

## II.—CONTRIBUTIONS TO THE STUDY OF SPIKE-DISEASE OF SANDAL (*SANTALUM ALBUM*, LINN.). PART VIII.

### Chemical composition of tissue fluids from the leaf.

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The tissue fluid of a living plant represents its dynamic or physiologically active component, while the residue is composed of the static reserves incorporated with the inert structural units of the plant. Specific alterations in the composition of animal blood are brought about with the progressive onset of specific diseases and are often utilised to diagnose and determine the stage of the malady. In plants also marked changes may be expected to accompany the pathological state and a means may thus be found to recognise plant diseases by a biochemical analysis of its tissue fluids long before the external symptoms manifest themselves.

In some diseases prominent changes may take place in leaf tissue, while in others there may be profound disturbances in stem or root. The type of the main metabolic disturbance in the plant may vary with the nature of the disease. In some cases it is the carbohydrate metabolism that may be disturbed, while in other instances the nitrogen or mineral nutrition may have to be considered. In devising methods for diagnosis and for following the progress of plant diseases, it is therefore essential to consider (1) the choice of a suitable tissue that is most affected and (2) the type of metabolism that is most disturbed.

While the changes revealed in the tissue are specific, it should not be overlooked that the causal agency may lie in a different tissue, remote from that under examination, or may even be located externally to the plant. The biochemical changes effected in the diseased tissue fluids are therefore secondary in character, and a closer knowledge of these changes is essential for effectively combating the disease. An attempt has been made to analyse the tissue fluids of sandal in health and disease.

### MATERIALS AND METHODS.

The material was collected periodically from two areas, Uttarahalli and Ragihalli, and a full description of its collection, storage, transport and treatment in the laboratory appears in our previous contributions (*J. Indian Inst. Sci.*, 1928, 11A, 23). The expressed tissue

fluid was centrifuged at 3000 r. p. m. and the clear centrifugate used for analysis. Aliquot portions were used for the determination of total solids, ash, total nitrogen and reducing sugars. Phosphorus and calcium were determined in the ash by the official methods of the A. O. A. C.

TABLE I A.

*Yield of tissue fluids from leaves (Uttarahalli).*

Date	Percentage yield on weight of green material		
	Healthy	Spiked	Difference
27-4-27	54.0	41.3	+ 12.7
16-6-27	67.1	56.6	+ 10.5
5-8-27	63.2	54.5	+ 8.7
20-4-28	49.0	46.7	+ 2.3
30-4-28	60.6	57.5	+ 3.1
8-5-28	62.4	61.6	+ 0.8
22-5-28	62.5	65.4	- 2.9
8-6-28	57.0	62.7	- 5.7
22-6-28	49.8	56.0	- 6.2
9-7-28	57.1	54.9	+ 2.2
Mean ...	58.2	55.7	+ 2.5

TABLE I B.

*Yield of tissue fluids from leaves (Ragihalli).*

Date	Percentage yield on weight of green material		
	Healthy	Spiked	Difference
8-7-27	63.7	57.1	+ 6.6
22-7-27	50.4	52.2	- 1.8
29-7-27	55.5	47.3	+ 8.2
Mean ...	56.5	52.2	+ 4.3

TABLE II A.

*Grams per 100 c.c. of tissue fluid (Uttarahalli).*

Date	HEALTHY			SPIKED		
	Total solids	Ash	Per cent. of ash in solids	Total solids	Ash	Per cent. of ash in solids
19-5-27	13.13	2.21	16.78	13.79	2.20	15.99
16-6-27	10.53	1.93	18.35	12.23	1.45	11.83
23-6-27	11.53	2.04	17.66	10.21	1.52	14.84
15-7-27	12.20	1.92	15.75	12.24	1.66	13.53
5-8-27	13.39	2.91	21.72	12.84	1.98	15.41
18-8-27	15.36	3.62	23.54	14.07	2.26	16.03
15-9-27	13.39	2.91	21.72	12.84	1.98	15.41
27-10-27	16.79	3.63	21.64	14.92	2.18	14.57
9-1-28	15.67	3.71	23.64	16.38	2.12	12.93
11-4-28	17.72	4.46	25.66	14.31	2.41	16.84
20-4-28	20.20	4.53	22.42	17.40	3.77	21.66
30-4-28	15.89	3.59	22.57	14.61	3.08	21.06
7-5-28	17.67	3.73	21.12	13.20	2.94	22.27
22-5-28	16.42	3.83	23.32	13.06	3.42	26.20
8-6-28	21.25	4.58	21.55	13.49	3.38	25.15
22-6-28	17.48	3.79	21.65	15.52	2.99	19.25
Mean ...	...	3.33	...	...	2.46	...
Standard deviation ...	...	±0.28	...	...	±0.22	...

TABLE II B.

*Grams per 100 c.c. of tissue fluid (Ragihalli).*

Date	HEALTHY			SPIKED		
	Total solids	Ash	Per cent. of ash in solids	Total solids	Ash	Per cent. of ash in solids
8-7-27	10.90	1.76	16.18	14.95	1.52	10.15
22-7-27	10.36	2.28	13.94	19.24	1.42	7.38
29-7-27	19.57	3.06	15.64	22.55	1.80	7.97
11-8-27	16.09	2.11	13.10	20.10	1.96	9.75
6-9-27	19.62	3.46	17.64	19.85	1.48	7.43
14-10-27	18.81	3.54	18.81	21.90	1.55	7.07
7-11-27	20.92	3.90	18.65	18.92	1.57	8.31
15-6-28	16.71	2.72	16.27	17.36	1.70	9.81
Mean ...	...	2.85	...	...	1.62	...
Standard deviation ...	...	±0.26	...	...	±0.21	...

TABLE III A.

