Studies on the Nodule Bacteria VI.

Influence of different parts of plant on the growth of nodule bacteria.

By

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In the previous paper[1], it was reported that the growth of nodule bacteria is stimulated by the addition of plant extracts in general and it was especially marked when the extracts of the leguminous plants are added. Now in this paper, it was investigated as to the influence of different parts of the same plant and the results are reported as follows.

Experimental.

The cultures used were the same ones as used in the previous experiments, namely the nodule forming bacteria from Genge (Astragalus sinicus) strains A, B and C; bean and clover.

Preparation of plant extracts.

Since the chemical composition of plants vary by the different parts and also by the season, it was decided to collect the plants at their flowering period. Genge was chiefly used thgether with white clover, and their seeds were tried also. The nitrogen content of these materials was determined by KJELDAHL method and found, as shown in Table I.

Table I.

Nitrogen Contents of diffcrent Parts of Plants.

Samples.	Genge.	White clover.
	(%)†	(%)
Seeds.	5.931	4.850
Stems & leaves.	3.447	4.117
Roots.	3.140	2.171
Nodules.	7.125	6.937

Note: † percent of dried matter.

Table I indicates that the largest amount of nitrogen was found in the nodules, and the smallest in the roots while the seeds contained a considerable amount of nitrogen which was followed by the stems. That is more nitrogen was found in the parts above the ground than those under the ground, as has been generally reported by others.

Procedure :

The extracts were prepared by both directly from the fresh plants right after they were collected, and the others were dried in shade and used as the powder.

Part I. Experiments with the fresh plants.

1. Preparation of the fresh plant extracts :

As it was described in the previous paper, the collected plants were thoroughly washed with the top water first and again well washed with the distilled water repeatedly; after the water was drained off, each part viz. stems, roots and nodules was separated; the stems and roots were cut into small pieces and crushed in a mortar and made up into ten percent solution by adding the distilled water; heated for 30 minutes over the steam bath and filtered, and the filtrate was added to the culture medium in various concentration.

2. Culture medium and inoculation :

The mannit agar medium was used to which the extract was added in various concentration. For inoculation, the culture which was grown for four days at 28°C was taken and suspended in the sterile water, and made the streak on the agar slant as evenly as possible.

3. Examination for growth :

The cultures were kept in an incubator at 28°C and examined at different intervals by the naked eyes for the general growth and finally by staining with ZIEHL's carbol fuchsin, the morphological changes were noted.

The results are given in the following tables:

(See Table II on next page.)

Table II indicates that the best growth was obtained by 50 percent nodule extract while Genge seeds, stems and leaves and root depressed the growth. As a whole better growth was obtained by the nodule and bad growth, by the seeds. Morpholsgically the cells vary greatly although the rod forms were prevalent and the coccic forms were rarely found especially in Genge 10 percent extract. Among the rods, the forms varied from short to long rods which sometimes exceded over 2 μ having two to three granules, as shown in the photograph. (Plate I.)

The results obtained with Genge nodule bacteria, strain B are given in Table III.

(See Table III on 338 page.)

Table II.

Influence of Fresh Plant Extracts on Growth and Morpholcgy of Genge Nodule Bacteria A.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.					14 days old culture.	
			2	4	7	14	Sum of +	Forms.	Size. (μ)
Control.	—	—	—	+	++	++	5	Short rod.	0.3×0.8—0.5×1.0
Yeasts.	—	10.0	—	+	+++	+++	9	„	0.3×0.5—0.5×0.8
Genge.	Seeds.	Stock.	—	—	+	++	3	Coccic & Short rod.	0.2×0.2—0.5×0.8
		50	—	+	++	++	5	Short rod.	0.3×0.4—0.5×1.0
		10	—	—	++	+++	5	Rod (granulated).	0.4×0.8—0.8×2.0
	Stems & leaves.	Stock.	—	—	+	++	3	Short rod.	0.3×0.4—0.5×0.8
		50	—	—	+++	+++	7	„	„
		10	—	+	+++	+++	9	Coccic.	0.2—0.4
	Roots.	Stock.	—	—	+	++	3	Rod.	0.2×0.5—0.5×1.2
		50	—	—	+++	+++	7	Rod (granulated).	0.4×0.5—0.8×1.8
		10	—	—	+++	+++	8	„ „	0.5×0.8—0.8×2.2
	Nodules.	Stock.	—	+	++	++	6	Rod.	0.3×0.5—0.5×1.2
		50	—	+++	++++	++++	13	Rod (granulated).	0.5×0.8×0.7×2.5
		10	—	++	+++	++++	11	Coccic.	0.2—0.4
Clover.	Stems & leaves.	50	—	—	+++	+++	9	Rod (granulated).	0.3×0.5—0.6×1.5
		10	—	+	++	+++	8	„ „	0.3×0.6—0.8×2.0
	Roots.	50	—	—	+++	+++	7	Rod (granulated).	0.3×0.8—0.6×2.0
		10	—	+	+++	+++	8	„ „	0.3×0.6—0.6×2.0
	Nodules.	50	—	+	++++	++++	11	Short rod.	0.4×0.5—0.5×1.0
		10	+	++	++++	++++	13	Rod (granulated).	0.3×0.6—0.5×1.8

N.B. + indicates the rate of growth.

