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[Vol. 13A, Part XII, pp. 147 to 158.]

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CONTENTS.

I. THE SPIKE-DISEASE OF *DODONAEA VISCOSA*.

BY

B. N. Sastri and N. Narayana.

**II. STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS
PART III. THE GLOBULINS OF BENGAL GRAM
(*CICER ARIETINUM*, LINN.) AND HORSE
GRAM (*DOLICHOS BIFLORUS*).**

BY

Nuggihalli Narayana.

V. FORSTER, F.R.S., CHAIRMAN OF EDITORIAL BOARD.

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N30.13A.12

172-19

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THE SPIKE-DISEASE OF *DODONAEA VISCOSA*.

By B. N. Sastri and N. Narayana.

Dodonaea viscosa (Kanarese—*Bandarika*) is a shrub occurring wild in the *maidan* forests of Mysore. It grows to a height of about 4 ft. and possesses stipulate, lanceolate leaves with entire margin. The plant is regarded as a febrifuge in Ayurvedic medicine, but has no economic importance otherwise. It has been known to occur as a host for sandal.

This plant suffers from a disease similar to the spike of sandal (Coleman, *Dept. of Agriculture in Mysore, Mys. Bull.*, 1917, 3, 19), prevalent in areas where sandal spike occurs. As in the case of sandal, the diseased leaves diminish in size and the internodes become shortened giving the whole plant a characteristic bushy appearance: flowering and reproduction cease and vegetative growth is accelerated.

In view of observations previously made on healthy and diseased tissues of sandal (*J. Indian Inst. Sci.*, 1928, 11A, 23, 97 and 241), it was considered that a similar study of *Dodonaea viscosa* might throw light on the nature of sandal spike. The investigation was expected to be less complicated than in the case of sandal because the plant is not a parasite and being a shrub, presents no difficulties in the collection of samples for study.

EXPERIMENTAL.

Representative specimens of healthy and diseased plants were collected from different areas where sandal occurs, and were separated into leaves, stems and roots after thorough washing. The various tissues were dried quickly over calcium chloride in vacuum desiccators at about 0° to prevent changes due to enzyme action (Link, *J. Amer. Chem. Soc.*, 1925, 47, 470). The dry tissues were powdered to pass a 40-mesh sieve and preserved in glass-stoppered bottles.

Methods of analysis.—Total nitrogen was determined by Gunning's modification of Kjeldahl's method to include the nitrogen of nitrates, proteins by Stutzer's copper hydroxide method and free and saline

ammonia by Graffe's method (*Z. Physiol. Chem.*, 1906, **48**, 300). For the last determination, 20 g. lots of the material were treated with 100 c.c. of ammonia-free water, 100 c.c. of 95 per cent. alcohol, and 25 c.c. of saturated sodium chloride solution. Saturated sodium carbonate solution (25 c.c.) was added to the mixture which was then distilled *in vacuo* at 37° for 4 hours, ammonia being determined in the usual way.

Nitrate was estimated by Scale's method (*J. Biol. Chem.*, 1916, **27**, 327). The material (40 g.) was extracted with 80 per cent. alcohol for 36 hours and the extract after removal of alcohol, clarified with lead acetate and made to a definite volume. The nitrate in aliquot parts was reduced by a zinc-copper couple and the ammonia estimated. The Griess-Ilosway method (qualitatively) was used for nitrites.

Distribution of non-protein nitrogen.—Preliminary experiment showed that the material contained large quantities of mucilaginous matter hindering filtration of the hot-water extracts; 60 per cent. alcohol was therefore used. Weighed quantities were extracted repeatedly with boiling alcohol and the combined extracts concentrated by distillation under reduced pressure at a temperature not exceeding 40°. The concentrate was taken up with water, filtered and freed from fat by extraction with ether; it was then treated with dialysed iron, centrifuged, and the clear extract made up to a known volume.

The extract contained polypeptides, amino-acids, amides, ammonia and other forms of non-protein nitrogen. A definite volume was hydrolysed for 12 hours with sufficient hydrochloric acid to bring the concentration to 20 per cent. The insoluble melanin was separated and its nitrogen estimated. The filtrate, after distilling the hydrochloric acid was diluted, and the distribution of nitrogen determined by Plimmer's modification of the Van Slyke method (*Biochem. J.*, 1925, **19**, 1004).

