steam or water distillation.<sup>6</sup> Most rosin producing countries use large centralised and continuous stills using steam under pressure,<sup>2,4,8</sup> a situation clearly not suitable for Sri Lanka at this early stage. An alternative method is the use of a simple fire still,<sup>4,7,8</sup> which was popular in the early part of the century. Unfortunately, the critical factors involved in the operation of a fire still are not clearly documented.

\*Some of this work has been carried out as a part of the M. Phil. Thesis of L. A. Goonetillake.

## 2. Experimental

### 2.1. Determination of Turpentine Content of Oleoresin

Oleoresin (25 to 100 g) was water distilled using 150 ml water for 4 hours, and the turpentine collected in a light oil (clavenger) arm. During 4 hours nearly all the turpentine is recovered (Figure 1), but the method has an error of about 5%.

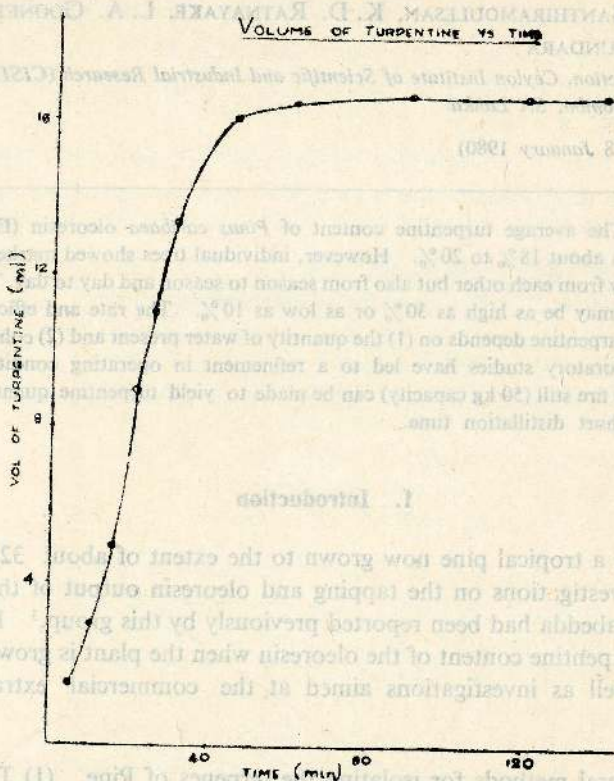


Figure 1. Time Courses of Turpentine Recovery. Weight of Rosin, 60 g ; Volume of water, 150 ml.

### 2.2. Factors Affecting Efficient Recovery of Turpentine

Experimental details are given in legends of figures.

### 2.3. Pilot Plant stills

#### (a) Model 1.

Designed for steam distillation and the production of high quality rosin, this is a single container where the oleoresin chamber is separated from the boiler by a partition that allows only the passage of steam. As a result, the temperature of the oleoresin does not rise above 100°C. The boiler is constructed of iron and the rosin chamber of aluminium.

## (b) Model 2.

This is a copper fire still, where the rosin is in the boiling compartment (Figure 2). By controlling the amount of water, the rate of turpentine recovery can be optimised.

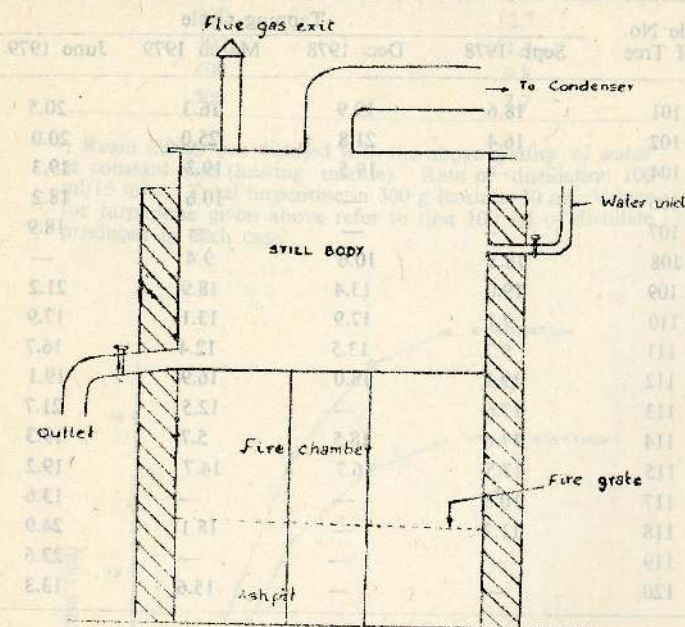


Figure 2. Turpentine, Fire Still Maximum capacity = 50kg.

Appropriate Technology Services  
 121, POINT-PEDRO ROAD  
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 No.

The still comprised a still body 1M by 0.7M containing an inlet (2 cm diameter) for the entry of cohobate and exit (25 cm diameter) for hot rosin. The lid contained the exit to the multitubular condenser. The still body is heated at the base by direct firing. The furnace contained a fire chamber and ash-pit separated by a fire grate and a flue gas exit.

### 3. Results

#### 3.1. Turpentine Content of the Oleoresin.

The mean turpentine content of the oleoresin of *Pinus caribaea* (of Erabedda) is about 18% to 20%. Turpentine content of the oleoresin varies markedly: (a) from tree to tree, (b) tapping cycle to tapping cycle and (c) day to day.

The variation from tree to tree is illustrated in Table 2. There appears to be a distinct direct relationship between yield of oleoresin and turpentine content of oleoresin.

Studies also showed the turpentine content of the oleoresin also markedly varied with tapping cycle (Table 1).

TABLE 1. Variation of Turpentine Content of Oleoresin

Code No. of Tree	Tapping Cycle			
	Sept. 1978	Dec. 1978	March 1979	June 1979
101	18.6	19.9	16.3	20.5
102	16.4	21.8	25.0	20.0
104	—	19.5	19.3	19.3
106	—	—	10.6	18.2
107	—	—	—	18.9
108	18.3	10.6	9.4	—
109	19.0	13.4	18.9	21.2
110	9.1	17.9	13.1	17.9
111	9.7	13.5	12.4	16.7
112	19.2	18.0	16.9	19.1
113	17.6	—	12.5	21.7
114	17.6	13.5	5.7	19.3
115	17.5	16.7	14.7	19.2
117	10.8	—	—	13.6
118	11.7	—	18.1	24.9
119	—	—	—	22.6
120	—	—	15.6	13.3

—, signifies not determined due to low oleoresin output.

The situation is further complicated by the fact that there is nearly always even a daily variation of turpentine content of oleoresin even in the same tree; turpentine content generally decreasing with time. In one tree (studied in detail), turpentine content which was 27.3% in the first day had declined to 16.5% by the fifth day.

### 3.2. Laboratory Studies on Recovery of Turpentine.

These studies were concentrated on two main lines (a) the effect of the oleoresin to water ratio and (b) the effect of cohobation on the rate of turpentine recovery.

#### 3.2.1. Effect on Oleoresin to water ratio.

Table 2 shows that large quantities of water result in a relatively inefficient rate of turpentine recovery. In this experiment, relative rate of turpentine recovery is measured by determining the quantity of turpentine recovered for every 100 ml water distilled. At limiting water levels, the temperature of the contents of the flask is higher than 100°C.

TABLE 2. Effect of Water to Rosin Ratio.

Volume of Water added (ml)	Vol. Turpentine per 100 ml distillate (ml)
150	18.0
200	12.7
300	11.4
400	9.8
500	7.7

Rosin (300g) was distilled with the above quality of water at constant rate (heating mantle). Rate of distillation 100 ml/15 min. Total turpentine in 300 g Rosin = 30 ml. Values for turpentine given above refer to first 100 ml of distillate produced in each case.

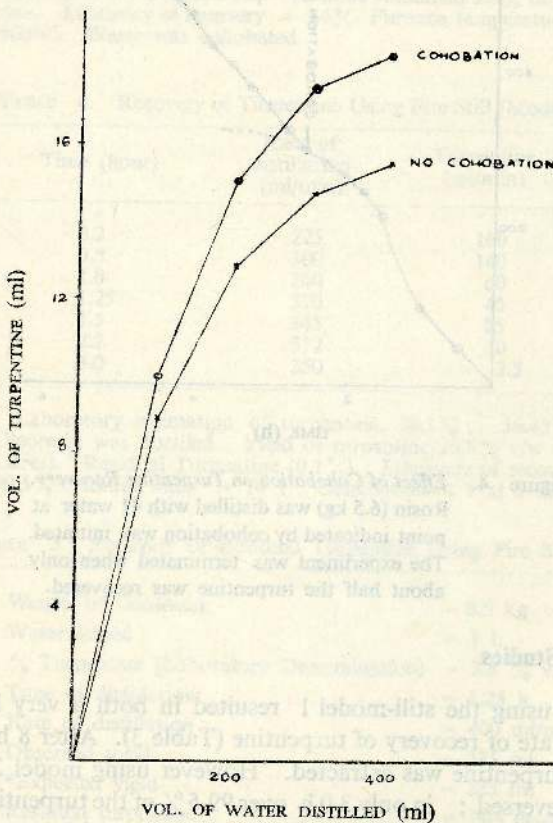


Figure 3. Effect of Cohobation on Turpentine Recovery. Rosin (150 g) was distilled with water (100 ml)  
 X — X, without Cohobation  
 O — O, with Cohobation.

### 3.2.2. Effect of Cohobation

At a temperature of 35°C to 40°C the turpentine forms an emulsion with water (containing 0.5 ml turpentine/100 ml water). Therefore by recycling the separated water it was possible to increase yields. Figure 4 shows the effect of cohobation on the distillation of turpentine. A similar experiment on a larger scale gave the same result (Figure 4).

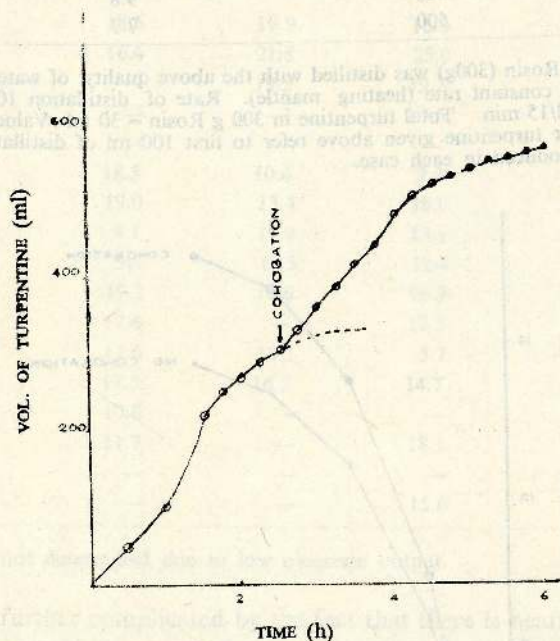


Figure 4. *Effect of Cohobation on Turpentine Recovery.* Rosin (6.5 kg) was distilled with 41 water at point indicated by cohobation was initiated. The experiment was terminated when only about half the turpentine was recovered.

### 3.3. Pilot Plant Studies

Steam distillation using the still-model 1 resulted in both a very low efficiency of recovery and low rate of recovery of turpentine (Table 3). After 8 hours distillation, only 84% of the turpentine was extracted. However using model 2, (the fire still) the situation was reversed; in only 3.0 h over 99.5% of the turpentine was recovered (Table 4). The efficiency of the second method was proved beyond doubt when the residual oleoresin from model 1 (containing 3.8% turpentine) was exhausted of turpentine in only 1h using model 2 (Table 5).

TABLE 3. Recovery of Turpentine Using Steam Distillation Still (Model 1).

Time (hours)	Water distilled (l)	Turpentine recovered (l)
1	12	0.75
1.5	21	1.025
2	27	1.225
3	42	1.550
4	58	1.775
5	75	1.935
6	92	2.095
7	109	2.245
8	127	2.355

Approx 13.89 kg of Oleoresin was used. Yield of Turpentine was 17.1 v/w. (2355 ml). Residue contained 3.8% turpentine. Efficiency of recovery = 84%. Furnace temperature = 900°C. Water was cohobated.