As shown in Table III, the best growth was obtained in 10 percent Genge nodule followed by that of 50 percent while the stock solution of Genge seeds and roots had ill effect. In all the cases, the stock solution gave weak growth. The different part of Genge and clover had the similar influence as in the previous

Table III.

Influence of Fresh Plant Extracts on Growth and Morphology of Genge Nodule Bacteria B.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.					14 days old culture.	
			2	4	7	14	Sum of +.	Forms.	Size. (μ)
Control.	—	—	—	+	+	+	3	Short rod.	0.3×0.5—0.5×0.8
Yeasts.	—	10	+	++	+++	+++	10	„	0.3×0.4—0.5×0.8
Genge.	Seeds.	Stock.	—	—	+	++	3	Short rod.	0.3×0.5—0.5×0.8
		50	—	+	++	+++	6	„	0.3×0.4—0.5×0.8
		10	—	+	+++	+++	8	Rod (granulated).	0.5×0.6—0.7×1.8
	Stems & leaves.	Stock.	—	—	++	++	5	Short rod.	0.3×0.4—0.5×0.8
		50	—	—	+++	++++	9	„	0.3×0.4—0.6×0.8
		10	—	+	+++	+++	9	Coccic.	0.2—0.4
	Roots.	Stock.	—	—	+	++	3	Rod.	0.2×0.5—0.3×1.5
		50	—	—	++	++++	7	Short rod.	0.3×0.5—0.5×1.5
		10	—	—	+++	+++	8	Rod (granulated).	0.3×0.5—0.7×1.2
	Nodules.	Stock.	—	+	+++	+++	7	Short rod.	0.2×0.4—0.3×0.7
		50	—	+++	+++	++++	12	Rod (granulated).	0.3×0.5—0.7×1.2
		10	—	+++	++++	++++	14	Short rod.	0.2×0.4—0.3×0.8
Clover.	Stems & leaves.	50	—	—	+++	++++	9	Rod (granulated).	0.3×0.5—0.6×1.2
		10	—	+	+++	+++	9	„ „	0.3×0.5—0.7×1.8
	Roots.	50	—	—	++	+++	7	Rod.	0.3×0.7—0.5×1.5
		10	—	++	+++	+++	9	„	0.3×0.8—0.6×1.8
	Nodules.	50	—	+	+++	++++	9	Coccic.	0.3×0.4—0.5×1.5
		10	—	++	+++	++++	11	Rod (granulated).	0.3×0.5—0.6×1.8

N.B. + indicates the rate of growth.

experiment. Morphologically strain B was the same as that of strain A.

The similar experiment was carried out with Genge nodule bacteria strain C and the following results were obtained as shown in Table IV.

Table IV.

Influence of Fresh Plant Extracts on Growth and Morphology of Genge Nodule Bacteria C.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.					14 days old culture.	
			2	4	7	14	Sum of +.	Forms.	Size. (μ)
Control.	—	—	—	+	+	++	4	Short rod.	0.2×0.3—0.4×0.8
Yeasts.	—	10	+	+++	+++	+++	11	„	0.3×0.4—0.5×0.8
Genge.	Seeds.	Stock.	—	—	+	++	3	Short rod.	0.2×0.3—0.5×1.0
		50	—	+	+	++	4	„	„
		10	—	+	+++	+++	8	Rod.	0.4×0.7—0.7×1.2
	Stems & leaves.	Stock.	—	—	+++	+++	7	Short rod.	0.3×0.4—0.5×0.8
		50	—	+	+++	+++	9	„	0.2×0.3—0.4×0.8
		10	—	+	+++	+++	9	Short rod & coccic.	0.2×0.3—0.4×0.8
	Roots.	Stock.	—	—	+	++	3	Rod.	0.3×0.6—0.5×2.0
		50	—	—	++	+++	6	Coccic & short rod.	0.3×0.4—0.4×1.0
		10	—	+	+++	+++	8	Rod (granulated).	0.3×0.7—0.8×1.8
	Nodules.	Stock.	—	+	+++	+++	9	Coccic & short rod.	0.3×0.5—0.5×0.9
		50	—	++	+++	++++	11	Rod.	0.3×0.5—0.8×1.2
		10	—	++	+++	++++	11	Short rod.	0.3×0.5—0.4×0.8
Clover.	Stems & leaves.	50	—	—	+++	+++	8	Short rod.	0.3×0.4—0.5×0.8
		10	—	+	+++	+++	9	„	0.3×0.4—0.5×1.2
	Roots.	50	—	—	+++	+++	8	Rod & coccic.	0.3×0.4—0.5×1.5
		10	—	+	+++	++	7	Rod.	0.3×0.4—0.6×1.5
	Nodules.	50	—	+	+++	++++	10	Rod (granulated).	0.3×0.5—0.5×1.5
		10	—	++	+++	++++	11	Rod & coccic.	0.3×0.5—0.5×1.8

N.B. + indicates the rate of growth.