*Milligrams per 100 c.c. of tissue fluid (Uttarahalli).*

Date	HEALTHY			SPIKED		
	Total N	P <sub>2</sub> O <sub>5</sub>	Ca	Total N	P <sub>2</sub> O <sub>5</sub>	Ca
19-5-27	395.2	84.1	...	517.6	81.1	...
16-6-27	357.3	52.3	300.5	659.7	89.3	126.5
23-6-27	405.1	88.1	290.0	496.7	120.1	226.5
15-7-27	379.1	104.5	254.7	533.9	137.4	87.3
5-8-27	429.1	...	...	546.4	...	...
18-8-27	450.8	74.6	257.5	453.0	110.5	52.5
15-9-27	342.0	103.5	165.0	362.6	166.0	107.5
27-10-27	305.8	...	...	512.9	...	...
9-1-28	408.4	...	...	585.2	...	...
11-4-28	332.8	177.8	462.0	623.7	191.8	142.0
20-4-28	466.6	158.4	750.0	659.4	364.6	154.0
30-4-28	400.1	128.2	634.0	616.2	236.0	154.0
7-5-28	328.4	197.1	668.0	542.3	256.0	118.0
22-5-28	327.2	102.7	558.0	543.8	231.0	154.0
8-6-28	246.8	84.2	940.2	499.9	94.9	120.7
22-6-28	307.1	85.5	540.4	464.7	96.8	96.8
Mean	367.8	110.7	485.0	538.6	167.3	128.3
Standard deviation	± 57.2	± 41.1	± 200.1	± 76.2	± 81.4	± 41.6

TABLE III B.

*Milligrams per 100 c.c. of tissue fluid (Ragihalli).*

Date	HEALTHY			SPIKED		
	Total N	P <sub>2</sub> O <sub>5</sub>	Ca	Total N	P <sub>2</sub> O <sub>5</sub>	Ca
8-7-27	215.6	...	146.3	316.1	...	49.0
22-7-27	135.0	...	86.2	389.8	...	68.8
29-7-27	135.5	122.4	121.7	377.5	112.0	66.4
11-8-27	174.2	95.5	90.9	294.0	29.5	51.4
8-9-27	166.6	104.0	...	370.2	96.4	...
14-10-27	140.0	116.3	131.4	344.9	83.3	48.9
7-11-27	178.0	...	...	253.9	...	...
15-5-28	62.7	...	...	238.6	...	...
15-6-28	271.7	106.7	165.9	561.6	88.9	91.3
Mean	164.3	108.9	123.7	349.6	82.0	62.6
Standard deviation	± 54.8	± 9.4	± 28.4	± 90.4	± 28.0	± 15.2

TABLE IV A.

*Milligrams per 100 c.c. of tissue fluid (Uttarahalli).*

Date	Healthy			Spiked		
	Reducing sugars	Sucrose	Maltose	Reducing sugars	Sucrose	Maltose
23- 6-27	45.0	135.4	291.7	289.8	337.5	191.3
27-10-27	29.5	500.4	125.0	100.5	604.3	287.5
9- 1-28	51.0	315.5	287.0	220.5	537.8	450.0

TABLE IV B.

*Milligrams per 100 c.c. of tissue fluid (Ragihalli).*

Date	Healthy			Spiked		
	Reducing sugars	Sucrose	Maltose	Reducing sugars	Sucrose	Maltose
8- 7-27	177.7	291.0	478.5	210.0	163.5	528.7
22- 7-27	210.0	305.0	920.0	338.0	180.0	360.0
14-10-27	...	575.0	705.0	117.5	265.0	870.0

## DISCUSSION OF RESULTS.

Table I gives the percentages of the tissue fluid that can be expressed under definite standardised conditions and calculated on the weight of the green material. In the majority of cases, healthy leaves yield higher percentages of fluid than spiked leaves and the mean value for healthy is higher than that of spiked.

The total solids and ash per 100 c.c. of tissue fluid, with percentage of ash on the weight of the total solids, have been tabulated in Tables II A and B. It has been pointed out that, especially in view of the parasitic nature of sandal, it is misleading to compare the samples brought from one area with those of another. We have accordingly divided the results under two heads and comparison of the tables does not indicate any consistent variation in the total solids; but there does exist a remarkably striking uniformity with regard to the lower ash in the tissue fluids of spiked leaf. In the case of the Uttarahalli area, the average ash of healthy leaf fluid is about 30 per cent. higher, while the

corresponding figure for Ragihalli is 75 per cent. The differences in this respect are more pronounced in the Ragihalli samples.

Table III gives the values for nitrogen, phosphorus pentoxide and calcium, calculated in milligrams per 100 c.c. of the tissue fluid. Nitrogen is high in the case of the tissue fluids from spiked leaf, while calcium is low. The mean value for nitrogen in healthy leaf is 367.9 while that for spiked leaf is half as much again. Calcium in healthy leaf is nearly four times that of the spiked leaf fluid in the Uttarahalli area. In the Ragihalli area, the mean nitrogen value of spiked leaf is a little more than twice that of healthy leaf fluid, while the calcium value for spike is half that of healthy. The phosphorus pentoxide values by themselves are not very significant, but when combined with other values, as will be shown in a later communication, the results attain great importance.

Tables IV A and B give the direct reducing sugars, as well as sucrose and maltose, in 100 c.c. of the tissue fluids. It is seen that in most of the cases, there is higher concentration of these in the diseased tissues.

#### SUMMARY.

The tissue fluids derived from the healthy and diseased sandal have been examined for their content of total solids, ash, nitrogen, phosphorus, calcium and sugars.

A lower ash, a higher nitrogen, a lower calcium, a higher maltose content and a higher content of reducing sugars characterise the tissue fluids of the spiked leaf.

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