TABLE 4. Recovery of Turpentine Using Fire Still (Model 2).

Time (hour)	Rate of distillation (ml/min)	Turpentine (ml/min)
0.2	225	160
0.5	300	140
1.0	240	60
1.25	370	45
1.5	345	25
2.2	512	10
3.0	350	2.5

Laboratory estimation of turpentine, 20.1% ; 36.45 kg Oleoresin was distilled. Yield of turpentine 20.8% v/w (7.6 Litres). Residual Turpentine (0.1%). Efficiency of recovery 99.5%. Initial water = 10 l. Cohobation, 4 l at a time.

TABLE 5. Recovery of Residual Turpentine Using Fire Still.

1. Weight of Oleoresin	= 8.5 kg
2. Water added	= 3 L
3. % Turpentine (Laboratory Determination)	= 3.8 % v/w
4. Time of distillation	= 1.25 h
5. Rate of distillation	= 400 ml/min
6. Observed yield	= 335 ml
7. *Expected yield	= 323 ml
8. Residual turpentine	= Not detected
9. % $\alpha$ -Pinene—Laboratory Distilled	= 66%
10. % $\alpha$ -Pinene—Fire Still	= 67%

\*Based on sample drawn for laboratory estimation.

#### 4. Discussion

Results show that the average turpentine content of oleoresin is about 20%. However, there is wide variation (1) between trees, (2) from tapping cycle to tapping cycle and (3) day to day. There appeared to be some relation between turpentine content of oleoresin and oleoresin yield. However, other unidentified factors probably also affect yield. The relationship is interesting as the turpentine content of the oleoresin will affect fluidity.

Results of pilot plant studies were highly encouraging. By controlling water content of the distillation and using cohobation, it is possible to obtain turpentine (of the same quality as laboratory distilled material) not only in a very short distillation time but also at nearly quantitative yields by using a simple fire still.

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## Urea as a source of non-protein nitrogen for ruminants

### II. Effect of urea as a non-protein source on digestibility and voluntary intake by sheep fed alkali treated rice straw

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**Abstract :** Rice straw, the largest annually harvested agricultural by-product in Sri Lanka, is essentially an energy feed high in cell wall material, but because of its low digestibility, the intake of digestible energy is often inadequate even to prevent live weight loss in mature ruminants. In recent years, chemical procedures have been elaborated for improving low quality forages and rice straw has been successfully upgraded in feeding value, to a medium quality hay, by spray treatment with NaOH solution. Low nitrogen content is a predominant factor limiting efficient utilization of roughage material but non-protein nitrogen supplementation has been successfully employed to overcome this problem. In order to assess the influence of non-protein nitrogen supplementation on its feeding value, rice straw, variety H<sub>4</sub>, treated with 4% (w/w) NaOH solution (at the rate of 1.2 l/kg of straw) was fed *ad libitum* to growing sheep along with 100 g ground maize/animal/day, with or without urea. Urea represented 0 to 2.65% of the total dry matter consumed. Urea supplementation up to 1.87% resulted in a significant increase in digestibility. Beyond 1.87% there was no significant influence on digestibility, but voluntary intake increased. At 2.65% level of supplementation voluntary dry matter intake was 30% higher than the dry matter intake of diet containing no urea. It appears that 1.8 to 2.0% of the total dry matter consumed is the optimum level of urea supplementation for 4% NaOH (w/w) treated rice straw. Since insufficient sulphur could have influenced urea nitrogen utilization by rumen micro-organisms, ammonium sulphate may be a better source of npn for ruminants.

#### 1. Introduction

Many low quality forages are essentially energy feeds high in cell wall material. Even so, they are little used as animal feeds because of their low energy yields. They are generally low in digestibility and intake and the intake of digestible energy is often inadequate to even prevent live weight loss in mature ruminants.

Alkali treatment can markedly increase the nutritive value of many poor quality roughages,<sup>3,8,12,17,25,27</sup> and sodium hydroxide treatment has been claimed to be the most effective.<sup>14,22</sup> Recent trials, in Sri Lanka, have indicated that rice straw, one of the largest annually harvested agricultural by-products, can be successfully up graded in feeding value, to a medium quality hay, by spray treatment with sodium hydroxide solution.<sup>15,16</sup>

A further factor limiting the efficient utilization of roughage material by ruminants is their low nitrogen content which is generally regarded as too low to sustain adequate activity of the cellulose and hemicellulose digesting microorganisms in the rumen. For efficient breakdown of lignocellulose in the rumen a nitrogen content of 1% in the DM of diets, the energy contents of which are less than 50% digestible is considered essential. In more digestible lignocellulose based diets nitrogen requirements may increase up to 1.5% to 2% of the DM.<sup>23</sup>

The usefulness of non-protein nitrogen (npn) compounds in the nutrition of the ruminants has been well established.<sup>4,5,9,10,24</sup> However, despite the voluminous research carried out,<sup>5</sup> practical progress in this field has been relatively small, partly because of the complexity of nitrogen metabolism in the rumen, and partly because of the lack of data on applied research in the field.

The objective of the work reported here was to determine the effect of npn supplementation of NaOH treated rice straw on digestibility and voluntary intake, using growing sheep and thereby quantify the optimum level of supplementation, for the preparation of practical rations. Urea was selected as the npn source as it is probably the commonest npn compound of choice as a protein replacer for ruminant feeding. The work was carried out at the Department of Animal Husbandry, Faculty of Agriculture, University of Peradeniya.

## 2. Experimental

Rice straw, variety H<sub>4</sub>, chopped into 20 to 50 mm lengths was treated with 40 g NaOH dissolved in 1.2 litres of water per 1 kg of straw, according to the technique previously described.<sup>16</sup> Treated straw dried in an Unitherm oven at 98°C for 6 hours, was stored in bags until required for the feeding trial.

The experiment was designed as a complete randomized block experiment with seven treatments. All animals were offered *ad libitum* treated straw + 100 g ground maize with or without urea. The quantity of urea in the concentrate (0, 5, 8, 10, 12, 15 or 18 g urea/100 g concentrate, representing 0 to 2.65% of total DM consumed) represented the seven treatments (Table 2). Concentrate and treated straw was offered separately, twice daily, in equal amounts. Water and a standard mineral mixture were available *ad libitum* through out the feeding trial.

Growing Jaffna × Bikaneri female sheep of average live weight 21.7 kg (range 20.5 to 25.5 kg) were used to measure the apparent digestibility of the treated straw rations. Three animals were used for each treatment. All animals were housed in individual digestion cages designed to enable the separate collection of faeces and urine.

The feeding period was of 20 days duration, the first 12 days to allowing for adjustment to the ration with voluntary intake and digestibility measured over the last 8 days. Voluntary intake was measured by feeding daily, an amount of ration to insure an excess of at least 10% over previous days intake and determining actual intake by daily weighing of refused feed.

Daily faecal collection for each sheep during the collection period was weighed, sampled and frozen for later analysis. Urine was collected daily in plastic buckets containing 25 ml of 1N HCl; the volume determined, a 2% aliquot was refrigerated for later analysis.

Proximate constituents,<sup>2</sup> acid detergent fibre (ADF), cellulose and silica<sup>13</sup> were determined for treated straw and the concentrate.

The digestibility of straw organic matter was calculated by assuming the organic matter digestibility of concentrate to be 85%.

### 3. Results

The average chemical composition of treated straw and ground maize used in the feeding trial is shown in Table 1. The composition, energy value and crude protein content of the experimental diets are shown in Table 2. The consumption of treated straw and increasing levels of urea had no ill effects on the health of animals.

Addition of urea up to 1.87% of the total DM consumed, (0 to 10 g urea/100 g concentrate) significantly ( $P < 0.01$ ) increased both dry and organic matter digestibility of NaOH treated straw diets. Beyond this level however, there was no further increase in digestibility (Table 3).

Dry matter intake remained constant up to 1.87% level of urea supplementation. Above this however, an increase in the level of supplementation increased the DM intake markedly. At 2.65% level of supplementation, voluntary DM intake was 30% higher than the diet containing no urea. This also resulted in an increase in digestible energy intake (Table 3).

All urea fed animals showed a positive nitrogen balance compared to the control treatment without any urea. Urea had no influence on voluntary intake of water and output of urine.

TABLE 1. The average chemical composition of concentrate and treated straw used in the feeding trial

Component	Treated straw	Concentrate
	g/100 g dry matter*	
Ash	16.5	1.3
Crude protein	4.2	8.4
Crude fibre	30.8	2.2
Acid detergent fibre (ADF)	56.3	5.1
Cellulose	38.9	4.2
Silica	9.9	—

\*dry matter content — straw — 96.6

dry matter content — concentrate — 93.4

TABLE 2. Composition and energy value of rations used in the feeding trial.

Ration number	1	2	3	4	5	6	7
Amount of NaOH (g/kg straw)	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Volume of water (l/kg straw)	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Amount of concentrate fed with straw (g/animal/day)	100	100	100	100	100	100	100
Quantity of urea added to concentrate (g/100g concentrate)	0	5.0	8.0	10.0	12.0	15.0	18.0
Crude protein content of ration (g/100 g DM consumed)	5.04	7.76	8.16	10.42	10.78	10.81	12.56
Intake of urea (%) (intake as a % of total DM consumed)	0	0.94	1.33	1.87	2.02	2.18	2.65
Estimated ME content of ration (MJ/kg DM)	6.18	7.56	7.46	8.60	8.43	8.22	8.48

TABLE 3. Intake and apparent digestibility of dietary constituents

Ration number	1	2	3	4	5	6	7	S.E. of difference between means
<i>Intake</i>								
Urea (%) (intake as a % of total DM consumed)	0	0.94	1.33	1.87	2.02	2.18	2.65	—
Digestible energy (MJ/day)	4.16	4.96	5.52	5.67	6.18	6.99	7.10	—
Straw- <i>ad libitum</i> (g/DM/kg W 0.75/day)	44.8	43.6	41.3	43.9	49.98	54.0	58.1	± 2.72
Water (Voluntary intake l/day)	1.386	1.563	1.823	1.832	1.492	1.897	2.261	± 0.316
<i>Digestibility of dietary constituents (%)</i>								
Dry matter (DMD)	41.1	51.1	51.2	58.4	57.3	57.2	59.0	± 2.56
Organic matter (OMD)	46.7	56.9	56.5	64.9	63.8	62.4	64.3	± 1.98
Digestible organic matter in dry matter (DOMD)	40.2	49.1	48.5	55.9	54.8	53.4	55.1	± 2.48
<i>Derived digestibility of straw organic matter</i>								
Output	37.4	49.9	50.2	59.9	59.1	58.1	60.5	± 3.53
Urine (l/day)	0.911	0.762	0.926	1.033	0.739	1.219	1.434	± 0.133
N balance (g/day)	-3.71	+ 1.56	+ 2.18	+ 3.90	+ 4.40	+ 6.60	+ 5.35	—

#### 4. Discussion

Addition of urea (0 to 2.65% of total DM consumed) increased the crude protein equivalent of the straw diets from 5.0 to 12.5%, but this increase was not proportionate to the increase in the urea content of the ration. This was due to the comparatively higher intake of straw DM at the upper levels of urea supplementation.

The results of the present experiment are in general agreement with work reported elsewhere on both treated and untreated cereal straw rations supplemented with urea. With untreated straw, there had been many instances where the addition of urea resulted in increases in digestibility and voluntary intake of poor quality roughages.<sup>1,11,19</sup> There were other instances, however, where urea supplementation had no influence on the utilization of low quality roughages.<sup>6,18,20,21</sup> Similar results have been obtained with alkali treated roughages as well. Bramen and Abe<sup>7</sup> found no improvement in digestibility of NaOH treated (4% NaOH, w/w) wheat straw when it was supplemented with 1.0 or 2.0% urea. Donefer *et al*<sup>12</sup> observed no significant effect on digestibility, but voluntary intake increased three fold when 2.5% urea was added to oat straw treated with 8% (w/w) NaOH solution. They explained this observation on the basis that nitrogen was a limiting factor and that adequate supply of nitrogen by way of urea resulted in an increase in the rate of microbial digestion and thus voluntary consumption of forage by the animal. The work of Rexen *et al*<sup>26</sup> also supports the view that urea supplementation need not necessarily increase the utilization of treated straw diets.