As shown above, the best growth was obtained in 10 and 50 percent Genge nodule extract and also in 10 percent clover nodule extract. The growth was worst in the stock solution of Genge seeds while in other cases the results were similar to those obtained with strain A and B.

As the foregoing results indicate, all three strains of Genge nodule bacteria showed the similar tendency in all the cases and grew well in the nodule extracts

of Genge and clover while the leaves and stems were less effective, and the seed extracts depressed the growth. Morphologically they varied somewhat but no definite correlation was found between their forms and their growth although more short rods were observed in the stem and leaf extracts.

Next the nodule bacteria of beans and clover were experimented and the results are shown in the following table:

Table V.

Influence of Fresh Plant Extracts on Growth and Morphology of Bean Nodule Bacteria.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.					14 days old culture.	
			2	4	7	14	Sum of +.	Forms.	Size. (μ)
Control.	—	—	—	+	+	+	3	Rod (granulated).	0.2×0.6—0.5×1.1
Yeasts.	—	10	+	++	+++	+++	12	Rod.	0.2×0.4—0.5×1.2
Genge.	Seeds.	Stock.	—	—	+	++	3	Short rod.	0.3×0.5—0.5×1.0
		50	—	+	++	+++	6	„	0.3×0.5—0.5×1.0
		10	—	+	++	+++	6	„	0.3×0.4—0.5×0.8
	Stems & leaves.	Stock.	—	—	+	++	4	Rod.	0.3×0.5—0.5×1.5
		50	—	—	++	++++	8	„	„
		10	—	+	+++	++++	10	Coccic.	0.2—0.4
	Roots.	Stock.	—	—	+	++	3	Rod.	0.3×0.5—0.4×1.2
		50	—	—	++	+++	6	„	0.4×0.5—0.6×1.2
		10	—	+	+++	+++	9	Rod (granulated).	0.5×0.6—1.0×2.0
	Nodules.	Stock.	—	+	++	++	7	Short rod.	0.2×0.4—0.4×1.0
		50	—	+	+++	+++	9	Rod (granulated).	0.7×0.8—1.0×2.0
		10	—	+	++++	++++	11	„ „	0.5×0.8—0.8×1.5
Clover.	Stems & leaves.	50	—	—	+++	++++	9	„ „	0.3×0.5—0.8×2.2
		10	—	++	+++	++++	11	Rod.	0.4×0.8—0.8×2.0
	Roots.	50	—	+	+++	+++	9	Short rod.	0.3×0.4—0.5×0.8
		10	—	+	++++	++++	11	Rod.	0.4×0.7—0.8×1.2
	Nodules.	50	—	+	+++	++++	10	„	0.3×0.5—0.5×1.5
		10	—	++	++++	++++	12	Coccic & rod.	0.2—0.3—0.5×1.3

N.B. + indicates the rate of growth.

Table V indicates that good growth of bean nodule bacteria was obtained in 10 percent clover nodule extract followed by that of Genge; the stems and roots of clover. In other cases the results were similar to those of Genge nodule bacteria. Also morphologically they were similar to Genge nodule bacteria viz. a majority of cells were rod and the coccic forms were observed only in ten percent Genge stem and clover nodule extract.

The results obtained with the clover nodule bacteria are given in Table VI.

Table VI.

Influence of Fresh Plant Extracts on Growth and Morphology of Clover Nodule Bacteria.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.					14 days old culture.	
			2	4	7	14	Sum of +.	Forms.	Size. (μ)
Control.	—	—	—	+	+	++	4	Rod.	0.2×0.5—0.5×1.2
Yeasts.	—	10	+++	+++	++++	++++	17	„	0.3×0.7—0.5×1.2
Genge.	Seeds.	Stock.	—	++	+++	++++	9	Short rod.	0.3×0.4—0.5×0.8
		50	+	++	+++	+++	10	Coccic.	0.3—0.5
		10	—	+++	+++	+++	11	Rod (granulated).	0.3×0.8—0.4×1.2
	Stems & leaves.	Stock.	—	+++	+++	++++	11	Rod.	0.4×0.8—0.5×1.2
		50	+	+++	++++	++++	13	„	0.3×0.5—0.5×1.5
		10	—	+++	+++	+++	10	Coccic & Short rod.	0.3×0.5—0.5×0.8
	Roots.	Stock.	—	+	+++	+++	9	Rod.	0.3×0.5—0.5×1.5
		50	—	+	+++	++++	10	„	0.6×0.8—0.8×2.5
		10	—	++	+++	++++	10	Rod (granulated).	0.3×0.5—0.5×1.2
	Nodules.	Stock.	+	++	+++	++++	12	Short rod.	0.3×0.5—0.5×1.0
		50	+	+++	++++	++++	15	Rod (granulated).	0.6×0.7—0.8×2.5
		10	—	+++	+++	++++	12	Rod.	0.5×0.7—0.7×1.2
Clover.	Stems & leaves.	50	—	+++	+++	++++	13	Rod.	0.5×0.8—1.0×2.2
		10	+	++	+++	+++	10	„	0.3×0.5—0.4×2.0
	Roots.	50	+	+++	+++	++++	13	Rod (granulated).	0.4×0.8—0.6×1.8
		10	—	+++	+++	+++	11	Rod.	0.3×0.5—0.8×1.8
	Nodules.	50	+	++++	++++	++++	16	Rod (granulated).	0.5×1.0—0.7×2.3
		10	—	+++	++++	++++	13	„	0.3×0.5—0.5×1.2

