Journal of the Indian Institute

of Science

Vl2 A. Part XVII pp 233 to 252

Verified
B

JOURNAL

OF THE

INDIAN INSTITUTE OF SCIENCE

Volume I included 21 issues during 1914–18. Thereafter one volume was published annually until 1925, when Volumes VIII (A) and (B), followed by IX (A) and (B) (1926) and X (A) and (B) (1927) appeared.

VOLUME XI (A)—1928.

Price, Rs. 1-4.] [Vol. 12A, Part XVII, pp. 233 to 252.

JOURNAL

OF THE

Indian Institute of Science.

CONTENTS.

TABLE I.

Activity of Dialysed Enzyme Extracts.
(Ragihalli, November 27, 1929).

Reaction mixture, 50 c.c. each of enzyme and 4 per cent. soluble starch : temperature, 30°. U = Undialysed. D = Dialysed.

Time in minutes	Mgms. of maltose per 20 c.c. of reaction mixture							
	Healthy		Spiked		Relative concentration of spiked to healthy		Percentage loss of activity by D	
	U	D	U	D	Before D	After D	Healthy	Spiked
30	6·3	4·4	16·5	6·3	2·6	1·4	30·1	61·8
60	8·1	4·8	18·0	7·6	2·2	1·5	40·7	57·7

It is clear from the table that (1) loss in activity is higher in spiked than in healthy extract and (2) the diastase content of the spiked leaf extract is 1·5 times that of the healthy leaf extract.

STUDY OF ACTIVATORS.

Table I shows that the loss of activity in the diseased leaf extract is about 60 per cent., while the corresponding figure for healthy leaf extract is about 35. This is largely due to the escape of diffusible activators during dialysis and a change in the hydrogen-ion concentration. The results of analysing extracts are incorporated in Table II.

TABLE II.

Analysis of the Enzyme Extracts (per 100 c.c.).

	Ragihalli 27—11—27		Uttarahalli 9—11—28	
	Healthy	Spiked	Healthy	Spiked
Total solids ...	3·81 g.	4·80 g.	4·54 g.	4·42 g.
Ash ...	740 mg.	450 mg.	880 mg.	440 mg.
Phosphate (P_2O_5) ...	20·8 ,,	37·7 ,,	47·0 ,,	62·0 ,,
Calcium (Ca) ...	...	...	139·0 ,,	17·5 ,,
Total Nitrogen ...	60·3 mg.	101·5 mg.	235·8 ,,	425·0 ,,
Amino-nitrogen ...	22·4 ,,	42·3 ,,	34·7 ,,	43·2 ,,