Carbohydrates.—Alcoholic extracts of the material were clarified with basic lead acetate and used for the estimation of sugars. Sucrose was determined after hydrolysis with 10 per cent. citric acid, reducing sugars by Bertrand's method and starch in the residue after alcoholic extraction by hydrolysis with taka-diastase according to Davis and Daish (*J. Agric. Sci.*, 1914, **6**, 152).

Ash constituents were estimated by the A.O.A.C. methods (1925). The results of analysis are shown in Tables I–VII, the specimens having been collected from the Laggare forest area on 14th August, 1928, unless otherwise stated.

TABLE I.

Percentage of Dry Weight

	LEAVES		STEMS		ROOTS	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Total nitrogen	1.86	1.86	0.38	0.51	0.37	0.17
Protein nitrogen	1.76	1.58	0.36	0.48	0.32	0.14
Non-protein nitrogen (by difference)	0.10	0.28	0.02	0.03	0.05	0.03
Ash	5.75	6.34	3.91	3.80	6.15	2.49

TABLE II.

Percentage of total Nitrogen

Form of Nitrogen	LEAVES		STEMS		ROOTS	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Nitrate	0.16	0.38	2.37	4.38	1.40	2.00
Free ammonia	0.40	0.45	1.29	1.58	0.50	2.64
Nitrite	Nil.	Traces	Nil.	Nil.	Nil.	Traces

TABLE III.

Distribution of Non-protein Nitrogen.

Percentage of total Non-protein Nitrogen

Form of Nitrogen	LEAVES		STEMS		ROOTS	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Melanin { Insoluble	10.02	11.12	8.14	11.57	9.37	14.45
Soluble	5.56	4.25	4.07	4.63	4.48	1.53
Amide	15.50	20.54	17.66	19.50	13.75	15.16
Basic	25.41	19.33	24.53	19.17	15.00	14.85
Non-basic	47.50	47.59	44.75	43.87	55.90	55.45

TABLE IV.

Carbohydrates.

Percentage of Dry Weight						
	LEAVES		STEMS		ROOTS	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Free reducing sugars as—						
Glucose ...	0.78	2.34	0.24	0.49	0.41	0.27
Sucrose ...	0.50	0.60	0.48	0.26	0.23	0.17
Starch ...	2.49	4.61	3.76	3.81	2.71	2.86

TABLE V.

Ash Constituents.

Percentage of Dry Weight							
Date of collection	Locality	Tissue— condition	Ash	Total nitrogen	P ₂ O ₅	Ca	K ₂ O
14—11—27	Laggare	... Leaf, H	6.20	1.85	0.10	0.38	0.47
15—4—28	Denkanikota	... " D.	9.65	1.76	0.15	0.15	0.55
		... " H.	4.96	1.95	0.11	0.77	0.45
15—7—28	"	... " D.	6.27	2.29	0.15	0.10	0.46
		... " H.	6.09	2.36	0.52	0.95	0.32
23—8—28	Uttarahalli	... " D.	6.43	1.91	0.53	0.77	0.48
		... " H.	6.57	2.41	0.37	0.59	...
14—9—28	Laggare	... " D.	5.17	1.82	0.39	0.56	...
		... " H.	5.75	1.86	0.52	0.56	0.14
"	"	... " D.	6.34	1.85	0.32	0.37	0.12
"	"	... Stem H.	3.91	0.38	0.39	0.32	0.04
"	"	... " D.	3.80	0.51	0.29	0.27	0.03
"	"	... Root H.	6.15	0.37	0.25	0.63	0.19
"	"	... " D.	2.49	0.17	0.24	0.36	0.15

H=healthy; D=diseased; not determined.

Discussion.—It may be seen from Tables I, II and III, that the different forms of non-protein nitrogen, such as nitrate, amide and ammonia are higher in the diseased tissues than in the healthy ones. Bourquet obtained similar results in his study of the virus diseases, the mosaic of tobacco, the curly-top of sugar beet and the leaf-roll of potato (*J. Amer. Chem. Soc.*, 1916, 38, 2572). The present observations suggest that the spike of *Dodonaea viscosa* is allied to the above and also caused by a virus.

Table IV shows that, as in the case of sandal spike (*J. Ind. Inst. Sci.*, 1928, 11A, 99) diseased leaves have higher sugar and starch contents than the healthy ones. Similar results were obtained in another series of experiments in which tissue-fluids were expressed from the leaves, as may be seen from Table VI. The diastatic activity of the juice was measured and found to be higher for the diseased plant as in the case of sandal.

TABLE VI.