As pointed out by Balch,<sup>4</sup> in spite of more than half a century of experimentation with npn compounds, there is still much doubt about the response to npn supplementation of diets of cattle and sheep. As he rightly pointed out, extra nitrogen in the form of urea or any other npn compound, cannot be expected to give responses, if energy becomes a limiting factor.

The results of the present experiment shows that even with alkali treated straws, energy can become a limiting factor for urea utilization. Alkali treatment increases the digestible energy content of the treated material and thereby its ME content. This will encourage the utilization of npn by rumen micro-organisms. But beyond a certain level any further addition of npn will not give higher responses as energy becomes a limiting factor. It is thus evident that with 4% NaOH treated rice straw, the optimum utilization of urea occurs at 1.87% level of supplementation at which stage the metabolizable energy availability of treated material is 8.60 MJ/kg DM.

The higher intake achieved beyond 1.87% level of urea supplementation could have been due to an increased rate of passage of digesta through the digestive tract. The increase in intake was not associated with an increase in digestibility but it influenced the digestible energy intake.

Sulphur could have been a limiting mineral element in npn utilization by rumen micro-organisms as no additional sulphur was given with the diet in the reported work. In this respect ammonium sulphate could be a better source of npn for ruminants but its role as a npn source has to be first ascertained before it could be used in practical rations. Thus, more research is required before any conclusions could be drawn on the optimum level of npn supplementation for alkali treated rice straw.

Cost analyses have been done on data from many feeding trials conducted in various countries to establish the economic feasibility of feeding alkali treated straw to farm animals. While in Europe the substitution of treated straw for hay and silage appears to be profitable, it does not seem to be so in North America even when the straw is produced in the same farm. However in countries where straw is traditionally fed to livestock, alkali treatment is clearly profitable, especially when straw has no value other than the cost of collecting it and treating it on the farm. In Sri Lanka, the main cost would be that of the cost of alkali, if family labour of the small farm unit is not taken into account. The economic feasibility of treated straw as a substitute for fodder in Sri Lanka is however yet to be established under field conditions.

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## Sterols of Some Sri Lankan Marine Algae

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**Abstract :** Eighteen species of Sri Lankan marine algae belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae were examined for sterols. 28-isofucoesterol is the major sterol in green algae belonging to Order Ulvales. Green algae belonging to orders Siphonales, Siphonocladales and Dasycladiales contain 24-ethyl cholesterol as the major sterol. Fucosterol is the major sterol in all the brown algae examined. The red algae contained cholesterol as the major sterol. The work indicates that there is no significant difference in the major sterols found in the marine algae of Sri Lankan, Japanese, British, French and Canadian waters.

### 1. Introduction

The sterols of marine algae<sup>11</sup> are distinctly different from division to division and in some cases within divisions. The major sterol of most of the red algae examined is recognised as cholesterol.<sup>1,5,7,10</sup> It was found previously<sup>4</sup> that Japanese red algae could be classified into three groups based on sterol profiles. One group contains cholesterol as the major sterol. The sterol mixtures of the second group contains cholestanol as the major sterol. The third group contained dehydrocholesterol as the principal sterol. Another sterol which usually accompanies cholesterol in red algae is desmosterol<sup>1,5</sup> and is the major sterol in several species.

Major sterol of the brown algae is fucosterol.<sup>3,9</sup> Cholesterol, 24-methylene cholesterol and saringosterol are also found in brown algae, but in small quantities.

The sterols of green algae are usually complex mixtures. Gibbons et al<sup>8</sup> identified 28-Isoufucoesterol as the major sterol of British green algae *Enteromorpha intestinalis* and *Ulva latuca*.

Very little information is available on the sterol composition of the Indian Ocean algae.

### 2. Results and Discussion

Eighteen species of Sri Lankan marine algae were examined for sterols using gas chromatography-mass spectrometry (GC-MS). The identification of each sterol was based upon its GC relative retention time and its MS fragmentation pattern. The sterol profiles of the algae are summarized in the table given below.

TABLE I. Sterol Profiles of Sri Lankan Marine Algae

GREEN ALGAE	Order	Family	Species	Locality									Sterol								
				1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
GREEN ALGAE	Ulvales	Ulvaceae	<i>Ulva reticulata</i>	Galle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
			<i>Ulva lactuca</i>	Galle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
			<i>Ulva fasciata</i>	Galle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	Cladophorales	Cladophoraceae	<i>Chaetomorpha crassa</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
			<i>Halimeda opuntia</i>	Galle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
			<i>Bryopsis corticilang</i>	Galle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	Siphonales	Bryopsidaceae	<i>Caulerpa taxifolia</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
			<i>Caulerpa clavata</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
			<i>Valoniopsis pachynema</i>	Mt. Lavinia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Siphonocladales	Valoniaceae	<i>Valoniopsis pachynema</i>	Mt. Lavinia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
		<i>Acetabularia cranulata</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Dasycladiales	Dasycladiaceae	<i>Valoniopsis pachynema</i>	Mt. Lavinia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
		<i>Acetabularia cranulata</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
		<i>Acetabularia cranulata</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
BROWN ALGAE	Fucales	Sargassaceae	<i>Sargassum tenerrimum</i>	Galle	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
			<i>Cystophyllum muricatum</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
			<i>Cystostera triquetra</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
RED ALGAE	Gelidiales	Gelidiaceae	<i>Gelidium corneum</i>	Galle	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
			<i>Gracilaria lichenoides</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	Rhodomelales	Rhodomelaceae	<i>Gracilaria confervoides</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
			<i>Gracilaria opuntia</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
			<i>Laurencia papillosa</i>	Jaffna	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

\*Major sterol; + Minor sterol

Cholesterol is the most common sterol in red algae, all species presently examined contained cholesterol as the major sterol. Fucosterol is the major sterol of brown algae. All the species of brown algae contain small amounts of cholesterol in addition to fucosterol. The green algae examined could be classified into two groups based on the sterol profiles. (One group three species) contained 28-isofucosterol as the major sterol. All these species belong to the family Ulvaceae. The present identification of 28-isofucosterol in Sri Lankan *U. lactuca*, *U. reticulata* and *U. faciata* in addition to its presence in *E. linza*<sup>15</sup> and British green algae *E. intestinalis* and *U. lactuca*<sup>7</sup> confirms that this is characteristic of Ulvaceae. The other group of green algae (six species) contain 24-ethylcholesterol as the major sterol.

Our work indicates that there is no difference in the major sterols found in marine algae of Sri Lanka, Japanese, British and Canadian waters.

### 3. Experimental

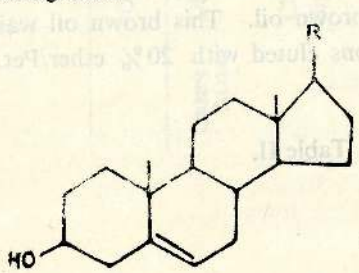
The algae were collected along the coast of Colombo, Galle and Jaffna, Sri Lanka. The air dried algae (ca 100g) was extracted with refluxing methanol (500 ml) for 3h. Extracts were saponified in a mixture of 40% KOH (1 ml) and methanol (25 ml).

An aliquot (ca 1 mg) of the unsaponifiable material was treated with trimethylsilylimidazole (100  $\mu$ l) in a sealed tube at 70° for 30 mins. Resulting trimethyl silyl ether of the sterol were analyzed by GC-MS on 1.5% OV. 17(1.5 m  $\times$  3 mm, 250°C.). Identification of each sterol was based upon the comparison with the respective authentic sample in respect of GC retention time and MS fragmentation pattern as previously described.<sup>2,3,8,11</sup> RR'<sub>s</sub> of the TMSi derivatives of standard samples were ; Cholestane, 1.0 ; 22-dehydrocholesterol, 1.84 , cholesterol, 1.97 brassicasterol, 2.26 ; stigmasterol, 2.79, sitosterol, 3.18 ; fucosterol, 3.38 isofucosterol 3.51, 24-methyl cholesterol, 2.57 ; saringosterol ; 4.85. A different procedure was adopted for the isolation of sterols from *Sargassum* and *Laurencia*. Air dried algae (2kg) were extracted with refluxing methanol (2l). The methanol extract was concentrated, water was added to the concentrate and extracted with ether. Removal of ether under reduced pressure gave a brown oil. This brown oil was chromatographed on alumina and the sterol fractions eluted with 20% ether/Pet. ether were combined and analyzed by GC-MS.

The MS fragmentations of the sterols are given in Table II,

KEY TO STEROLS

Sterol	Number	Chemical Structure
Cholesterol	1	
24 - Ethylcholesterol	2	
24 - Methylcholesterol	3	
24 - Methylcholesta - 5, 22 - diene 3 β - ol	4	
Fucosterol	5	
28 - Isofucosterol	6	
24 - Methylenecholesterol	7	
24 - Ethylcholesta 5, 22 - diene - 3 β - ol	8	
Saringosterol	9	



\* Indicates the point of attachment of R.

TABLE (II)—Mass spectral Fragmentation of TMSi Derivatives of Sterols.

STEROLS	NUMBER	MS FRAGMENTATIONS (Major fragmentations at m/e)
Cholesterol	1	458 ( $M^+$ ), 443 ( $M^+ - CH_3$ ), 368 ( $M^+ - TMSOH$ ), 358 ( $M^+ - TMSOH - CH_3$ ), 329 ( $M^+ - 129$ ), 255 ( $M^+ - TMSOH - Sidechain$ ), 129
24 $\xi$ -Ethylcholesterol	2	486 ( $M^+$ ), 471 ( $M^+ - CH_3$ ), 396 ( $M^+ - TMSOH$ ), 381 ( $M^+ - TMSOH - CH_3$ ), 357 ( $M^+ - 129$ ), 255 ( $M^+ - TMSOH - Side chain$ ), 129.
24 $\xi$ -Methylcholesterol	3	472 ( $M^+$ ), 457 ( $M^+ - CH_3$ ), 382 ( $M^+ - TMSOH$ ), 367 ( $M^+ - TMSOH - CH_3$ ), 343 ( $M^+ - 129$ ), 255 ( $M^+ - TMSOH - Sidechain$ ), 129.
Fucosterol	5	484 ( $M^+$ ), 469 ( $M^+ - CH_3$ ), 394 ( $M^+ - TMSOH$ ), 386 ( $M^+ - 98$ ), 379 ( $M^+ - TMSOH - CH_3$ ), 355 ( $M^+ - 129$ ), 255 ( $M^+ - TMSOH - Sidechain$ ) 129.
Isofucosterol	6	484 ( $M^+$ ), 469 ( $M^+ - Me$ ), 386 ( $M^+ - 98$ ) 355 ( $M^+ - 129$ ), 296 ( $386 - TMSOH$ ), 281 ( $296 - Me$ ), 257, 129.
24-methylenecholesterol	7	470 ( $M^+$ ), 386, 296.
Saringosterol	9	572 ( $M^+$ ), 557 ( $M - Me$ ), 529 ( $M - iPr$ ) 439 ( $529 - TMSOH$ ), 349 ( $439 - TMSOH$ ) 295, 255, 253, 171, 129.

### Isolation of Isofucosterol

The algae (1.5 kg) was defatted with petroleum ether (60° to 80°) and then extracted with acetone. The acetone extract was concentrated under reduced pressure. The residue was diluted with water and extracted with ether. Removal of ether gave a brown oil. This brown oil was chromatographed on alumina and the fraction eluted with 25% benzene in petroleum ether yielded 28-isofucosterol (1.5g) as a white crystalline solid m.p. 135° to 136° (Lit<sup>14</sup> m.p. 135.5—136.4°). (Found : C, 84.29 ; H, 11.56;  $C_{29}H_{48}O$  requires C, 84.47 ; H, 11.40). IR (KBr) $cm^{-1}$ : 812, 840, 1065, 1580, 3440.  $^1H$ NMR ( $CDCl_3$ ) :  $\delta$  5.29 (m, 1H) C-6 proton, 5.05 (q,  $J \approx 6.9Hz$ ) C-28 proton ; 3.40 (m, 1H) C-4 proton ; 1.54 (d, 3H) C-29 protons, 2.80 (m, 1H) C-25 proton. I.R. and n.m.r. of this white solid were identical to those reported<sup>8</sup> for the compound. Further confirmation came from GC-MS analysis as its trimethylsilyl ether.