N.B. + indicates the rate of growth.

As Table VI indicates the best growth was obtained with 50 percent clover nodule extract which is followed by that of Genge. In general, the clover nodule bacteria behaved similarly to those which have been investigated in the foregoing experiments.

Thus, throughout the foregoing experiments, the growth of nodule bacteria was influenced most strongly by the extract of nodules of plants used, followed by that of the stem and leaf and the roots in the order. However the concentrated extracts depressed the growth in all the cases although the nodule extract alone was relatively effective. Morphologically it is noteworthy that the bacteroides of rare forms were found especially in the extracts of nodules and roots.

Part II. Experiments with the dried plants.

The influence of the extracts of fresh plants was investigated in Part I. while in this series, the air-dried plant material was used since the supply of fresh materials is limited to a certain season as well as their composition varies from time to time.

1. Preparation of plant materials :

The fresh plants collected for the previous experiments were dried in a rapid drying oven at about 60°C and used as powder. The moisture contents of the powdered materials are given in Table VII.

Table VII.

Moisture Contents of Plant Materials.

Plant materials.	Genge.	Clover.
	%	%
Seeds.	12.507	10.496
Stems & leaves.	8.361	6.276
Roots.	8.848	7.524
Nodules.	7.980	6.185

As Table VII indicates, the moisture content was highest in the seeds and lowest in the nodules.

2. Experimental procedure :

The same procedure as used in the preceding series was followed by using the powdered materials which were added to the culture medium. The inoculation was made on the agar slant with an equal amount of culture suspension carried by platinum needle; incubated for four days at 28°C., and the growth was examined as follows: (a) the growth was examined by the naked eyes at different intervals; (b) the weight of bacterial cells was determined by Mueller's method[4] which was originally used for diphtheria culture. That is, the bacterial

growth is scraped off carefully with a platinum loop and suspended in 0.05 percent acetic acid; centrifuged for 15 minutes, pipett off the supernatant liquid and suspend the sediment again in the acetic acid, and centrifuge; finally transferred into an evaporating dish and after evaporation, dried in hot air oven at 110°C and weighed.

At the sametime, strains B and C of Genge nodule bacteria were examined morphologically by stainning with ZIEHL's carbol fuchsin.

The results are presented in the following tables:

Table VIII.

Influence of Dried Genge on Growth and Cellular Weight of Genge Nudule Bacteria A.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.				Wt. of bacterial cells in mg.
			2	4	7	Sum of +.	
Control.	—	—	+	+	+	3	0.4
Yeasts.	—	10	+	++	++++	9	6.8
Genge.	Seeds.	1.0	—	—	—	0	—
		0.5	—	—	—	0	—
		0.2	—	—	+	1	0.3
		0.1	—	++	++	4	0.4
	Stems & leaves.	1.0	—	—	—	0	—
		0.5	—	—	—	0	—
		0.2	—	++	++++	7	1.6
		0.1	—	++	++	6	0.5
	Roots.	1.0	—	++	++++	7	1.4
		0.5	—	++	++++	7	1.5
		0.2	+	++	++	7	1.8
		0.1	+	++	++	7	1.4
	Nodules.	1.0	+	++++	+++++	12	11.7
		0.5	+	++++	++++	10	6.8
		0.2	+	++++	++++	10	5 6
		0.1	+	++++	++++	9	4.4

N.B. + indicates the rate of growth.

Table VIII indicates the influence of Genge on Strain A of Genge nodule bacteria, and the best growth was obtained in 1.0 percent nodule and no growth in 1.0 and 0.5 percent of seeds and stem. The weight of bacterial cells agreed fairly well with the results of observations made by the naked eyes. The weight in 1.0 percent nodule was 11.7 mg. while that of yeast agar was 6.8 mg. indicating a marked beneficial influence of nodule. On the other hand only 0.4 mg was obtained in the control.

The results obtained with Genge on clover nodule bacteria are given in Table IX.

Table IX.