*Specimens collected from the Uttarahalli forest area
on 23-8-1928.*

Carbohydrate	Healthy	Diseased
Free reducing sugars in 100 cc. of fluid ...	301 mg.	341 mg.
Sucrose " ...	114 "	225 "
Diastatic activity mg. of maltose*	6.7 "	24.0 "
Starch in residue ...	6.1 per cent.	8.4 per cent.

* Vide *J. Ind. Inst. Sci.*, 1928, 11A, 25.

The figures for nitrogen, phosphorus and potassium (Table V) do not appear to depend upon the condition of the plant. Those for calcium are, however, more significant, being invariably lower in the case of diseased tissues. It is well known that calcium assists the hydrolysis and translocation of starch and prevents the accumulation of organic acids such as oxalic, which would otherwise prove poisonous. The growing parts of plants require large quantities of calcium, and serious metabolic disturbances are caused by a shortage of the element. The high N/Ca ratio in the diseased tissues is indicative of pathological disturbances (Parker and Truog, *Soil Sci.*, 1920, 10, 49).

To determine whether the differences in mineral composition observed in the cases of healthy and diseased tissues of sandal and *Dodonaea viscosa* are borne out in the case of *Zyzyphus aenoplia*, known also to suffer from a similar disease, specimens of the latter were analysed for their nitrogen, phosphorus and calcium contents, percentage on the weight of dry leaves being shown in Table VII.

TABLE VII.

Analysis of Leaves of Zyzyphus ænoplia.

Date	Locality	Condition of the leaf tissue	N	P ₂ O ₅	Ca
5— 1—28	Aiyur	Healthy	2.47	1.53	0.89
" "	" "	Diseased	3.68	1.87	0.38
5— 3—28	Denkanikota	Healthy	3.20	1.07	1.15
" "	" "	Diseased	3.87	1.28	0.47

Calcium is again lower in the diseased tissues, and measurements of diastatic activity showed that this factor was higher than in the healthy leaf fluid.

SUMMARY.

The non-protein nitrogen constituents of the tissues of *Dodonæa viscosa* in healthy and diseased conditions were determined. The nitrate, nitrite, ammonia and amide contents of the diseased tissues were invariably higher than those of the corresponding healthy ones.

As in the case of sandal, sugars and starch tend to accumulate in the diseased tissues. The diastatic activity of the latter was also higher than that of the healthy tissues.

The diseased condition is further characterised by deficiency in calcium. This is true also of *Zyzyphus ænoplia* which suffers from a similar disease.

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

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STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS.

PART III. The Globulins of Bengal Gram

(*Cicer Arietinum*, Linn.)

and

Horse Gram (*Dolichos Biflorus*).

By Nuggihalli Narayana.

Bengal gram is an important Indian foodstuff belonging to the order *Leguminosæ*. The vernacular names are:—Sanskrit, *Chenaka*; Hindi, *Chana*; Tamil, *Kadalay*; and Telugu, *Senagalu*. The crop is raised in all parts of India, the upper basins of the Ganges and the Indus being the chief gram-producing areas. Agricultural statistics show that this pulse accounts for about 13 per cent. of the total area (including States) under food crops. It is consumed by all classes of people and is believed to be highly nutritious. In many parts of the country any surplus material is fed to cattle.

Horse gram is one of the cheapest among Indian pulses and is chiefly employed as food for horses and cattle. The vernacular names are:—Sanskrit, *Kulattha*; Hindi, *Kulthi*; Kanarese, *Hurali*; and Tamil, *Kollu*. It is grown in all parts of the country as a catch-crop and thrives with a minimum rainfall. As it enriches the soil with nitrogen, the plant, after harvesting the seed, is generally ploughed in as green manure.

The present investigation of the composition of the globulins which form the chief proteins of the seeds, was undertaken in view of the importance of the pulses in relation to nutrition.

EXPERIMENTAL.

The seeds were sun-dried until crisp and easy to handle. The husk was removed from the Bengal gram and the kernel ground to obtain the flour. The horse gram was ground whole because (a) it was not possible to remove the husk and (b) the whole seed is invariably fed to animals. Flours passing the 60-mesh sieve were used in the

TABLE III.