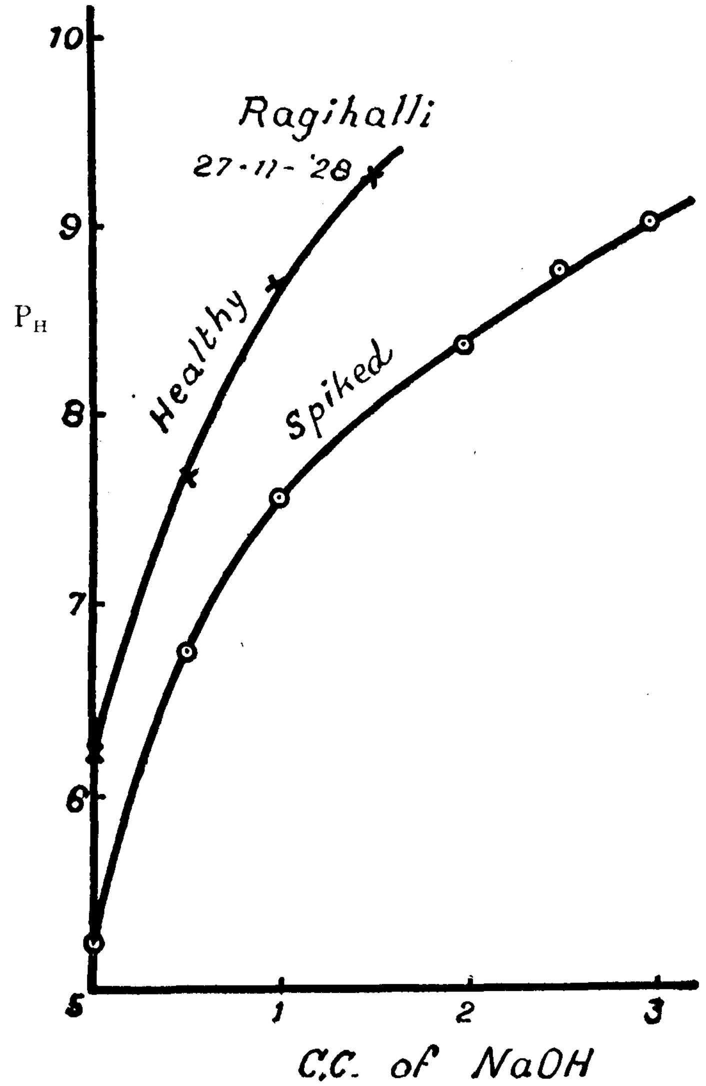

FIG. 1.

A lower ash content, a higher proportion of phosphorus, a higher total and amino-nitrogen, and a lower calcium content characterises the enzyme extract from the spiked leaves. The nitrate nitrogen is practically absent from the spiked leaf extract, but is present in the healthy extracts. The chloride content is the same in both cases.

The higher proportion of phosphorus and the higher amino-nitrogen content of the spiked leaf extract accelerate the diastatic activity, while the low calcium content is also favourable.

INFLUENCE OF P_H ON THE DIASTATIC ENZYMES.

The hydrogen-ion concentration and the buffer value of the original extracts are tabulated below.

TABLE III.

Buffering Capacity of Leaf Extracts.

(Ragihalli, November 27, 1927).

Electrometric titration of extract (5 c.c.) with 0·1 *N* NaOH.

NaOH, c.c. ...	0·0	0·5	1·0	1·5	2·0	2·5	3·0
Healthy P_H ...	6·24	7·65	8·70	9·39	...	...	...
Spiked P_H ...	5·25	6·75	5·75	...	8·36	8·77	9·05

The results are represented in Fig. I. Spiked leaf extract has a lower P_H and a higher buffer value, as can be observed from the curves.

TABLE IV.

Change of P_H on Dilution.

(Ragihalli, November 27, 1927).

Extract	Original P_H	Dilution with equal volume of water	Dilution with equal volume of 4 per cent. starch
Healthy ...	6·24	6·00	5·90
Spiked ...	5·25	5·30	5·30

Table IV clearly shows that the addition of an equal volume of starch does not alter the P_H in the case of spiked extract, while in the healthy extract the fall is 0·3 unit.

Acetate buffers were used to equalise the P_H values of the two extracts during starch hydrolysis. Mixtures employed for the experiment and the results obtained have been tabulated below and also graphically represented (Fig. II).

TABLE V A.

Influence of P_H *on Diastatic Action.*

(Ragihalli, November 27, 1927). Reaction mixture, 50 c.c. enzyme, 20 c.c. water or buffer and 30 c.c. of 6·66 per cent. starch solution : temperature, 30°.

Time in minutes	Mgms. of maltose per 20 c.c. of reaction mixture			
	Healthy		Spiked	
	P_H 5·9	P_H 5·85	P_H 5·3	P_H 5·6
30	6·3	...	16·5	...
35	...	6·7	...	10·2
60	8·1	9·7	18·0	17·2
105	11·0	13·0	24·8	25·0
150	18·7	17·0	36·0	28·7
210	20·5	20·8	50·8	39·5
345	23·4	...	58·5	...
360	...	24·4	...	57·0
450	29·7	...	69·1	...

TABLE V B.