Influence of Dried Genge on Growth and Cellular Weight of Bean Nodule Bacteria.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.				Wt. of bacterial cells in mg.
			2	4	7	Sum of +	
Control.	—	—	+	+	+	3	0.3
Yeasts.	—	10	+	++	++++	9	7.7
Genge.	Seeds.	1.0	—	—	—	0	—
		0.5	—	—	—	0	—
		0.2	—	±	+	1.5	0.5
		0.1	+	++	++	7	1.1
	Stems & leaves.	1.0	—	—	—	0	—
		0.5	—	—	—	0	—
		0.2	—	++	++	7	2.6
		0.1	+	++	++	8	2.2
	Roots.	1.0	—	++	++	6	1.2
		0.5	—	++	++	6	3.5
		0.2	+	++	++	7	3.7
		0.1	+	++	++	6	1.7
	Nodules.	1.0	+	++	++++	10	10.5
		0.5	+	++	++++	10	9.0
		0.2	+	++	++++	10	8.5
		0.1	+	++	++	8	4.2

N.B. + indicates the rate of growth.

As Table IX indicates, the best growth was obtained in yeast agar, followed by 1.0 and 0.5 percent nodule while no growth was observed in the seeds and stem.

The influence of Genge on clover nodule bacteria is shown in Table X.

Table X.

Influence of Dried Genge on Growth and Cellular Weight of Clover Nodule Bacteria.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.				Wt. of bacterial cells in mg.
			2	4	7	Sum of +.	
Control.	—	—	++	+++	+++	8	1.2
Yeasts.	—	10	+++	++++	++++	14	14.4
Genge.	Seeds.	1.0	—	—	—	0	—
		0.5	—	—	—	0	—
		0.2	+	+++	+++	8	1.9
		0.1	+++	+++	+++	10	2.9
	Stems & leaves.	1.0	—	—	—	0	—
		0.5	—	+	+++	4	2.5
		0.2	++	+++	+++	9	2.9
		0.1	++	+++	+++	8	1.3
	Roots.	1.0	+	++++		11	6.1
		0.5	+	+++	+++	7	3.0
		0.2	++	+++	+++	9	4.2
		0.1	+++	+++	+++	11	3.2
	Nodules.	1.0	+++	++++	++++	13	13.9
		0.5	+++	++++	++++	13	8.9
		0.2	+++	+++	+++	11	4.9
		0.1	+++	+++	+++	11	2.3

N.B. + indicates the rate of growth.

The best growth was obtained in the yeast followed by 1.0 and 0.5 percent of nodule, and no growth in either 1.0 and 0.5 percent seeds or 1.0 percent leaves. The influence of different part of plants on Genge and bean nodule bacteria was similar in general. The weight of bacterial cells was 14.4 mg in yeast and 13.9 mg

in 1.0 percent nodule, and the least 1.2 mg in the control. From the foregoing results, the influence of different parts of Genge plant on three nodule bacteria is summarized by the weight of bacterial cells in the following table:

Table XI.

Relation between different Parts of Genge and Cellular Weight of Bacteria.

Materials.	Cellular weight of bacteria.			Total weight. (mg.)	Average,† weight. (mg.)
	Genge A. (mg.)	Bean. (mg.)	Clover. (mg.)		
Control.	0.400	0.300	1.200	1.900	0.633
Yeasts.	6.800	7.700	14.400	28.900	9.633
Seeds.	0.175	0.400	1.200	1.775	0.592
Stems & leaves.	0.525	1.200	1.675	3.400	1.133
Roots.	1.525	2.525	4.125	8.175	2.725
Nodules.	7.125	8.050	7.450	22.625	7.542

N.B. † the average weight was obtained by dividing the total weight by the numbers of experiments carried out on the same plant.

Table XI indicates that the weight of bacterial cells was greatest in yeast which was followed by the nodule although Genge strain A and bean bacteria grew best in the nodule. In practice, an addition of optimum amount of nodule gives better growth than where yeasts are added. The growth was very poor and sometimes no growth was obtained where the seeds are used so that the average of cell weight is less than that of the control.

The results obtained with Genge nodule bacteria, strain B are presented in Table XII.

(See Table XII on next page.)

From the data given above, it is evident that the best growth was obtained in the seeds, which was better than that in the yeast. In other cases, the results are similar to those obtained with strain A. Morphologically the rod forms were prevalent and the coccic forms were present besides the rods where the vigorous growth took place. A majority of rod cells were granulated, and the cells were largest in 0.1 percent seeds where some cells were longer than 3.5 μ and club shaped in some cases. Also in 0.5 percent root, some large cells of 3.0 μ were found.

Table XIII presents the results obtained with Genge nodule bacteria strain C.

(See Table XII on 348 page.)

Table XII.