	From Bengal gram by			From Horse gram by	
	(1)	(2)	(3)	(1)	(2)
Nitrogen as :—					
Acid Insoluble melanin ...	0.32	0.32	0.48	1.32	1.30
Soluble melanin (absorbed by lime)	0.38	0.30	0.29	0.05	0.05
Amide	10.40	10.40	10.47	10.09	10.78
Basic :—					
Arginine	19.23	19.24	19.47	13.61	11.88
Histidine	1.75	1.12	1.82	1.13	1.75
Cystine	0.24	0.27	0.24	0.41	0.45
Lysine	8.45	8.80	8.22	9.56	8.93
Non-basic :—					
Amino	57.80	58.43	58.45	63.64	63.84
Non-amino	0.49	0.47	0.46	1.88	1.65
Total	99.06	99.35	99.90	100.69	100.59
Arginine direct	22.51	22.50	23.17	14.09	14.01
Free amino-nitrogen, direct ...	4.99	5.23	...	4.81	4.27
Half Lysine, N.	4.23	4.40	...	4.78	4.47
Sulphur in basic fraction ...	0.55	0.59	0.54	...	...
Sulphur in non-basic fraction ...	1.60	1.44	1.44	...	...

TYROSINE, TRYPTOPHAN AND CYSTINE.

Tyrosine was estimated by the method of Zuwerkalao (*Z. physiol. Chem.*, 1926, **163**, 185) and tryptophan by that of Komm (*Z. physiol. Chem.*, 1926, **163**, 202). The figures for cystine obtained by the Van Slyke method cannot be taken as correct, since, owing to racemisation during hydrolysis, the amino-acid is incompletely precipitated by phosphotungstic acid. Independent determinations of cystine were therefore carried out by the method of Folin and Looney (*J. Biol. Chem.*, 1922, **51**, 421). Tryptophan in the globulin of horse gram could not be estimated by the method of Komm, as the colour obtained with the globulin was different in shade from that with pure tryptophan. The results were as follows :—

TABLE IV.

Percentages of ash and moisture-free protein.

			Globulin from	
			Bengal gram	Horse gram
Tyrosine	...	...	4.90	6.68
Cystine	...	...	0.88	1.81
Tryptophan	...	...	0.41	present
Arginine (Van Slyke)	...	...	10.27	6.02
Arginine (direct)	...	...	12.09	7.11
Histidine	...	...	0.99	0.84
Lysine	...	...	7.57	7.64

DISCUSSION OF RESULTS.

The figures for the distribution of nitrogen show that the different preparations of each globulin are identical in composition. Arginine, as estimated in the protein hydrolysate, is 2–3 per cent. higher than when estimated in the diamino-fraction, showing that the amino-acid is not completely precipitated by phosphotungstic acid (Plimmer and Rosedale, *loc. cit.*).

The ratio of sulphur in the basic and non-basic fractions of the globulin from Bengal gram is nearly 1 to 3; if all sulphur in the protein had been present as cystine, the ratio should have been 1 to 1.5 (Plimmer and Lowndes, *loc. cit.*). Moreover, since cystine estimated by the method of Folin and Looney (*loc. cit.*) accounts for only two-thirds of the total sulphur, it may be inferred that in addition to cystine, one or more sulphur-containing compounds are present in the non-basic fraction.

The close agreement obtained between the figures for free amino-nitrogen and half the lysine nitrogen in both globulins indicates that the free amino-group of lysine accounts for all the nitrogen in that form so that practically no other free amino-groups are present in the proteins. This result is a contrast with an unpublished observation of the author that in the case of the prolamins of Ragi (*Eleusine coracana*), only one-seventh of the free amino-nitrogen is due to lysine.

Both the globulins contain sufficient amounts of tyrosine, arginine and lysine, while cystine and tryptophan contents are comparatively

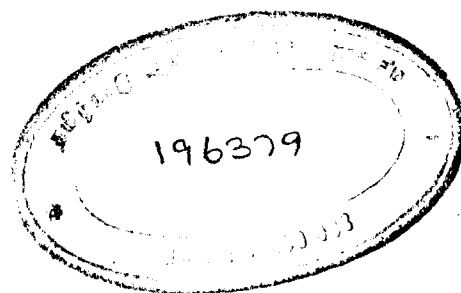
low. A comparison with the composition of similar proteins present in other important Indian pulses (*J. Indian Inst. Sci.*, 1929, 12A, 193; *J. Biol. Chem.*, 1920, 42, 59) shows that the globulin of the Bengal gram is characterised by a high percentage of arginine and a low one of non-amino nitrogen (proline?).

SUMMARY.

The globulins of Bengal and horse gram were isolated and analysed by the method of Van Slyke. Tyrosine, tryptophan and cystine were estimated. The arginine content of the Bengal gram, was much higher than those usually met with in the globulins of other Indian pulses. Both proteins were found to contain sufficient amounts of arginine, tyrosine and lysine, but were deficient in cystine and tryptophan.

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