(Uttarahalli, January 9, 1928).

—	P_H 6·02	P_H 5·95	P_H 5·45	P_H 5·75
30	21·2	27·0	61·0	59·7
75	31·5	38·2	90·5	82·5
135	38·5	48·6	113·8	103·7
210	45·0	59·6	142·5	126·2

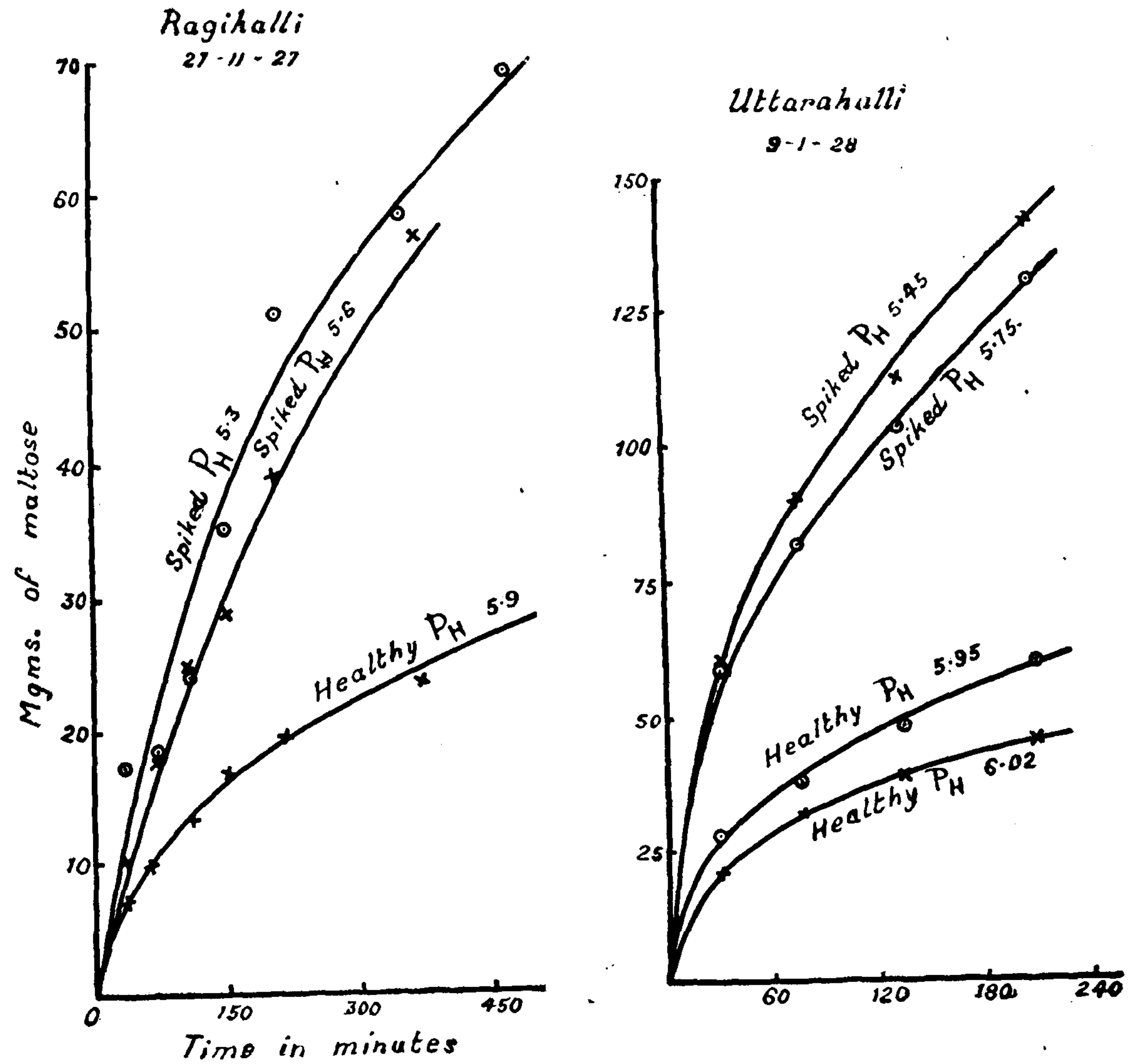

FIG. 2.

