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

Factors influencing Diastatic Activity.

By B. N. Sastri and M. Sreenivasaya.

It was pointed out (*J. Indian Inst. Sci.*, 1928, **11A**, 23) that the higher diastatic activity of the spiked leaves of sandal was not due only to the higher concentration of diastatic enzymes, but was possibly enhanced by (1) the low P_H value approximating to the optimum reaction of the plant amylases, (2) the lower calcium content and (3) the higher amino-nitrogen content.

It is generally known that phosphates, nitrates, chlorides and amino-acids accelerate the diastatic activity (Lintner, *J. pr. Chem.*, 1887, **36**, 481 ; Effront, *Compt. rend.*, 1892, **115**, 1324 ; Cole, *J. Physiol. Chem.*, 1904, **30**, 202–281), while calcium is supposed to exert an inhibiting influence. An attempt has been made to study (1) the relative concentration of the diastatic enzymes in the healthy and diseased leaves, (2) the enhancement of activity due to various activators and (3) the possible presence of inhibitors in healthy leaves.

Preparation of the Enzyme Extract.

Healthy and diseased leaves were obtained from the two areas, Ragihalli and Uttarahalli, referred to in our previous communication, desiccated over calcium chloride, powdered to pass through a 40-mesh sieve and preserved in stoppered bottles. The enzyme extracts were prepared in the same way as already described (*J. Indian Inst. Sci.*, 1928, **11A**, 27).

Dialysis and Determination of Relative Concentration of Diastatic Enzymes.

Definite volumes of the extracts were dialysed in collodion bags against distilled water at room-temperature for 48 hours. The activity of the dialysed enzyme extract, freed from a large proportion of the crystalloid activators, was measured in the usual way, and the relative concentration of the diastase in the two extracts tabulated, assuming that the amount of maltose produced in equal intervals of time is proportional to the concentration of the enzymes at initial stages of action.

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		Porcentage 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 ,,

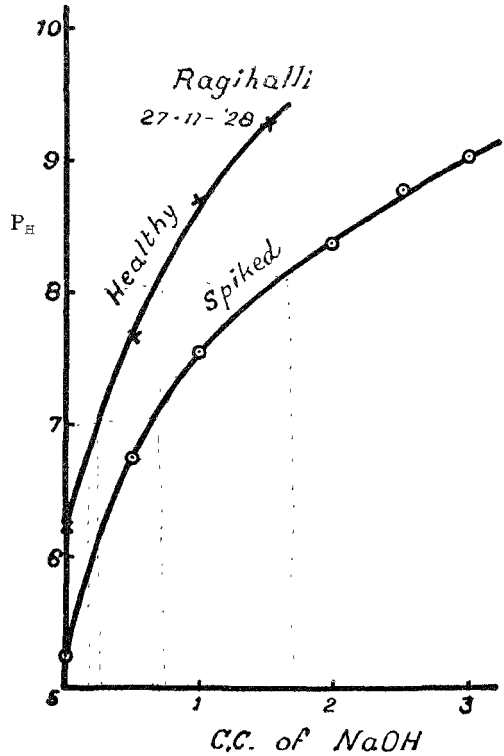

FIG. 1.

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

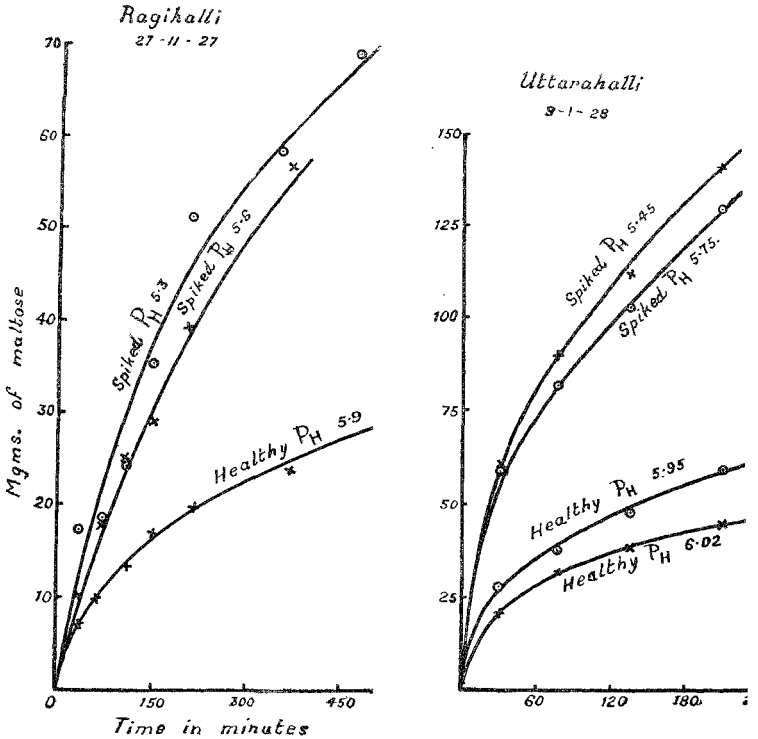
Ragikalli
27 - 11 - 27
Mgms. of maltose
Time in minutes
Spiked PH 5·3
Spiked PH 5·6
Healthy PH 5·9
Uttarahalli
9 - 1 - 28
Spiked PH 5·45
Spiked PH 5·75
Healthy PH 5·95
Healthy PH 6·02

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.

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