### Acknowledgements

We thank the National Science Council of Sri Lanka and the University of Colombo, Sri Lanka for financial assistance. We thank Professor C. Djerassi, Stanford University, for the analysis of sterol fraction of *Sargassum*. The authors thank Professor Hisashi Takei, Tokyo Institute of Technology, for his advice and encouragement. Our sincere thanks are due to Mrs. S. Medis for the illustrations.

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## Geology of Sri Lanka in relation to Plate Tectonics

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**Abstract :** The crystallines of the Island of Sri Lanka is divided into Highland series and Vijayan series and the contact between these two series is a possible Paleo plate boundary. The geological evidence supporting this interpretation includes :—

The presence of paired metamorphic belts—the granulite facies and the amphibolite facies.

The presence of basic and ultrabasic rocks of probable igneous origin closer to the Highland-Vijayan contact.

The copper-magnetite-apatite body and its related rocks. It is possible that mineralisation has taken place at the boundary between the two main rock groups.

The hot springs of Sri Lanka mainly confined to Vijayan series and are mostly along the contact.

The Submarine Canyon with wall heights 1,350 meters off the coast of Trincomalee contiguous with the Highland-Vijayan contact.

The interpretation is that the boundary between the Highland-Vijayan series was an old subduction zone; where the two plates were moving together. Active volcanism continuous subduction and upliftment have caused the present day Highland series. The rocks of the Highland series comprises of quartzites, crystalline limestones, garnetiferous granulites and charnockites. The volcanic rocks are not present today, as they have been eroded away. Both groups are intensely metamorphosed and metasomatised. The Highland series was metamorphosed to the granulite facies and the Vijayan series to the almandine-amphibolite facies.

### 1. Introduction and General Geology of Sri Lanka

Sri Lanka though an island today, is an integral portion of the carnatic gneissic terrain of the Deccan Peninsula of India only recently severed from the mainland. An attempt has been made in this paper to interpret the Geology of Sri Lanka in terms of plate tectonics.

The major geological divisions of Sri Lanka are given by (Figure 1).<sup>3</sup> The greater part of the country is underlain by crystalline rocks of Pre-Cambrian age; which are divided into two groups on the basis of metamorphic rank and age. The Highland Series metamorphosed to granulite facies<sup>6</sup> which occupies the central part of Sri Lanka consisting of a succession of gneisses, garnet-sillimanite-graphite-gneisses, quartzites and marbles together with charnockites. Migmatites and granite gneisses are less developed in the Highland Series.

Geology of Sri Lanka in relation to Plate Tectonics

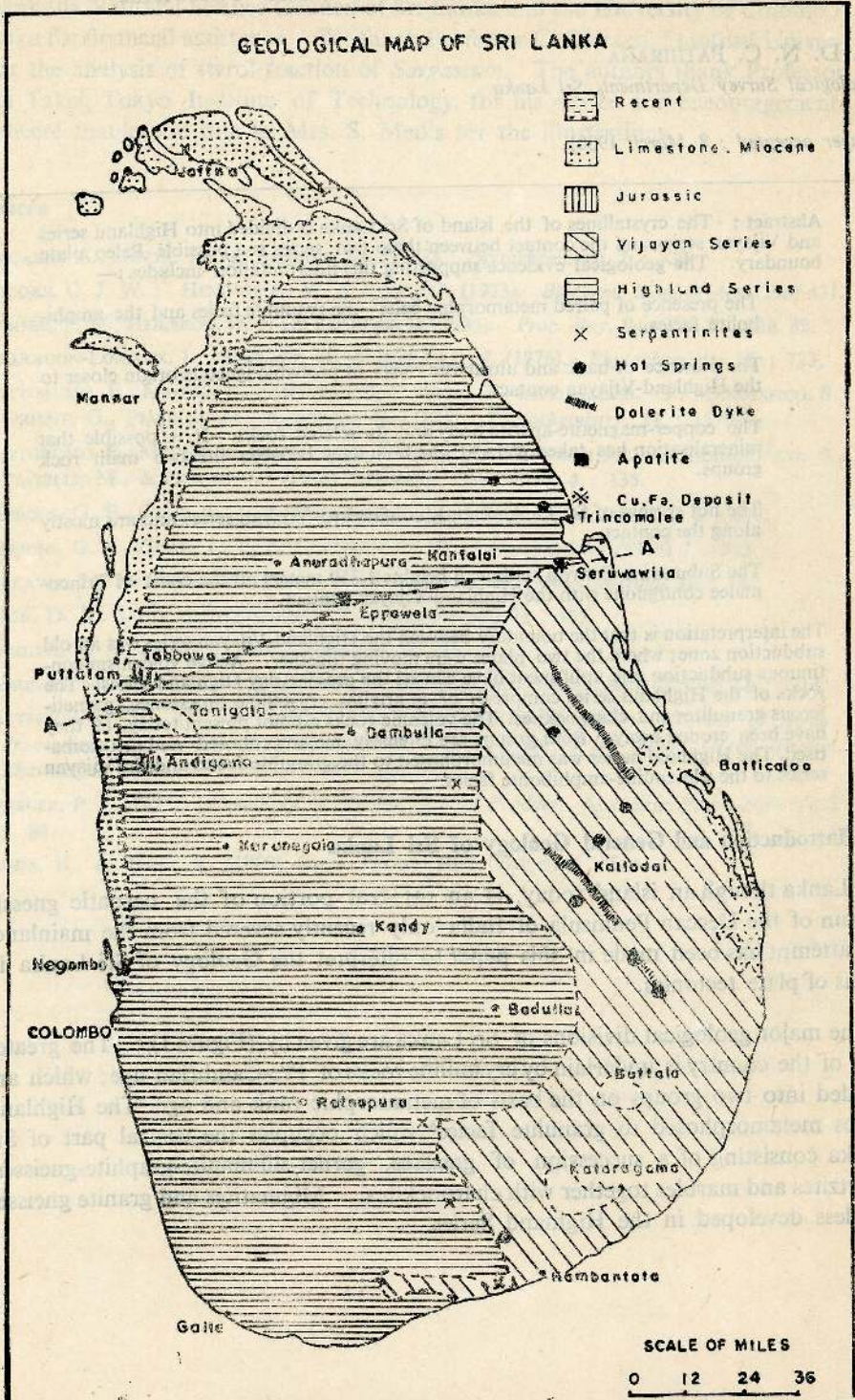


Figure 1. Geological Map -- Sri Lanka

The Vijayan series metamorphosed to the amphibolite facies<sup>6</sup> which consist mainly of microcline-biotite and hornblends biotite-gneisses, granites and granite gneisses, and occupies the eastern and south-eastern Lowlands.

Mesozoic rocks, mainly sandstones, shales and siltstones of Upper Gondwana age (Wayland, 1925) are preserved in faulted basins at Tabbowa and Andigama near Puttalam. In the north-western part of the country the dominant rock is the sedimentary limestone which underlies the whole of Jaffna Peninsula and the surrounding Islands. This limestone belt extends southwards along the west coast of the Island as a gradually narrowing belt.

## 2. Previous Work

There is no direct evidence to show the origin of the Highland series Adams<sup>1</sup> considered the peneplain which they represent, to be produced by ordinary denudation of dip slopes. Wadia (1945) impressed by the "Stupendous Mural Scarps" emphasised the horst like nature of the central Sri Lanka massif and suggested block uplift of the Highlands through powerful dislocations and block faults of the normal type. King<sup>11</sup> points out that direct faulting has not been forthcoming and the mighty rock faces are seemingly true "Erosion Scarps". More recently Vithanage (1972) postulated that the regional morphotectonic and morphological features can be explained by normal erosion acting on the Precambrian terrain which has been subjected to prolonged differential warping along a broad SSW-ENE zone. The predominant deformations were by series of vertical differential uplifts with a culminating phase around Jurassic (Gondwana and Miocene early to end of Tertiary). Hatherton *et al*<sup>7</sup> predicts that the central Highlands appear to be completely uncompensated isostatically. The largest scale anomaly in the island runs along the western part of the Vijayan series and parallel to its junction with Highland series (see Figure 2).

## 3. Geological Processes at Plate Boundaries

According to the plate tectonic hypothesis, the outer layer of the earth consists of lithosphere, 80 to 100 km thick divided into a number of rigid plates moving relatively to each other over a plastic asthenosphere. There are twelve major plates and thirty minor plates recognised at present. Volcanism, mountain building, and mineralization are concentrated at the boundaries of these plates. Plate boundaries are three types, constructive, destructive and conservative. At constructive plate boundaries new lithosphere is consisting of a layer of oceanic crust overlying the

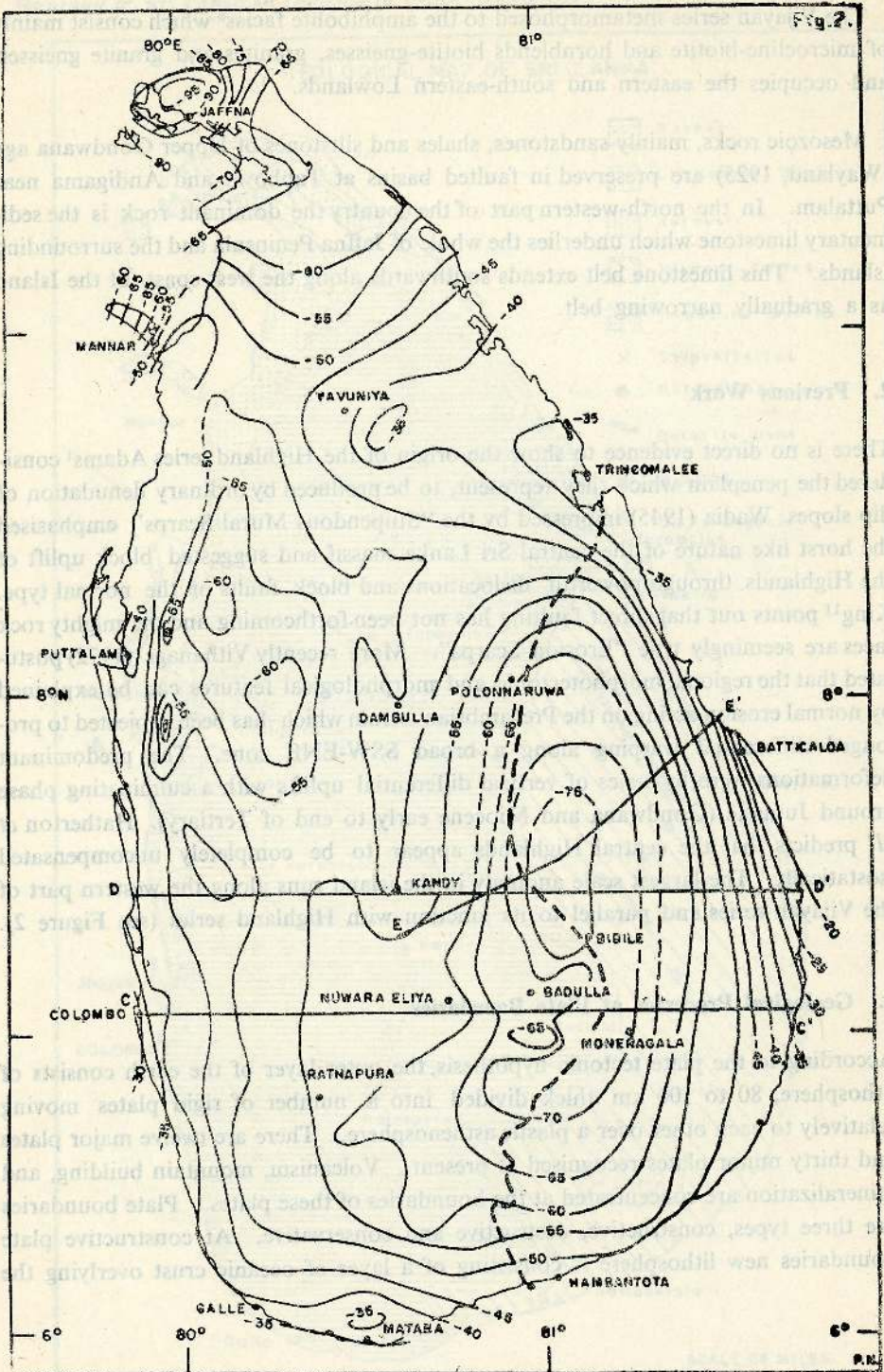


Figure 2. Hybrid gravity anomaly map, Sri Lanka. (After Hatherton *et al*)

upper mantle, and it is created either continuously or intermittently at the rate of spreading of about 10 cm per year along the axis of an oceanic spreading ridge system. At constructive plate boundaries the plates move away from each other (Figure 3). At destructive plate boundaries the plates move opposite to each other (Figure 4) and the collision result due to continuous subduction of the oceanic crust, for example the collision of Indian plate with the Asian plate in the Tertiary following subduction and closure of Tethys ocean. At conservative plate boundaries the plates move parallel to each other along a transform fault. Transform faults are linked with ridges trench systems and at times large faults on lands, for example the San Andras fault in the U.S.A. and Alpine fault in the South Island of New Zealand. The spreading ridges, trenches associated with subduction zones and transform faults form a continuous linked network around the Earth Surface.