It is clear from tables and figures that the hydrogen-ion concentration has influenced the activation, and that the higher buffer action of the spiked leaf extract tends to maintain the optimum P_H and resists the action of appreciable quantities of acetate buffer.

PRESENCE OF INHIBITORS.

Experiments were made to reveal any change in activity by employing mixtures of tissue fluids from the healthy and spiked leaves. The presence of inhibiting agents in healthy leaf extracts would bring about a measurable reduction of activity of the spiked leaf extract on adding boiled extract of healthy leaves. The results have been tabulated below. The results are given in c.c. of $KMnO_4$ (1 c.c. = 10 mg. of copper) required for the estimation of sugars by Bertrand's method produced in 20 c.c. of the reaction mixture after incubation at 30° for 20 hours.

TABLE VI.

Influence of Boiled Extracts on Active Extracts.

(Uttarahalli, August 15, 1927).

Reaction mixture	c.c. of $KMnO_4$	Coloration with Iodine
25 c.c. of 2 per cent. starch, 2 c.c. spike extract and 2 c.c. water	9·30	Red
25 c.c. of starch, 2 c.c. boiled spike extract and 2 c.c. water	1·50	Blue
25 c.c. of starch, 2 c.c. healthy extract and 2 c.c. water	3·30	Reddish violet
25 c.c. of starch, 2 c.c. boiled healthy extract and 2 c.c. water	1·60	Blue
25 c.c. of starch, 2 c.c. boiled healthy extract and 2 c.c. spike extract	10·80	Red
25 c.c. of starch, 2 c.c. boiled healthy extract and 2 c.c. boiled spike	3·05	Blue
25 c.c. of starch, 2 c.c. healthy extract and 2 c.c. boiled spike	6·15	Red

Healthy leaf diastase is activated by the addition of boiled spiked leaf extracts, and addition of boiled healthy leaf extracts to active spiked leaf extract does not inhibit the activity of the latter. The experiments, while definitely establishing the presence of activators in the spiked leaf extract, do not indicate the presence of inhibitors in the healthy leaf extract.

The diastatic activity is considerably retarded by the presence of oxidases, as has been shown by True, Black, Kelly and others (*J. Agric. Res.*, 1918, **15**, 369). The leaf extracts have been examined for their oxidase content, and it has been found that the spiked leaf extracts have a decidedly higher oxidase activity. The high starch-splitting activity of spiked leaf extracts, in spite of their high oxidase content, is noteworthy.

Maltase.

The high cupric-reducing power of the reaction mixture containing spiked leaf extracts might be due to the presence of glucose, produced by the action of maltase on the maltose which is a product of starch hydrolysis by diastase. Extracts were tested for maltase by the usual polarimetric method, and although present, it did not exist in any significant quantities in either of the extracts. The healthy extracts showed a slightly higher maltose-splitting activity than the spiked leaf extracts.

SUMMARY.

The higher diastatic activity of spiked tissues, as compared with the corresponding healthy tissues, is due partly to a higher concentration of the enzyme, partly to the presence of activators like phosphates and amino-acids, and partly to their higher acidity approximating to the optimum P_H of plant diastase.

The lower diastatic activity of the healthy tissues as compared with the corresponding spiked tissues is not due to the presence of inhibitors which retard their amyloclastic capacity.

In conclusion, our thanks are due to Prof. Roland V. Norris for his keen interest and many helpful suggestions in the course of the work.

Department of Bio-Chemistry,
Indian Institute of Science,
Bangalore.

[Accepted, 7–10–29.]

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

Chemical composition of tissue fluids from the leaf.

