

Studies on Nodule Bacteria VII.

Influence of the extract of nodules on the growth of nodule bacteria.

By

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In the preceding papers, it was reported that the leguminous plants with nodules stimulate the growth of nodule bacteria better than those without the nodules¹⁾ and also the nodules were more effective than the other portions. Further enquiry was made as to the agents in the nodules which caused the stimulation by preparing various extracts from the nodules, and the results are reported in this paper.

Experimental.

1.) *Cultures used :*

The same strains of nodules bacteria as in the previous experiments were used, namely Genge nodule bacteria, strain A, B and C together with the bean and clover cultures.

2.) *Collection of nodules :*

The Genge nodules which were used in the previous experiment together with those of beans collected from the field were taken.

Since no experiment has previously been undertaken with the different part of bean plants, it was investigated at this time in the same manner as adapted in case of Genge.

As it was done previously, the nitrogen contents of different part of bean plants were determined, as shown in Table I.

(See Table I on next page.)

As shown above, the nitrogen content was largest in the seeds and least in the roots while the nodules were intermediate.

The influence of different part of bean plants on the nodule bacteria is noted in Table II.

Table I.
Nitrogen Contents in different Parts of Bean Plant.

Names of Parts.	Air-dried matter. (%N)	Dry matter. (%N)
Seeds.	6.542	6.876
Stems & leaves.	2.792	2.956
Roots.	0.846	9.944
Nodules.	4.963	5.335

Table II.
Influence of different Parts of Bean Plant on the Growth of
Nodule Bacteria.

Names of Parts.	Conc. (%)	Names of Nodule bacteria.									
		Genge A.		Genge B.		Genge C.		Bean.		Clover.	
		Growth on 4th day.	Wt. of cells on 7th day.	Growth on 4th day.	Wt. of cells on 7th day.	Growth on 4th day.	Wt. of cells on 7th day.	Growth on 4th day.	Wt. of cells on 7th day.	Growth on 4th day.	Wt. of cells on 7th day.
Control.	-	++	(mg.) 0.5	++	(mg.) 0.6	++	(mg.) 0.4	##	(mg.) 1.2	++	(mg.) 0.2
Yeasts.	10	##	2.1	##	2.4	##	1.1	###	4.9	##	1.5
Seeds.	Stock.	###	3.8	###	6.8	####	5.4	####	12.2	###	5.4
	50	###	4.6	##	4.4	###	4.6	###	8.5	##	3.1
	10	##	1.4	##	1.8	##	1.6	##	3.2	##	0.8
Stems & leaves.	Stock.	-	0.0	-	0.0	+	0.4	++	1.2	-	0.0
	50	##	1.2	##	5.0	###	5.8	###	8.7	+	1.9
	10	##	2.8	##	1.3	##	0.9	##	4.4	##	2.9
Roots.	Stock.	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0
	50	-	0.0	-	0.0	-	0.0	+	1.8	-	0.0
	10	##	1.4	##	1.1	##	1.3	##	2.6	##	1.5
Nodules.	Stock.	####	9.0	####	15.5	####	8.2	####	18.6	####	9.9
	50	####	5.7	###	7.0	####	6.3	####	14.1	####	8.3
	10	##	2.6	##	2.0	##	4.1	###	5.5	##	3.0

Notes: + indicates the rate of growth.

The above data indicate that the bean plants differ somewhat from the results reported with Genge and white clover in that the order of beneficial influence was as follows: nodules, seeds, stems and leaves, and roots where the seeds were relatively beneficial. This may be due to the composition of bean. On the other hand, the nodules were most effective as the other plants, and also no parallelism was noted between the nitrogen contents and the rate of beneficence.

3.) *Preparation of extracts:*

The following seven solvents were used: ethyl-ether, alcohol, acetone, chloroform, benzene, petroleum benzine and water.

After the nodules were separated from the host plants, washed in water, dried and powdered, the extracts were made with Soxhlet extraction apparatus except for water by using the solvents named above, and the duration of extraction varied by different solvent from 24 hours to a week. After the extracts were made, the distilled water was added and boiled until the odor of solvent completely disappeared, and filtered before used.

4.) *The culture medium used and the method of inoculation:*

Yeast mannit agar was used as the stock to which various nodule preparation was added in quantity equivalent to one gram of nodule per 100 cc of medium. The inoculation was made with a loopful of the bacterial suspension which is prepared from four days old culture kept at 28°C.

5.) *Method of examination:*

After the inoculation, incubated at 28°C and examined for the growth by naked eyes and morphologically examined microscopically staining with ZIEHL's carbol fuchsin, and the cellular weight was determined as done previously. Recently VITA³⁾, HARITANTIS⁴⁾ and GIRTSCHANOFF⁵⁾ reported in regard to the fixation of nitrogen by the leguminous plant and the presence of alkaloids. In this investigation, the extracts were examined for the presence of alkaloids by using the following fourteen reagents: mercuric chloride, platonic chloride, platinum-potassium cyanide, tannic acid, I:KI, KI: CdI, HgI: KI, picronic acid, picric acid, phosphotungstate, phosphomolybdate, Bromine water, WENZELL'S, LUCHINL'S reagent and the coloration, precipitation and turbidity were noted. The reaction of these reagents on the pure solvents, is given in Table III.