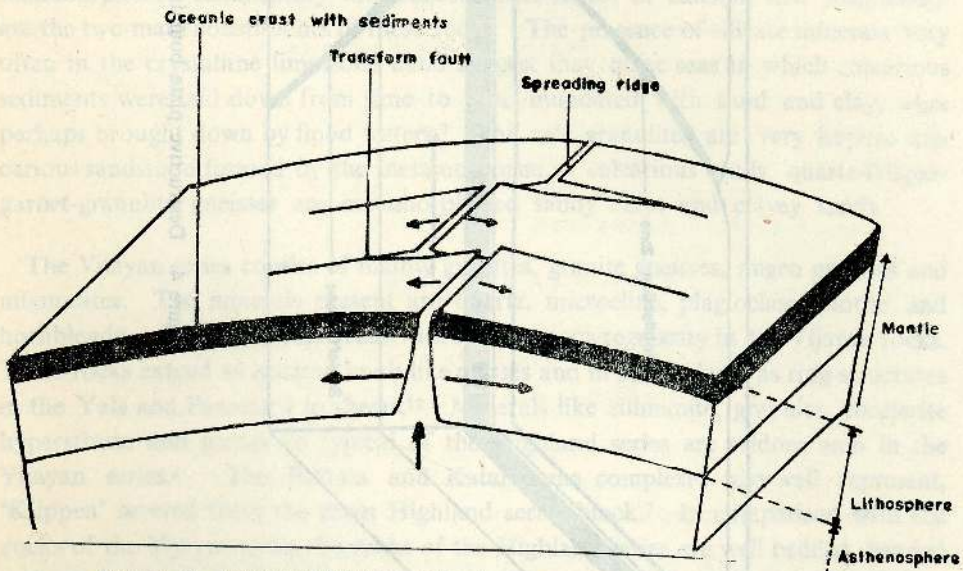


Figure 3. CONSTRUCTIVE PLATE BOUNDARY

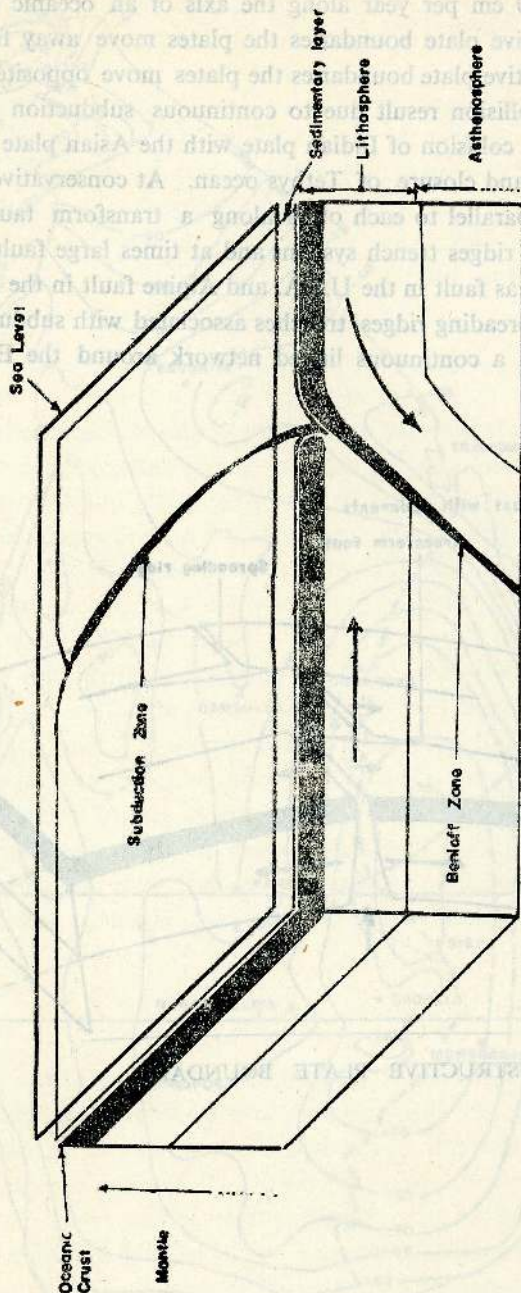


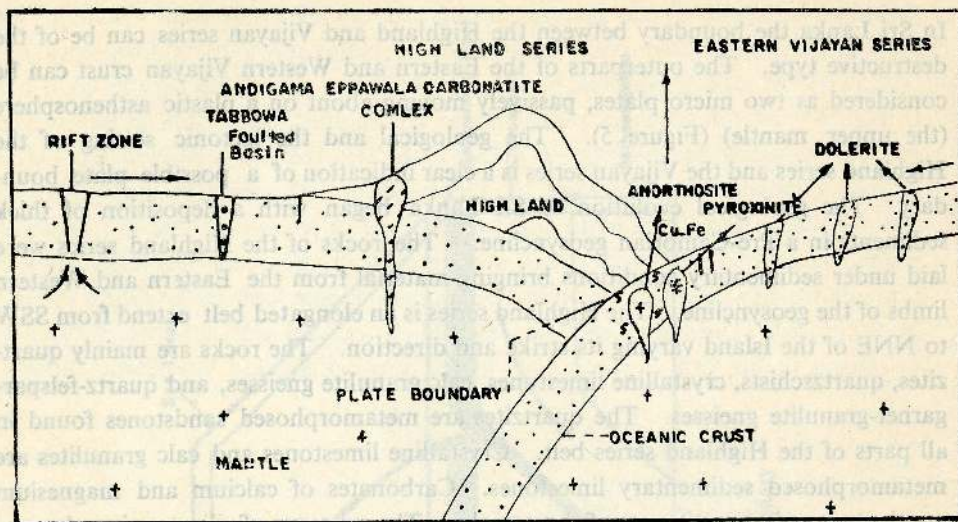
Figure 4. Destructive plate boundary

#### 4. Some Possible Evidence of the Plate Boundary

In Sri Lanka the boundary between the Highland and Vijayan series can be of the destructive type. The outerparts of the Eastern and Western Vijayan crust can be considered as two micro plates, passively moving about on a plastic asthenosphere (the upper mantle) (Figure 5). The geological and the tectonic setting of the Highland series and the Vijayan series is a clear indication of a possible plate boundary. The geological evolution of Sri Lanka began with a deposition of thick sediments in a Pre-Cambrian geosyncline.<sup>3</sup> The rocks of the Highland series were laid under sedimentary conditions bringing material from the Eastern and Western limbs of the geosyncline. The Highland series is an elongated belt extend from SSW to NNE of the Island varying its strike and direction. The rocks are mainly quartzites, quartzschists, crystalline limestones, calc granulite gneisses, and quartz-felspar-garnet-granulite gneisses. The quartzites are metamorphosed sandstones found in all parts of the Highland series belt. Crystalline limestones and calc granulites are metamorphosed sedimentary limestones. Carbonates of calcium and magnesium are the two main constituents of these rocks. The presence of silicate minerals very often in the crystalline limestone band suggest that quiet seas in which calcareous sediments were laid down from time to time inundated with sand and clay, were perhaps brought down by flood waters.<sup>3</sup> The calc granulites are very impure calcareous sandstone formed by the metamorphism of calcareous muds, quartz-felspar-garnet-granulite gneisses are metamorphosed sandy clays and clayey sands.

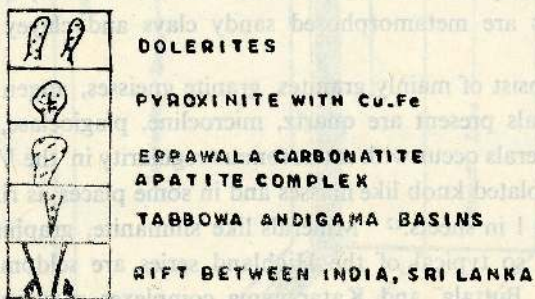
The Vijayan series consist of mainly granites, granite gneisses, augen gneisses and migmatites. The minerals present are quartz, microcline, plagioclase, biotite and hornblende. These minerals occur with monotonous regularity in the Vijayan rocks. These rocks extend as isolated knob like masses and in some places as ring-structures in the Yala and Panama 1 in sheets.<sup>12</sup> Minerals like silimanite, graphite, cordierite hypersthene and garnet so typical of the Highland series are seldom seen in the Vijayan series.<sup>1</sup> The Buttala and Kataragama complexes may well represent, 'Klippen' severed from the main Highland series block.<sup>7</sup> In comparison with the rocks of the Vijayan series the rocks of the Highland series are well bedded, banded and run continuously for miles along the strike with little variation and hardly any dislocation. The Vijayan rocks are seldom uniform. It is seen that these two groups are completely different in their mode of origin. It can be considered that these Highland series (the granulite facies) and Vijayan series (the amphibolite facies) are paired metamorphic belts with the relics eroded away.

## GEOLOGICAL SECTION OF SRI LANKA - ALONG LINE A - A'



SCALE 1" = 36.5 Kilometres

(Figure 5)



## 5. Basic and Ultrabasic Rocks

Basic and ultrabasic rocks are located along the Highland series and Vijayan series boundary. The basic rocks are fine grained quartz dolerites and albite dolerites; a very few medium grained basic rocks are also observed at a number of places in the Vijayan series. The dolerites and the ultrabasic rocks are shown in the Figure 1. Petrological and mineralogical studies of the basic rocks show two groups with diagnostic mineral assemblages as given below of which the first is dominant;

- apatite ilmenite  $\pm$  olivine clinopyroxene and plagioclase
- ilmenite clinopyroxene and plagioclase

Similar type of mineral assemblages are found in the basic and ultrabasic rock suite in the South West of England.<sup>4</sup> The greenstone belt in the South West of England are basic in character with quartz dolerites, albite dolerites and spilite dolerites, and the ultrabasic rocks are very few in this area and are serpentinised. It may be possible that the basic and ultrabasic rocks of the Vijayan series are akin to the rocks of the greenstone belt of the South West of England.

The most important occurrence is the serpentinite body at Udawalawe surrounded by migmatitic gneisses and crystalline limestone and is located in the South East quadrant of Sri Lanka near the Highland Vijayan boundary.<sup>10</sup> Katz considered that this ultramafic belt is an ancient Pre-cambrian suture of two plates.