Influence of Dried Genge on Growth and Cellular Weight of Genge Nodule Bacteria B.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.				7 days old culture.	
			2	4	7	Sum of +.	Forms.	Size. (μ)
Control.	—	—	+	+	+	3	Short rod.	0.8×0.5—0.5×0.8
Yeasts.	—	10	+	++	++++	9	Coccic & Short rod.	0.2×0.2—0.6×1.0
Genge.	Seeds.	1.0	—	—	—	0	—	—
		0.5	—	—	—	0	—	—
		0.2	—	—	+	1	Rod (granulated).	0.3×0.4 - 0.6×1.2
		0.1	—	+	++	3	„ „	0.5×0.8—1.0×3.5
	Stems & leaves.	1.0	—	—	—	0	—	—
		0.5	—	—	—	0	—	—
		0.2	—	++	+++	6	Rod.	0.2×0.5—0 6×1.2
		0.1	—	++	+++	7	Short rod.	0.3×0.5—0.6×1.0
	Roots.	1.0	+	++	++++	8	Rod.	0.3×0.5—0.8×1.5
		0.5	—	+++	+++	8	Rod (granulated).	0.3×0.5—0.8×3.0
		0.2	++	+++	+++	8	Coccic & Short rod.	0.2×0.2 - 0.6×1.0
		0.1	—	+++	+++	6	Rod (granulated).	0.3×0.5—0.8×1.8
	Nodules.	1.0	+	+++	++++	10	Coccic & Short rod.	0.2×0.2 - 0.5×1.0
		0.5	+	+++	++++	10	Rod (granulated).	0.3×0.5—0.7×1.3
		0.2	+	+++	++++	10	Rod & coccic.	0.2×0.2—0.7×1.2
		0.1	++	+++	+++	10	„ „	0.2×0.2—0.6×1.2

N.B. + indicates the rate of growth.

As the above data indicate, strain C behaved just about the same as strains A and B, and the largest cells of 4.0 μ were found in 0.1 percent seeds. The coccic cells were present together with the rods.

In general, the cells were small where the growth was vigorous while they were large where the growth was poor such as in the seeds, and in this regard, the results obtained with the raw plant extracts were different. But it was not possible to find any definite correlation between the growth and different parts of plants.

B. The results obtained as to the influence of white clover on different nodule bacteria are presented in the following tables.

Table XIII.

Influence of Dried Genge on Growth and Cellular Weight of Genge Nodule Bacteria C.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.				7 days old culture.	
			2	4	7	Sum of +.	Forms.	Size. (μ)
Control.	—	—	—	+	+	2	Short rod.	0.3×0.4—0.6×1.0
Yeasts.	—	10	+	|||	||||	10	Rod.	0.3×0.5—0.6×1.2
Genge.	Seeds.	1.0	—	—	—	0	—	—
		0.5	—	—	—	0	—	—
		0.2	—	+	++	3	Rod (granulated).	0.3×0.5—0.7×1.2
		0.1	—	++	|||	5	„ „	0.3×0.7—1.0×4.0
	Stems & leaves.	1.0	—	—	—	0	—	—
		0.5	—	—	—	0	—	—
		0.2	—	++	|||	6	Rod (granulated).	0.2×0.6—0.7×1.2
		0.1	+	|||	|||	8	Rod.	0.3×0.4—0.7×1.5
	Roots.	1.0	—	+	|||	5	Rod (granulated).	0.3×0.7—0.8×1.2
		0.5	+	|||	||||	10	Rod.	0.2×0.5—0.7×1.5
		0.2	+	|||	|||	8	Coccic & rod.	0.2×0.2—0.8×1.2
		0.1	+	|||	|||	8	Rod (granulated).	0.3×0.5—0.8×2.5
	Nodules.	1.0	—	|||	||||	8	„	0.3×0.5—0.5×1.2
		0.5	+	|||	||||	10	„	0.2×0.3—0.8×2.0
		0.2	+	|||	||||	10	Short rod & coccic (granulated).	0.2×0.2—0.7×1.0
		0.1	+	|||	||||	10	Rod (granulated).	0.3×0.5—0.7×1.5

N.B. + indicates the rate of growth.

The results obtained on Genge nodule bacteria, strain A are given in Table XIV.

(See Table XIV on next page.)

Table XIV indicates that the growth in 0.5 and 0.1 percent nodules was the best, and no growth was observed in 0.5 percent seeds. The root in general was second to the nodule although it seems to be worse than the stem in some cases.

The results on the bean nodule bacteria are given in Table XV.

Table XIV.

Influence of Dried White Clover on Growth and Cellular Weight of Genge Nodule Bacteria A.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.				Wt. of bacterial cells in mg.
			2	4	7	Sum of +.	
Control.	—	—	+	+	+	3	0.4
Yeasts.	—	10.0	+	|||	||||	10	7.4
Clover.	Seeds.	0.5	—	—	—	0	—
		0.1	+	++	++	5	0.4
	Stems & leaves.	0.5	—	+	|||	5	3.6
		0.1	+	|||	|||	7	1.1
	Roots.	0.5	+	|||	|||	8	2.1
		0.1	++	++	++	6	0.9
	Nodules.	0.5	+	||||	||||	11	7.6
		0.1	++	|||	||||	11	5.6

N.B. + indicates the rate of growth.

Table XV.