By M. Sreenivasaya and B. N. Sastri.

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	58·0	− 8·2
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—2 .	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	13·43	3·38	25·18
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	16·36	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
8—9—27	19·62	3·46	17·64	19·95	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	16·65	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_2O_5	Ca	Total N	P_2O_5	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	308·8	...	...	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_2O_5	Ca	Total N	P_2O_5	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 sandals 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.

Our grateful thanks are due to Prof. R. V. Norris for his many helpful criticisms.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

[Accepted, 7-10-29.]

III.—CONTRIBUTIONS TO THE STUDY OF SPIKE-DISEASE OF SANDAL (*SANTALUM ALBUM*, LINN.). PART IX.

Chemical composition of tissue fluids from the stem.

By M. Sreenivasaya and B. N. Sastri.

The tissue fluids from healthy and diseased sandal stems after expression in the usual way (*J. Indian Inst. Sci.*, 1928, **11A**, 23) were centrifuged at 3,000 r. p. m. and the respective centrifugates examined for their total nitrogen, total solids, ash, phosphorus and calcium. The results have been tabulated in the three following tables.

TABLE I A.

UTTARAHALLI AREA.

Yield of tissue fluid from stems (Uttarahalli).

Date	Percentage yield on weight of green material	
	Healthy	Spiked
30-4-28	28·9	29·9
7-5-28	33·0	27·5
22-6-28	25·3	24·2
Mean ...	29·1	27·2

TABLE I B.

(RAGIHALLI).

Date	Healthy	Spiked
8-7-27	23·0	13·0
22-7-27	19·8	12·9
29-7-27	15·1	17·0
15-5-28	12·6	16·7
Mean ...	17·6	14·9

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
5—8—27	11·88	0·71	5·99	6·46	1·40	21·60
27-10—27	11·77	1·22	10·38	11·77	1·63	13·83
9—1—28	15·30	1·26	8·23	9·93	1·63	16·41
11—4—28	9·58	1·30	13·56	8·94	1·84	20·58
20—4—28	8·54	1·23	14·39	8·78	1·89	21·57
30—4—28	7·87	1·05	13·31	9·35	2·13	22·74
7—5—28	7·98	1·29	16·15	11·08	2·60	23·47
22—5—28	6·83	0·93	13·64	7·86	1·93	24·56
8—6—28	10·73	1·52	14·12	12·71	3·18	25·04
22—6—28	10·40	1·40	12·47	9·27	2·16	23·34
Mean ...	...	1·19	...	...	2·04	...
Standard deviation ...	...	± 0·22	...	...	± 0·49	...

TABLE II B.

RAGIHALLI AREA.

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	10·75	0·70	6·51	11·30	0·38	3·37
22—7—27	16·33	0·70	4·31	13·79	0·83	6·02
29—7—27	14·41	1·01	6·98	21·52	0·97	4·51
14-10—27	13·78	1·04	7·57	14·42	0·97	6·70
7-11—27	11·18	1·08	9·69	13·91	0·99	7·17
23-11—27	12·16	2·00	16·44	14·70	1·60	10·88
19-12—27	13·59	1·21	8·90	13·54	1·18	8·71
15—5—28	12·58	0·76	6·08	13·81	1·92	13·86
15—6—28	15·59	0·83	5·33	13·98	1·23	8·81
Mean ...	...	1·04	...	...	1·12	...
Standard deviation ...	...	±0·39	...	...	±0·41	...

TABLE III A.