The presence of serpentinite peridotite and dolerites is strong evidence for a tectonic setting, the gravity evidence is consistent with a thrust contact between the two major rock groups. The low density at the boundary suggest that there was down-warping and this was due to the continuous subduction of the Eastern Vijayan crust.

## **6. Copper-Magnetite Body and its Related Rocks**

There were a number of attempts to relate ore deposit genesis to plate tectonics. The distribution and origin of some classes of mineral deposits have recently been interpreted in terms of the plate tectonics hypothesis, (Mitchell and Garson, 1976). In Sri Lanka an occurrence of a body of copper-magnetite was discovered in 1971 at the Highland Vijayan boundary. The copper magnetite body is located in the Trincomalee district and is about 30 miles east of Kantalai, at Seruwawila (Figure 1) and is in the North Eastern quadrant of Sri Lanka. Detailed mineralogical and petrological studies have indicated that copper mineralization is confined to a pyroxinite which is associated with granites. The constituent minerals of the pyroxinite detected by microscopic studies are tremolite, diopside, augite, scapolite, apatite, chalcopyrite and magnetite. Basic and ultra-basic rocks and anorthosite rocks are found to be erupted closer to the magnetite deposit prospect. The copper magnetite deposit is found to be associated with cherty rocks. The presence of chert in the deposit suggests that sea water was in contact with erupted lavas and became enriched in silica which would have been deposited as the temperature fell. The apatite in the magnetite body suggests that gaseous emanations have taken place in the formation of the ore body.

Two occurrences of anorthosite intrusions are exposed closer to the copper magnetite apatite body and is located at the 3rd milestone on the Seruwila-Toppur road. The rock is highly coarse-grained and composed mainly of plagioclase laths with scattered grains of garnet. This is the first recorded occurrence of anorthosites in Sri Lanka, and the most interesting fact is that the location is almost at the contact of the mineralized area with the country rocks to the West. The detailed petrological

and mineralogical studies carried out indicate that the plagioclases contained are andesine and labradorite with  $\text{Na}_2\text{O}$  content 6.15%. The anorthosite at Scruwila may not represent a deep crustal or upper mantle material but a reworked material intruded along a faulted contact zone.<sup>9</sup>

About 60 km to the West of the Highland Vijayan boundary west of the magnetite deposit is the carbonatite (apatite) complex. This is located in the North Central province of Sri Lanka and falls within the Western Vijayan series.<sup>2</sup> The closer examination of the lithology of the carbonatite area shows an association of rocks akin to the Highland series. Geochemical study of the carbonatite complex concludes that the limestones are of igneous origin that has equilibrated from a carbonatite magma. The geochemistry of the Eppawela apatite is similar to the East African type.<sup>8</sup> In most of the East African carbonatite the mineralization has taken place at the intra continental rift zone plate boundaries, (Mitchell and Garson 1976). The carbonatite at Eppawela may have resulted due to subduction of the Eastern Vijayan oceanic crust and the partial melting of the crust at depth, deeper in the Benioff zone have resulted a carbonatite magma which has later intruded into the meta sediments (The Highland Series).

#### 7. Mineral and Thermal Waters of Sri Lanka

Mineral and thermal water of Sri Lanka are described by J. P. R. Fonseka *et al.*<sup>5</sup> All known springs occur in the coastal plain of the East and South east section of the island where also dolerite dykes are best exposed (see Figure 1). At present, there are nine thermal springs ; six of the nine thermal springs are located in the East and South Eastern coastal plain of the island which is underlain by gneisses of the Vijayan series. The other three namely Mahapellessa, Kanniyai and Rankihitiya are in the area underlain by Highland series. All the springs seep out invariably at ground level, without any appreciable head, from hollows or sandy bottoms of ponds or pools. A few issue from the fissures in the bedrock.

The origin of the mineral and thermal water is probably due to deeply circulating ground water. The most interesting feature is that all these hot springs confine to the Eastern and South Eastern sections of the island.

#### Geological Evolution of Sri Lanka

It is now realized that collision is probably the most important single cause of mountain building and orogeny and ancient collision belts and suture zones have been recognized in a number of orogenic belts Precambrian to Pliocene in age. Many papers have been written on mineralization at plate boundaries and the hypothesis is shown to be useful in explaining the origin of the host rocks of the ore bodies.

Sri Lanka Highland series was once a minor orogenic belt. This was an elongated zone extending from S.S.W. to N.N.E varying in strike and direction. The thickness of the sediments are much thicker compared with the Eastern Vijayan rocks. The

elongated zone may have started as a geosyncline, accumulating sediments during Precambrian time. The Highland rocks have formed due to the continuous subduction of the Vijayan oceanic crust (Figure 5) and subsequent series of differential upliftment with a culminating phase around Jurassic to end of Tertiary. The strata were eventually folded and crumpled, faulted and overthrust and more or less intensely metamorphosed at different stages of historic evolution.

The Seruwila copper magnetite apatite body is another feature that shows mineralization has taken place in a plate boundary.

The presence of chert and apatite in the copper magnetite body shows that lavas have been contaminated with sea water and gaseous emanations had taken place.

The basic and ultrabasic rocks, anorthosites, granitic ring structures in the Vijayan series shows that there was active volcanism.

The submarine canyon with wall heights of 1,350 meters at Trincomalee and also the hot springs are contiguous with the Highland-Vijayan contact. The Eppawala apatite carbonatite complex is also a result of the converging plates, and the partial melting due to continuous subduction have resulted in carbonatite lavas, although there is no geochemical evidence.

The Andigama Tabbowa Gondwana basins are the tensional cracks formed as a result of the movement of the two plates. The rift valley between India and Sri Lanka (the cauvery basin) is also a result of the movement of the converging plates.

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# ලිපිවල සාරාංශ - සිංහල පරිවර්තන

පොල්වගා අතුරු බෝගයක් වශයෙන් වඩන ලද මංඤ්ඤාකා (මැනිහොට් එස්කියුලෙන්ටා ක්රාන්ටිස්) ගසේ වර්ධනය හා පලදාව කෙරෙහි රෝපණ පරතරය සහ නොටුපත් බලපාන ආකාරය.

එච්. පී. එම්. ගුණසේන; එන්. ටී. එම්. එච්. ද සිල්වා සහ එම්. පී. එල්. ඩී. මාර්ටින්

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පොල්වතු වල අතුරු බෝගයක් ලෙස වඩන ලද මංඤ්ඤාකා ගසේ වර්ධනය සහ පලදාව කෙරෙහි රෝපණ පරතරය හා නොටුපත් යෙදීම නිසා ඇතිවන ආවරණය ගැන හැදෑරීමට පුණ්ඩ්ල ශ්‍රී ලංකා පොල් පර්යේෂණ ආයතනයේදී පරීක්ෂණයක් පවත්වන ලදී. අල අමු බර පලදාව කෙරෙහි හෝ වියලි පදාර්ථ සමාවයනය කෙරෙහි හෝ නොටුපත් වලින් කිසිම බලපෑමක් ඇති නොවීය. අල අමු බර පලදාව කෙරෙහි රෝපණ පරතරය තදින් බලපාන බව පෙනීගිය අතර මීටර 0.91 x 0.61 රෝපණ පරතරය වඩා ඵලදායී විය. ඊට වඩා පරතරයක් ඇතිව පරීක්ෂණය සඳහා රෝපණය කරන ලද ගස්වල අස්වැන්න එතරම් සතුටුදායක නො විය. පත්‍ර, දඹු හා වියලි පදාර්ථ පලදාවෙන් හා පත්‍ර වර්ග වල සුචක (L) යෙන්ද දූන ගන්නට ලැබුනේ එම්යු 72 නමින් හැඳින්වෙන මංඤ්ඤාකා වර්ගය සිටුවා මාස 8 කට පසුව මෝරා අස්වනු නෙලා ගැනීමට සුදුසු අවස්ථාවට පත්වන බවයි. පත්‍ර වර්ග වල පැවැත්ම (D) සමග මුළු පලදාව සහ අල වියලි පදාර්ථ පලදාවද රේඛීය වශයෙන් සම්බන්ධව පැවති නිසා වගා පලදාවේ ප්‍රධාන නිර්ණායකය ලෙස පත්‍ර වර්ග ඵලය පවතින බවද පෙනී ගියේය.

## ශ්‍රී ලංකාවේ ආර්ථික වැදගත්කමක් ඇති ශාක :

3 වන කොටස: ආයුර්වේද බෙහෙත් කඩවලින් ලබාගත් ඖෂධ පැලෑටි සියයක් ශාක රසායන විශ්ලේෂණයට භාජනය කිරීම.

ඒ. ඒ. එල්. ගුණතිලක සහ එස්. ගෝතිශ්වරන්.

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ඇල්කොලොයිඩ්, සැපොනින්, ස්ටෙරොයිඩ්, ටෙර්පෙනොයිඩ්, කාර්බියැක්, ග්ලයිකෝසයිඩ් සහ ෆ්ලවොන්ග්ලොසයිඩ් යන රසායනික සංයෝග අඩංගු වී ඇති සැටි විමසා බැලීම සඳහා ආයුර්වේද බෙහෙත් කඩවලින් ලබාගන්නා ලද ඖෂධ ශාක සියයකට අධික ප්‍රමාණයක් පරීක්ෂාවට බඳුන් කරන ලදී. මේ සංයෝග වර්ගවල විකිණ්ඩිය වැදගත්කම පෙන්වාදී ඇති අතර එම ඖෂධ පැලෑටි වලින් ලබාගන්නා ලද ස්වාභාවික පදාර්ථයන්ට අයත් රසායනික සංයෝග වර්ග අනුසාරයෙන් එකී ශාක පරම්පරාගතව බෙහෙත් වශයෙන් යොදාගෙන ඇති සැටිද හැකිතාක් දුරට සාකච්චා කොට ඇත.

සසම්භාවී විචල්‍යයන්ගේ අසම්භාව්‍යශීලී අත්‍යනුක පද්ධති.

කේ. එල්. ඩී. ගුණවර්ධන

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ස්වයන්ත සසම්භාවී විචල්‍ය අනුක්‍රමයක් සනාථකිරීම සඳහා මෙහි ලක්ෂණ විභාග දක්වන ලද සසම්භාවී විචල්‍යයන්ගේ අසම්භාව්‍යශීලී අත්‍යනුක පද්ධතිහු සසම්භාවිතා සිද්ධාන්තයෙහි වැදගත් සසම්භාවී විචල්‍ය පන්තියකට අයත් වන්නාහුය. මේ නිබන්ධයෙහි අසම්භාව්‍යශීලී අත්‍යනුක පද්ධතීන්, පරායත්ත සසම්භාවී විචල්‍ය අනුක්‍රමයක් සනාථ කිරීම වස් ලක්ෂණ විභාගයකට බඳුන්කොට ඇති අතර ගුණ ලක්ෂණ කීපයක් ලබාගැනීමටද ප්‍රයත්න දරා ඇත.

ශ්‍රී ලංකාවේ මුද්‍රණ වර්ගයට අයත් ශාක පිළිබඳ අධ්‍යයනයක්.

I. ඇටවල අඩංගු එල්-ඩෝපා ප්‍රමාණය.

නිර්මලා පිරිස්, ඊ. ආර්. ජයන්ත සහ එච්. එම්. ධර්මසේ.

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තෝරාගන්නා ලද මුද්‍රණ වර්ගයට අයත් ශාක කීපයක අඩංගු එල්-ඩෝපා ප්‍රමාණය විමර්ශනයට ලක් කරන ලදී. මෙයින් ඇටවල 4.5% ක එල්-ඩෝපා ප්‍රමාණයක් අඩංගුවන මුද්‍රණ ඇටවර්ග වර්ගය විශේෂයෙන් ප්‍රයෝජනවත් වියහැකි බව පෙනී ගියේය. අමු ඇට පිටි බවට පත්කළ විගසම ප්‍රයෝජනයට ගන්නේ නම් 80%ක කාර්යක්ෂමතාවයකින් එල්-ඩෝපා ලබාගත හැකිය. එල්-ඩෝපා ප්‍රමාණයේ අඩුවැඩිවීම හෙරොහි දේශගුණික සාධක කොහෙන්ම බලපාන්නේ නැතැයි කිව යුතුය. ජනක ශාකය තුළ ඇති ලක්ෂණ සහ අනුරූප ශාක තුළ ඇති ලක්ෂණ සැලකිල්ලට ගත් කල්හි වුවද මේ පරාමිතිය නො වෙනස්ව පවතින බව පෙනී යයි.