Influence of Dried White Clover on Growth and Cellular Weight of Bean Nodule Bacteria.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.				Wt. of bacterial cells in mg.
			2	4	7	Sum of +.	
Control.	—	—	+	+	+	3	0.4
Yeasts.	—	10.0	+	|||	||||	10	5.6
Clover.	Seeds.	0.5	—	—	—	0	—
		0.1	+	|||	|||	7	0.5
	Stems & leaves.	0.5	—	+	|||	5	4.4
		0.1	+	|||	|||	7	2.5
	Roots.	0.5	+	|||	|||	9	3.6
		0.1	+	|||	|||	8	2.5
	Nodules.	0.5	+	||||	||||	11	9.7
		0.1	+	|||	||||	11	5.2

N.B. + indicates the rate of growth.

As the above data indicate, the bean nodule bacteria behaved similarly to the Genge bacteria as to their growth, and the cell weight was highest in 0.5 percent nodule being 9.7 mg and least in 0.1 percent seed weighing 0.5 mg.

Table XVI presents the results obtained with the clover nodule bacteria.

Table XVI.

Influence of Dried White Clover on Growth and Cellular Weight of Clover Nodule Bacteria.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.				Wt. of bacterial cells in mg.
			2	4	7	Sum of +.	
Control.	—	—	++	++	+++	7	1.4
Yeasts.	—	10.0	+++	++++	++++	13	5.3
Clover.	Seeds.	0.5	—	—	—	0	—
		0.1	++	+++	+++	10	1.3
	Stems & leaves.	0.5	++	++++	++++	12	4.4
		0.1	++	+++	+++	9	3.3
	Roots.	0.5	+++	++++	++++	13	5.4
		0.1	++	+++	++++	11	4.8
	Nodules.	0.5	+++	++++	++++	13	6.6
		0.1	+++	++++	++++	13	5.6

N.B. + indicates the rate of growth.

As it is noted in the above table, the clover bacteria showed the similar tendency as the foregoing organisms, weighing 6.6 mg in 0.5 percent nodule and 1.3 mg in the seed.

The cell weight of different bacteria in different materials which were noted in the foregoing tables, is summarized in Table XVII.

(See Table XVII on next page.)

As shown above, the weight was greatest in the nodule which was greater than that in the yeast culture, and it was least in the seed being less than that of the control. The leaf and root were the same and were similar to those results obtained with Genge plants as was shown in Table XI.

The results obtained with Genge nodule bacteria, strain B are given in Table XVIII.

Table XVII.

Relation between different Parts of Clover and Cellular Weight of Bacteria.

Materials.	Cellular weight of bacteria			Total weight. (mg.)	Ave.,† weight. (mg.)
	Genge A. (mg.)	Bean. (mg.)	Clover. (mg.)		
Control.	0.40	0.40	1.40	2.20	0.733
Yeasts.	7.40	5.60	5.30	18.30	6.100
Seeds.	0.20	0.25	0.65	1.10	0.367
Stems & leaves.	2.35	3.45	3.85	9.65	3.217
Roots.	1.50	3.05	5.10	9.65	3.217
Nodules.	6.60	7.45	6.10	20.15	6.717

N.B. † the average weight was obtained by dividing the total weight by the numbers of experiments carried out on the same plant.

Table XVIII.

Influence of Dried White Clover on Growth and Cellular Weight of Genge Nodule Bacteria B.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days				7 days old culture.	
			2	4	7	Sum of +.	Forms.	Size. (μ)
Control.	—	—	+	+	+	3	Coccic & rod.	0.2×0.2—0.5×1.2
Yeasts.	—	10.0	+	++++	++++	9	Rod (granulated) & coccic.	0.2×0.2—0.6×1.2
Clover.	Seeds.	0.5	—	—	—	0	—	—
		0.1	+	++++	++++	7	Rod (granulated).	0.4×0.5—0.6×1.8
	Stems & leaves.	0.5	±	+	++++	4.5	Rod.	0.4×0.5—0.6×1.8
		0.1	+	++++	++++	7	„	0.4×0.5—1.0×1.5
	Roots.	0.5	+	++++	+++++	10	„	0.3×0.5—0.6×2.0
		0.1	++	++++	++++	8	Rod (granulated) & coccic.	0.3×0.3—0.6×2.2
	Nodules.	0.5	+	+++++	+++++	11	Rod & coccic.	0.3×0.3—0.6×1.5
		0.1	++	++++	++++	10	Rod.	0.3×0.5—1.0×2.5

N.B. + indicates the rate of growth.

The above data show that the similar results were obtained in this case with other bacteria. The longest rods were 2.5 μ in 0.1 percent nodule.

The same experiment as above except strain C was used, and the results are given in Table XIX.

Table XIX.

Influence of Dried White Clover on Growth and Cellular Weight of Genge Nodule Bacteria C.