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

Date	Healthy			Spiked		
	Total N	P_2O_5	Ca	Total N	P_2O_5	Ca
5- 8-27	...	92·0	136·0	...	99·6	128·0
15- 9-27	145 0	101·8	144·0	258·5	184 0	117·0
27-10-27	171·0	58·0	125·0	481·6	164·0	125·0
9- 1-28	247·8	33·0	163·0	415·6	77·0	129·0
11- 4-28	239·6	130·2	...	365·1	176·7	...
20- 4-28	281·1	168·4	36·0	398·0	194·2	90·0
30- 4-28	378·3	223·6	72·0	467·2	321·2	132·0
7- 5-28	270·1	148·9	62·0	280·2	237·6	116·0
22- 5-28	198·8	92·6	64·0	375·4	150·0	74·0
8- 6-28	262·6	108·1	80·6	591·8	114·9	101·6
22- 6-28	214·5	86·2	82·1	298·6	94·3	126·3
Mean ...	240·8	112·9	96·5	393·2	164·7	113·9
Standard deviation ...	± 61·9	± 50·4	± 40·0	± 96·9	± 67·9	± 18·3

TABLE III B.

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

Date	Healthy			Spiked		
	Total N	P_2O_5	Ca	Total N	P_2O_5	Ca
8- 7-27	72·5	...	...	...	...	...
11- 8-27	...	45·6	103·0	...	68·1	89·0
14-10-27	95·0	41·0	133·0	162·0	66·0	129·0
7-11-27	96·3	109·3	121·0	147·3	153·1	114·0
23-11-27	153·6	68·0	94·0	176·8	35·0	94 0
19-12-27	128·0	7·0 (?)	104·0	214·0	32·0	82·0
15- 5-28	...	90·9	29·0	...	76·9	60·8
15- 6-28	188·2	78·8	77·5	254·7	100·1	64·5
Mean ...	122·3	72·3	94·5	190·9	75·9	90·5
Standard deviation ...	± 39·3	± 24·0	± 31·4	± 38·8	± 38·3	± 22·9

TABLE IV A.

UTTARAHALLI AREA.

Milligrams of calcium per 100 c.c. of tissue fluid
(Uttarahalli: healthy)

Date	Leaf	Stem
15—9—27	165·0	144·0
20—4—28	750·0	36·0
30—4—28	634·0	72·0
7—5—28	668·0	62·0
22—5—28	558·0	64·0
8—6—28	940·2	80·6
22—6—28	540·4	82·1
Mean	615·1	77·2
Standard deviation ...	±219·9	± 30·7

TABLE IV B.

(Uttarahalli: diseased).

Date	Leaf	Stem
15—9—27	107·5	117·0
20—4—28	154·0	90·0
30—4—28	154·0	132·0
7—5—28	118·0	116·0
22—5—28	154·0	74·0
8—6—28	120·0	101·6
22—6—28	96·8	126·3
Mean	129·0	108·1
Standard deviation ...	± 22·6	± 19·1

DISCUSSION OF RESULTS.

Tables I A and B give the yield of tissue fluids from stems. It has been observed that the yield is lower in the case of diseased stems and progressively decreases with the onset of the disease. The root ends and the haustorial connections are damaged by the disease, so that the absorption of water and nutrients from the soil solution is affected. The heavy accumulation of starch in the conducting tracts of the stem renders free passage of the sap extremely difficult. This can be experimentally demonstrated by selecting two four-inch lengths of twig having the same diameter, one from the healthy and the other from the diseased sandal and sucking water through them under a known negative pressure. It will be found that, while water can easily be sucked through the healthy twig, the diseased offers very great resistance.

Tables II A and B give the total solids, ash in grams per 100 c.c. of the tissue fluid and also the percentage of ash calculated on the weight of the total solids. In the case of the diseased stem fluid, the ash is distinctly higher for the Uttarahalli area. The corresponding average value in II B also shows a slight increase, but is not significant. It is however clear that the ash is not lower in the diseased condition, as was shown to be the case in the instance of the tissue fluids from diseased leaves.

Tables III A and B give the nitrogen, phosphorus pentoxide and calcium values expressed as milligrams per 100 c.c. of tissue fluid. Nitrogen figures are uniformly higher in the diseased condition, and in the majority of instances the phosphorus values are distinctly higher in the diseased state. The calcium content of the diseased tissue fluid is in many cases higher and in fact the average value of calcium for the Uttarahalli area is about 17 per cent. higher. At any rate, the distinctly lower calcium content which characterises the diseased leaf tissue fluid, is not to be found in the case of the diseased stem tissue.