නාලස්ථ රෝපණක්‍රම යටතේ සිදුවන ඇෆ්ලාටොක්සින් සංචායන ධාරිතාවේ ප්‍රමිතිගත මිනුමක් සඳහා යෝග්‍ය සංයුක්ත මාධ්‍යයක්.

එස්. එන්. අර්සකුලරත්න සහ එල්. ටී. වැලිආගේ.

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සන උපස්ථානුවල ඇෆ්ලාටොක්සින් උත්පාදනයවීම අධ්‍යයනය කිරීම පිණිස මේ දක්වා සම්ප්‍රදායානුකූලව යොදාගත් තනි බීජෝෂණ අවධි ක්‍රමයට එක්තරා අඩුවක් ඇත්තේය. එනම් කල් ගත වීමත් සමග බොහෝවිට ඇතිවන ධූලක ප්‍රමාණ විචලනයන් ගණන් ගැනීමට අපහසු වීමය. පිරික්සුම් රෝපනයේදී එකතුවන ඇෆ්ලාටොක්සින් ප්‍රමාණය නිර්ණය කිරීම සඳහා ද්විස්තර සංයුක්ත මාධ්‍යයකින් සමන්විත තනිපාද ක්‍රමයක් යොදාගන්නා හැටි මෙහි විස්තර කොට ඇත. මෙම ක්‍රමය ඇෆ්ලාටොක්සින් අධිශෝෂණය කිරීමට සිලිකාජෙල්වලට ඇති හැකියාව පදනම් කොට නිපදවා ඇති අතර එමගින් උපස්ථානුවේ ක්‍රියාකාරීවන - ඇතැම්විට අධිශෝෂක රචිත මාධ්‍යයක් තුළදී ධූලක ප්‍රමාණයේ කලා විචලනයකට තුඩු දෙන්නේ යයි සිතිය හැකි - භායන ක්‍රියාවලියේ බලපෑමද ඉවත් කළහැක. අධිශෝෂණය, උපස්ථානුව, ආමුකුලය, බීජෝෂණ අවස්ථා සහ නිස්සාරණ පරිපාටිය යන කරුණු ප්‍රමිතිකරණය කිරීමට ද මේ ක්‍රමයෙන් ඉඩ ලැබේ.

ශ්‍රී ලංකාවේ මූකලන්වල වැවෙන පයිනස් වර්ගයට අයත් ශාක පිළිබඳ අධ්‍යයනයක්.

**II. පයිනස් කැටිබියා ඔලියෝරේසින් නැමති ශාකයෙහි අඩංගු ටර්පන්ටයින් ප්‍රමාණය - වාණිජ පදනමක් යටතේ ටර්පන්ටයින් නිෂ්පාදනය කර ගැනීම පිළිබඳ අධ්‍යයනයක්.**

ඊ. ආර්. ජැන්ස්; එස්. සන්දිරමුලේසන්; කේ. ඩී. රත්නායක; එල්. ඒ. ශ්‍රේණිලක් සහ එන්. එස්. කේ. රාමසුන්දර.

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එරබැද්ද මූකලානේ වැවෙන පයිනස් කැටිබියා ඔලියෝරේසින් නැමති ශාකයෙහි අන්තර්ගත මධ්‍යක ටර්පන්ටයින් ප්‍රමාණය සාමාන්‍යයෙන් සියයට 18 සිට 20 අක්වා වෙයි. එහෙත් ඇතැම් ගස්වල අඩංගු ප්‍රමාණය ගසින් ගසටද ඉරිතුවෙන් ඉරිතුවටද, දවසින් දවසටද, විවලනයට භාජනයවන බව දැනගත හැකිවිය. ටර්පන්ටයින් ප්‍රමාණය 30% ක උපරිමයකටද 10% ක අවමයකට ද යටත්ව තිබිය හැක. අන්තර්ගත ජලය (1) හා සහවලනය (2) යන කරුණු දෙක මත ටර්පන්ටයින් පෙරා ගැනීමේ අනුපාතය හා කාර්යක්ෂමතාව රඳා පවතී. දීර්ඝ පරීක්ෂණ. භාර අධ්‍යයනයකින් පසුව නිෂ්පාදන ක්‍රියාවලිය බොහෝ දුරට සරල කිරීමට හැකිවිය. කිලෝ-ග්‍රෑම් 50 ධාරිතාව ඇති සරල ගිනි ආසවන යන්ත්‍රයක් මගින් ඉතා කෙටි ආසවන කාලයක් තුළදී උපරිම ටර්පන්ටයින් ප්‍රමාණයක් ලබාගැනීමට හැකිබව යොයා ගන්නා ලදී.

රෝමන්ටකයන්ට යෝග්‍ය ප්‍රෝටීන් රහිත නොවුණත් ප්‍රභවයක් වශයෙන් යූරියා යොදා ගැනීම.

**II. ක්ෂාර මිශ්‍රිත පිදුරු ආහාරයට දෙන ලද බැටළුවන්ගේ ආහාර රුචිය සහ පිරණතාව කෙරෙහි ප්‍රෝටීන් රහිත නොවුණත් ප්‍රභවයක් වශයෙන් යූරියා යොදා ගැනීමෙන් ඇති වූ බලපෑම.**

එම්. සී. එන්. ජයසූරිය.

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ශ්‍රී ලංකාවේ කෘෂිකාර්මික අතුරු නිෂ්පාදනයක් වන ගොයම් පිදුරු ශාකදී පොලයන්ගෙන් මනාව පිරිපුන් ශක්ති ජනක ආහාරයකි. එහෙත් පිදුරුවල පිරණතා ගුණය ඉතා අඩු බැවින් වැඩුණු රෝමන්ටකයන් උකහා ගන්නා දිරවිය හැකි ආහාර ප්‍රමාණය බොහෝ විට එම යතුන්ගේ ජීව බර අඩුවීම වලක්වා ගැනීමටවත් නො සෑහෙන තරම්ය. මෑතක දී රසායනික ක්‍රම අනුගමනය කිරීමෙන් කේවලක්වයෙන් අඩු සන්වාහාරවල පෝෂණ ගුණය වැඩිකොට ඇත. මේ අනුව NaOH ද්‍රාවණය යෙදීමෙන් පිදුරුවල පෝෂණ ගුණය සාමාන්‍ය මට්ටමේ සිට මධ්‍යම ප්‍රමාණයට උසස් කිරීමට හැකි වී ඇත. කෙඳි සහිත ද්‍රව්‍ය ආහාරයට යොදා ගැනීමට ප්‍රබල බාධාවක් වශයෙන් නොවුණත් උණුතාව පවතින නමුදු ප්‍රෝටීන් රහිත නොවුණත් ප්‍රතිපූරණය කිරීමෙන් මේ ප්‍රශ්ණය සාර්ථක ලෙස විසඳාගෙන ඇත. ප්‍රෝටීන් රහිත නොවුණත් යෙදූ ආහාරවල පෝෂණ ගුණය වැන බැලීම පිණිස, එව හතර වර්ගයේ ගොයම් පිදුරු 4% (w/w) NaOH ද්‍රාවණයක් සමඟ (පිදුරු කිලෝග්‍රෑම් 1.21 යන අනුපාතයට) කලවම් කොට දිනකට එක සතකට පිටි කළ බඩ ඉරිඟු ෧෮.෫ 100 ක් ද මිශ්‍ර කොට යූරියා රහිතව

හෝ සහිතව වැඩෙන බැටළුවන්ට රුචිපරිදි ආහාරයට ගැනීමට යලස්වන ලදී. ආහාරයට ගත් මුළු වියලි පදාර්ථයෙන් 0 - 2.65% ක් යුරියා විය. 1.87% දක්වා යුරියා ප්‍රතිපූරණය කළ විට ජීරණතාවේ සැලකිය යුතු වැඩිවීමක් ඇතිවිය. 1.87% ට වැඩියෙන් යුරියා එක්කළ විට ජීරණතාව කෙරෙහි විශේෂ බලපෑමක් ඇති නොවූන නමුදු ආහාර රුචිය වැඩිවිය. යුරියා ප්‍රතිපූරණය 2.65% දක්වා ඉහළ දැමූ විට ඇතිවූ වියළි පදාර්ථ ආහාරයට ගැනීමේ රුචිය යුරියා රහිත වියලි පදාර්ථ ආහාරයට ගැනීමේ රුචියට බඩා 30% කින් අධික විය. 4% NaOH (w/v) මිශ්‍රිත පිදුරු බැන සලකා බලන විට, ආහාරයට ගත් මුළු වියලි පදාර්ථ ප්‍රමාණයෙන් 1.8 සිට 2.0 දක්වා වූ අනුපාතය ප්‍රශස්ත යුරියා ප්‍රතිපූර්ණ මට්ටම වශයෙන් පවතින බව පෙනේ. ගෙන්දගම සෑහෙන ප්‍රමාණයක් නොතිබීම කරණයකට ගෙන රෝමන්ට්ගන ක්ෂුද්‍ර ජීවීන් විසින් යුරියා නොප්‍රජන් ප්‍රයෝජනයට ගත හැකි බැවින් රෝමන්ට්කයන්ට පුදුසු ප්‍රෝටීන් රහිත නොප්‍රජන් මූලයක් වශයෙන් ඇමෝනියම් සල්ෆේට් වඩා හොඳ වියහැක. ස්ථීර නිගමනයකට එළඹීමට පෙර මේ පිළිබඳව නඩුරුවන් පර්යේෂණ කිරීම අවශ්‍ය වේ.

**ශ්‍රී ලංකාවේ මුහුදු ඇල්ගා කීපයක ඇති ස්ටෙරෝල් වර්ග.**

මයිල්වානනම් මනේන්ද්‍රන්; ඩී. එම්. සිරිසේන; මධුබෝ මොරියානි; පුපානෝ ඝානෝ; නෙබ්‍රවෝ ඉනේකාඩා සහ ඒ. සිවපාලන්.

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ක්ලෝරෝෆයිසේ, පේයෝපයිසේ සහ රෝෂඩ්පයිසේ යන වර්ග වලට අයත් ශ්‍රී ලංකාවේ මුහුදේ ඇති ඇල්ගා පැලැටි වර්ග දහඅටක් ජීවයෙහි අඩංගු ස්ටෙරෝල් සොයා ගැනීම සඳහා පරීක්ෂණයට භාජනය කරන ලදී. උල්වාලේ ගණනයට පයන් කොළ ඇල්ගා වල පවතින ප්‍රධාන ස්ටෙරෝල් වර්ගය 28 - අයිසෝපුකෝස් ටෙට්‍රෝල් වෙයි. සිපොනාලේ, සිපොනෝක්ලාමේලේ සහ පඩිසික්ලාසියාලේ යන ගණවලට අයත් කොළ ඇල්ගාවල 24 - එනිල් කොලෙස්ටෙරෝල් ප්‍රධාන ස්ටෙරෝල් වර්ගය ලෙස පවතී.

පරීක්ෂණයට භාජනය කරන ලද සියළුම දුම්රු ඇල්ගාවල ප්‍රධාන ස්ටෙරෝල් පදාර්ථය වශයෙන් පුකොස්ටෙරෝල් අඩංගු වේ. රතු ඇල්ගාවල අඩංගු ප්‍රධාන ස්ටෙරෝල් පදාර්ථය කොලෙස්ටෙරෝල්ය. ශ්‍රී ලංකාවේ, ජපානය, ලීඩානයය, ප්‍රංශය සහ කැනඩාව යන රටවලට අයත් මුහුදේ වැඩෙන ඇල්ගාවල අඩංගු ප්‍රධාන ස්ටෙරෝල් පදාර්ථවල සංසන්දනාත්මක වශයෙන් සැලකිය යුතු වෙනසක් නැති බව අපේ පරීක්ෂණ වලින් අවබෝධ විය.