Materials.	Parts of plant.	Conc. (%)	Rate of growth by days.				7 days old culture.	
			2	4	7	Sum of +.	Forms.	Size. (μ)
Control.	—	—	+	+	+	3	Rod & coccic.	0.2×0.2—0.6×1.5
Yeasts.	—	10.0	+	++++	++++	11	Rod (granulated) & coccic.	0.2×0.2—0.7×1.8
Clover.	Seeds.	0.5	—	—	—	0	—	—
		0.1	+	+++	+++	7	Rod (granulated).	0.3×0.5—0.6×1.5
	Stems & leaves.	0.5	—	+	+++	4	Rod.	0.3×0.5—0.5×1.2
		0.1	+	+++	++++	8	„	0.3×0.5—0.8×2.0
	Roots.	0.5	+	++++	++++	9	Rod & coccic.	0.3×0.3—0.6×2.0
		0.1	+	++	+++	6	Rod (granulated).	0.3×0.5—0.6×2.0
	Nodules.	0.5	+	++++	++++	10	Rod & coccic.	0.3×0.3—0.6×1.0
		0.1	+	++++	++++	10	Rod (granulated).	0.3×0.3—0.7×2.0

N.B. + indicates the rate of growth.

The results shown in Table XIX resemble to those given in Table XVIII. The large cells were 2.0 μ in length and some bacteroids were found while the cells were small in the nodule where good growth took place.

So far as Genge nodule bacteria strains B and C are concerned, the cells were large in the root and nodule when their growth was poor as in the previous experiments with the fresh materials, but the dried Genge plants were different. Judging from these results, no definite relationship was found between the morphology of bacterial cells and the different part of plants. NOBBE and HILTNER[4] reported as to the variation of bacterial cells and the host plants, and noted that the bacteroids were beneficial while the rods were mere parasites without benefits to the host plants. It was attempted to find some relations between the morphological variation and the amount of nitrogen fixed, and also if the bacteroids could be produced experimentally. Regarding the relation between the morphological variation and the amount of nitrogen fixation will be reported in the future.

Summary and Canclusions.

In this investigation, the influence of different parts of plants, namely the seeds, nodules, roots and stems with leaves of Genge and white clover, in fresh and dried state, on three different strains of nodule bacteria of Genge, and a strain of each bean and clover, was tested. The results are summarized as follows:

1.) The fresh and dried plant materials influenced the growth of bacteria very similarly as a whole. In case of the fresh plants, the order of influence was the nodules, stems with leaves, roots and seeds, while with the dried materials, the roots were better than the stems with leaves otherwise the same. In both cases, the nodules were the best and the seeds, the worst while the stems with leaves and the roots were intermediate.

2.) The optimum quantity of nodules was from 0.1 to 1.0 percent as the dried matter, and the nodules were equal or sometimes better than the yeast extract.

3.) No relationship was noted between the rate of influence and the nitrogen content of different materials since the seeds which were the worst contained more nitrogen except the nodules.

4.) As a whole, the bacterial cells were large and the bacteroids were found where the plant materials were added but no definite relationship to the different part of plants was found.

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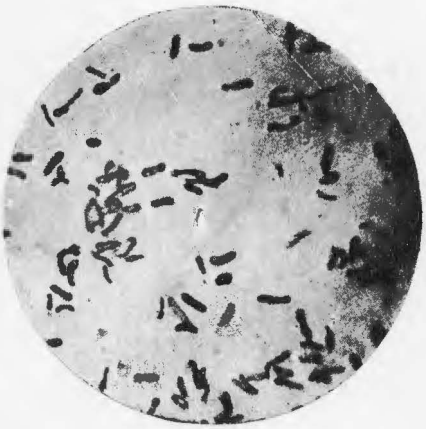
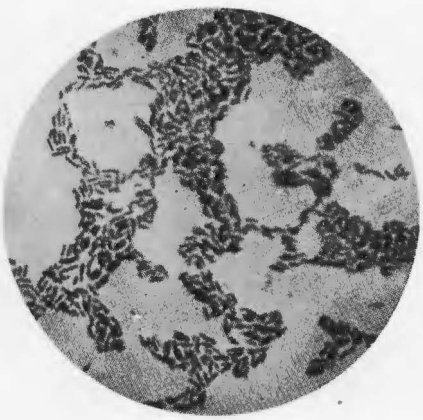
PLATE XVIII.

Influence of Plant Extract on the Morphology of Genge Nodule Bacteria.

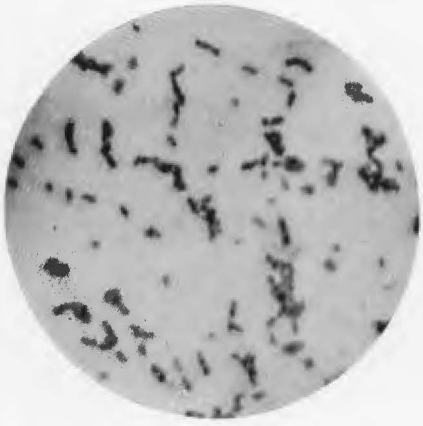
Bacteroids in nodule.



Cultured in yeast-mannit medium for 4 days.



Cultured in 50% stock Solution of Genge extract for 14 days.



Cultured in 10% stock Solution of Genge extract for 14 days.