The study of gradients of concentration of the several nutrient elements from the root to the growing tips will throw light on the nature of the distribution. In the case of the healthy sandal, it will be observed from Table IV A that there is a great difference between the calcium concentrations of the leaf and stem tissue fluids in the healthy state.

The average value for the stem fluid is only one-tenth of the value for the leaf. In the diseased condition, however, the calcium concentration of the leaf tends to fall to that of the stem and in advanced stages of the disease the concentration may fall far below that level (see Table IV B.)

Some investigators have found that pine trees grown in quartz sand and fed by a synthetic culture solution lacking calcium, exhibited irregularities in the transportation of starch, and a diminution in the size of the several organs (Loew, *U. S. Dept. of Agric. Bureau of Plant Industry*, 1903, Bull. No. 45). The low calcium concentration in the leaf, the main centre of photosynthetic activity, probably stimulates formation of starch, which in turn accumulates in the tissues of the diseased sandal.

SUMMARY.

The tissue fluids of healthy and diseased stems of sandal have been examined for total solids, total nitrogen, ash, phosphorus and calcium.

Nitrogen and phosphorus concentration of the tissue fluids from diseased stems is higher as compared with the values for the healthy stems, but the calcium content in the diseased state is higher.

A high gradient of calcium concentration exists from the stem towards the leaf which tends to flatten as the disease advances. It is suggested that the rapid transport of calcium through the stem is impaired with the incidence of spike; consequently, the leaf does not receive its adequate share of the calcium which, as shown by previous investigators, prevents abnormalities in the translocation of starch.

Our grateful thanks are due to Prof. Roland V. Norris for his many helpful criticisms and kind encouragement throughout this work.

Department of Bio-Chemistry,
Indian Institute of Science,
Bangalore.

[Accepted, 7-10-29.]

IV.—NOTE ON THE STARCH-LIQUEFYING ACTION OF SANDAL LEAF EXTRACTS.

By B. N. Sastri.

It is well known that the spike-disease brings about a heavy accumulation of starch in the diseased tissues. It has also been found that the diastatic or sugar-producing capacity of the leaf extracts and tissue fluids in the diseased state is higher than that of the healthy. It was suspected that there might exist in the two extracts a varying proportion of the two well-known diastatic components, the liquefier and the saccharifier. The abnormal accumulation of starch suggests that in the diseased condition there is poor translocation of the carbohydrate, possibly due to a deficient proportion of the liquefying fraction. Experiments have been described to determine the proportion of the two enzyme components in the leaf extracts.

Potato starch paste (25 c.c. of 2 per cent.) was treated with 2 c.c. of the tissue fluids, expressed from leaves as described in a previous paper (*J. Indian Inst. Sci.*, 1928, **11A**, 24); the mixture, with about 0·5 c.c. toluene, was incubated at 30° for 20 hours, and 20 c.c. then added to 120 c.c. of 95 per cent. alcohol. The precipitate was filtered on a prepared Gooch crucible, previously dried and weighed, and was washed first with 95 per cent. alcohol, then with ether and dried in a steam oven to constant weight. The sugars in the filtrate were estimated by Bertrand's method, after removing the alcohol by evaporation and treatment with dialysed iron. The following table gives the results of estimation, the usual controls being run; difference in the weights of precipitate between control and experimental gives the weight of starch liquefied.

Hydrolysis of Potato starch by leaf extracts (Uttarahalli).

Milligrams of	8-8-28		23-8-28	
	Healthy	Spiked	Healthy	Spiked
Starch liquefied ...	93·1	225·9	52·0	162·0
Maltose produced ...	46·5	172·5	26·1	120·3

It is clear from the table that for equal weights of sugar produced, more of starch is liquefied by the healthy leaf extract than by the