**ස්තර භූකාරක හේතූන් හා ශ්‍රී ලංකාවේ භූ විද්‍යාව අතර ඇති සම්බන්ධය**

එම්. ඩී. එන්. සී. පතිරණ.

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ශ්‍රී ලංකාවේ ඇති ස්ඵටිකරූපී පර්වත, උස් බිම් ශ්‍රේණිය හා විප්ලානු ශ්‍රේණිය යනුවෙන් කොටස් දෙකකට බෙදනු ලැබේ. මේ ශ්‍රේණි දෙක යාවත ස්ථානයෙහි පුරා ස්තර මායිම් පිහිටා ඇතැයි සැලකිය හැකිය. මෙම අර්ථනිරූපණය සනාථ කරන සාක්ෂි වශයෙන් පහත දැක්වෙන කරුණු ගෙන හැර දක්විය හැකිය :—

ගේට්ටුලයිට් පරිදර්ශ හා ඇම්ප්ලිෆයින් ලයිට් පරිදර්ශ වලින් සමන්විත දෙබීඩී විපරිත කලාප පිහිටා තිබීම.

උස්බිම් - විජයානු ස්පර්ශයට ආසන්නව පාඨපෝය පාඨානවලින් හටගත් භාස්මික හා අති-භාස්මික පර්වත පිහිටා තිබීම.

කොපර්-මැග්නීසියම් - ඇප්ටයිට් ස්කන්ධය හා ඊට නැතම කියන පර්වත පිහිටා තිබීම. ප්‍රධාන පර්වත කාණ්ඩ දෙක අතර ඇති මාලමේ බිනිජායනය සිදුවන්නට ඇතැයි අනුමාන කළ හැක.

විජයානු ශ්‍රේණිය තුළ ප්‍රධාන වශයෙන් දක්නට ලැබෙන ශ්‍රී ලංකා උණුදිය උල්පත් ඉහත සඳහන් ශ්‍රේණි දෙක ස්පර්ශවන තීරුව දිගේ පිහිටා තිබීම.

උස් බිම් - විජයානු ශ්‍රේණියට යාබදව ත්‍රිකුණාමලය වෙරළින් ඔබ්බෙහි මීටර 1,350 ක් උස් වූ බිත්තියෙන් යුත් සමුද්‍ර කැනියමක් පිහිටා තිබීම.

උස්බිම් - විජයානු ශ්‍රේණි දෙක අතර ඇති මායිම එම ස්තර දෙක එකට වලනය වූ පැරණි අවපාත කලාපයක් වශයෙන් විග්‍රහ කළ හැකිය. ගිනිකඳු පුපුරා යාමත් අනවරත අවපාතය හා උත්පාතයක් නිසා වර්තමානයෙහි පවතින උස්බිම් ප්‍රේණිය ඇති වී තිබේ. උස්බිම් ශ්‍රේණියට අයත් පර්වත, ක්වාසයිට්, ස්ඵටිකරූපී හුණුලේ, ගානට්ටර් ගේට්ටුලයිට් සහ වාර්තෝකයිට් වලින්ද සැදී ඇත. සමහල් (ගිනිකඳු) පාඨාන අද දක්නට තැන්පත් ඒවා බාදනය වී ගොස් ඇති නිසාය. මේ කාණ්ඩ දෙකම අතිගයින් විපරිත වී හා දේහාන්තරණය වී ඇත. උස්බිම් ශ්‍රේණිය ගේට්ටුලයිට් පරිදර්ශයක් වශයෙන් ද විජයානු ශ්‍රේණිය ඇල්මික්බයිට් - ඇම්ප්ලිෆයින් ලයිට් පරිදර්ශයක් වශයෙන් ද විපරිත වී ඇත.

Appropriate Technology Services  
121, POINT-PEEFO ROAD  
NALLUR, JAFFNA  
No. ....



## இந்த இதழின் கட்டுரைகளின் சுருக்கங்கள்

தென்னந் தோப்புகளில் இடைப்பயிராகச் செய்கை பண்ணப்படும் மரவள்ளி (மனிகொற் எசுக்கியுலன்ரு கிருன்றசு) செடியின் வளர்ச்சி மீதும் விளைச்சல் மீதும் நடுகை இடை வெளியும் நைதரசனும் ஏற்படுத்தும் விளைவு.

எச். பி. எம். குணசேனா; என். டி. எம். எச். த சில்வா; எம். பி. எல். டி. மாட்டின்,

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தென்னந் தோப்புகளில் இடைப்பயிராகச் செய்கை பண்ணப்பெறும் மரவள்ளிச் செடியின் வளர்ச்சி மீதும் விளைச்சல் மீதும் நடுகை இடைவெளியினதும் நைதரசன் உபயோகத்தினதும் தாக்கத்தினை ஆய்வு செய்வதற்கு லுணுவிலையில் உள்ள இலங்கைத் தெங்கு ஆராய்ச்சி நிறுவகத்தில் சோதனை யொன்று மேற்கொள்ளப்பட்டது. கிழங்குத்தாய்நிறை விளைச்சலின் மீது அல்லது உலர்ப்பொருள் திரட்சியின் மீது நைதரசன் எவ்விதத்திலும் தாக்கம் உண்டுபண்ணவில்லை. கிழங்குத் தூய நிறை விளைச்சல் மீது நடுகை இடைவெளியின் தாக்கம் பெரிதாக இருந்ததோடு  $0.91 \times 0.61$  மீற்றர் நடுகை இடைவெளி சிறந்த பலனளித்தது. இவ்வளவினைக் கடந்த இடைவெளியுடன் பரீட்சார்த்தமாக நடுகை செய்யப்பட்ட செடிகளின் விளைச்சல் அவ்வளவு குறிப்பிடத்தக்கதாக அமையவில்லை. இலை, தண்டு, உலர்ப்பொருள் விளைச்சல் வீதத்தாலும் இலைப்பரப்பளவுச்சுட்டி (L) யாலும் எம்யூ 72 எனப்படும் மரவள்ளி இனம் நடுகை செய்யப்பட்டு 8 மாதங்க ளுக்குப் பின்னர் முதிர்வுற்று அறுவடைக்குத் தயாரான நிலைக்கு வரு மென்பது அறியக்கூடியதாகவிருந்தது. இலைப் பரப்புக் காலவளவோடு (D) மொத்த விளைச்சலும் கிழங்கு உலர்ப்பொருள் விளைச்சலும் ஏகபரிமாண முடையதோர் இணைபு கொண்டிருந்தமையால் பயிர் விளைச்சல் சார் முக்கியத் துணிகோவையாக இலைப்பரப்பளவு இருக்குமென்பதும் தெரிகிறது.

பொருளாதார வகையில் சிறப்புமிக்க இலங்கைத் தாவரங்கள்.

3. ஆம் பாகம்: ஆயுள்வேத மருந்துக் கடைகளிலிருந்து பெறப்பட்ட நூறு மருந்துப் பூண்டுகளின் தாவர இரசாயனப் பகுப்பாய்வு.

ஏ. ஏ. எல், குணதிலக்கா; எஸ். சோதீஸ்வரன்.

J. Natn. Sci. Coun. Sri Lanka 1980 8(1): 11-29

அற்கலோயிட்டு, சாப்போனின், இசுத்திரோயிட்டு, தெர்பெனோயிட்டு, கார்டியாக்குக் கிளைக்கோசைட்டு, பிளேவனோயிட்டு ஆகிய இரசாயனச் சேர்வைகள் அமைந்துள்ளவற்றைக் கண்டறிதல் பொருட்டு ஆயுள்வேத மருந்துக் கடைகளிலிருந்து பெறப்பட்ட நூற்றுக்கு அதிகமான மருந்துப் பூண்டுகள் சோதனைக்குள்ளாக்கப்பட்டன. இச் சேர்வைகளின் நோய்த் தீர்ப்புச் சிறப்பினை விளக்கியுள்ளதோடு அம்மருந்துப் பூண்டுகளிலிருந்து கண்டெடுக்கப்பட்ட இயற்கைப் பதார்த்தங்களுக்குரிய இரசாயனச் சேர் வைகளின் உதவியைக் கொண்டு அத்தாவரங்கள் வாழையடி வாழையாக சிகிச்சைக்குப் பயன்படுத்தப்பட்டவாறும் இயன்றவாறு ஆயப்பட்டுள்ளது.

எழுமாற்றுமாறிகளின் நிபந்தனைக்குரிய நுண்ணெண் தொகுதிகள்.

கே. எல். டி. குணவர்த்தன.

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சாரர் எழுமாற்று மாறித் தொடரியொன்றை நிரூபிக்குமாக இங்கு வரை விலக்கணங் காட்டப்பெற்ற, எழுமாற்று மாறிகளின் நிபந்தனைக்குரிய நுண்ணெண் தொகுதிகள் நிகழ்தகவுக் கொள்கையின் முக்கியமான எழுமாற்று மாறி வகுப்பொன்றிற்குரியனவாக அமைந்துள்ளன. இக் கட்டுரையில் சார் எழுமாற்றுமாறித் தொடரியொன்றை நிரூபிக்கும் பொருட்டு நிபந்தனைக்குரிய நுண்ணெண் தொகுதிகள் வரைவிலக்கணங் காட்டலுக்கு ஆளாக்கப்பட்டுள்ளன. அவற்றின் உடைமைகளைக் கண்டு பிடிப்பதில் வெற்றி கண்டுள்ளது.

இலங்கையில் வளரும் முகுன இனம் சார்ந்த தாவரம் பற்றிய ஆய்வு.

I. விதையில் காணப்படும் எல்-டோபா அளவு.

நிர்மலா பீறீஸ்; ஈ. ஆர். ஜான்ஸ்; எச். எம். தர்மதாஸா.

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முகுன இனத்தைச் சேர்ந்த சில தேர்ந்தெடுக்கப்பட்ட தாவரங்களில் உள்ள எல்-டோபா அளவு நுண்ணுய்வுக்கு உட்படுத்தப்பட்டுள்ளது. இவற்றுள், விதையில் 4.5% வீத எல்-டோபா பதார்த்தம் கொண்ட முகுன அற்றைரிமா என்னும் இனம் பல சிறப்புக்கூறுகளைத் தன்னகத்திற் கொண்டவொரு தாவரமாகும். பச்சையான விதைகளை மாவாக்கியவுடனே எல்-டோபாப் பிரித்தெடுக்கப் பயன்படுத்திக் கொண்டால் 80% வீத வினைத்திறமையினைப் பெறலாம். எல்-டோபா அளவினைப் பாதிக்கும் ஒரு கருவியாகக் காலநிலை எந்த விதத்திலும் இருப்பதில்லை என்று கூற வேண்டும். தாய்த் தாவரத்திலுள்ள சிறப்புக்கூறுகளையும் அதன் செய்த்தாவரங்களிலுள்ள குணவியல்புகளையும் ஆய்ந்து பார்த்தாலும் கூட இப்பிரமானம் வித்தியாச முறாமல் இருப்பது குறிப்பிடத்தக்கதாகும்.

குழாயகப் பெருக்க முறைகளின் கீழ் நிகழும் அப்லாடொக்சின் திரள் திறன் சார்ந்த தர அளவிட்டுக்கான சேர்த்தி ஊடகம்.

எஸ். என். அர்சகுலரத்தின; எஸ். வீ. வெளியங்கே.

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திண்ம ஆதாரத்தளங்களில் அப்லாடொக்சின் பெருகுவதை ஆராயும் பொருட்டு இதுவரை வழக்கமாகப் பயன்படுத்தப்பட்டு வருகின்ற தனி நுண்மப் பெருக்கக்கால முறையில் ஒரு குறை உண்டு. அஃதாவது நச்சுப் பதார்த்தங்களில் காலப்போக்கில் பெரும்பாலும் ஏற்படுகின்ற மாறல்களைக் கணக்கிடவியலாமையாகும். பெருக்கப் பரிசோதனைகளின் போது

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