(See Table III on next page.)

Again the residues from the extraction were analysed for the nitrogen content by KJELDAHL method.

Results of Experiments.

The results obtained with the Genge extract and the residue on the growth

Table III.
Reaction of Alkaloids Reagents on the Solvent.

Names of reagents.	Ethyl ether.		Alcohol.		Acetone.		Chloroform.		Benzene.		Petroleum benzine.		Water.	
	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.
Mercuric chloride.	N	--	N	--	N	--	N	--	N	--	N	--	N	--
Platinic chloride.	Y	--	Y	--	Y	--	Y	--	Y	--	Y	--	Y	--
Platinum-potassium cyanide.	O	--	O	--	O	--	O	--	O	--	O	--	O	--
Tannic acid.	O	--	O	--	O	--	O	--	O	--	O	--	O	--
I: KI.	R.B	--	R.B	--	R.B	--	R.B	--	R.B	--	R.B	--	R.B	--
KI: CdI.	L.Y	--	L.Y	--	L.Y	--	N	--	N	--	N	--	N	--
HgI: KI.	L.Y	--	L.Y	--	L.Y	--	N	--	L.Y	--	L.Y	--	N	--
Picrolonic acid.	Y	--	Y	--	Y	--	Y	--	Y	--	Y	--	Y	--
Picric acid.	Y	--	Y	--	Y	--	Y	--	Y	--	Y	--	Y	--
Phosphotungstate	M.W	+	W	--	W	--	M.W	+	M.W	+	M.W	+	N	--
Phosphomolybdate.	M.W	++	M.W	++	M.W	++	M.W	+	M.W	+	M.W	+	M.W	+
Bromine water.	R	+	R	--	R	--	R.B	--	R.B	--	R	+	O	--
WENZEL'S.	Bl	--	Bl	--	Bl	--	Bl	--	Br	+	Br	--	O	--
LUCHINI'S.	P	--	P	--	Y.P	--	P	--	P.B	--	L.O	--	P	--

Notes: + indicates the degree of reaction; C—Color; T—Turbidity; P—Precipitate;

(N)—colorless; (M.W)—milky white; (L.Y)—light yellow; (Y)—yellow; (Y.P)—yellowish purple; (R.O)—red orange; (O)—orange; (G)—green; (L.G)—light green; (D.G)—dark green; (L.O)—light orange; (Bl)—blue; (Br)—brown; (P.B)—purplish brown; (R.B)—red brown; (P)—purple; (R.P)—red purple; (Y.B)—yellowish brown; (L.B)—light brown; (R)—red.

of nodule bacteria are given in Table IV, using ten percent yeast extract as the control, and also one percent Genge nodule powder was used.

(See Table IV on next page.)

Table IV indicates that the growth and the cellular weight are in parallel. The best growth was obtained in the nodule which was followed by yeasts, acetone and ethyl ether residue, and in general the growth in residues was better than that in the extracts. Among the extracts, the growth in the alcoholic extract was the best, weighing 4.3 mg but ranked ninth, on the whole, which was worse than the worst among the residues. The worst growth was obtained in the petroleum benzine.

Table IV.
Growth Test on Genge Nodule Bacteria, Strain A with Genge Nodules.

Solvents.	Condition of solvents.	Rate of growth by days.				Wt. of bacterial cells, 7 days old.	Order by the weight.
		2	4	7	Sum of +.		
Control.	—	+	+	+	3	(mg.) 0.2	14
Yeasts.	10%	++	###	####	13	9.8	2
Ethyl ether.	E.	—	##	##	6	2.8	10
	R.	+	####	####	13	9.6	4
Alcohol.	E.	+	##	##	8	4.3	9
	R.	+	##	###	10	6.2	8
Acetone.	E.	—	++	++	4	0.4	13
	R.	++	###	###	12	9.7	3
Chloroform.	E.	+	##	##	9	2.2	11
	R.	++	###	###	12	9.0	5
Benzene.	E.	—	++	++	4	0.4	13
	R.	++	###	###	12	8.5	6
Petroleum benzine.	E.	+	++	++	5	1.3	12
	R.	+	##	###	10	6.3	7
Nodule.	1%	++	####	####	14	10.1	1

Notes: +—rate of growth; E—extract; R—residue.

The same experiment was undertaken with strain B culture and the results are given in Table V.

Table V.
Growth Test on Genge Nodule Bacteria, Strain B. with Genge Nodules.

Solvents.	Condition of solvents.	Rate of growth by days.				Wt. of bacterial cells, 7 days old.	Order by the weight.
		2	4	7	Sum of +.		
Control.	—	+	+	+	3	(mg.) 0.4	14
Yeasts.	10%	++	##	###	11	8.0	7

Table V. (Continued).
Growth Test on Genge Nodule Bacteria, Strain B. with Genge Nodules.

Solvents.	Condition of solvents.	Rate of growth by days.				Sum of +.	Wt. of bacterial cells, 7 days old. (mg.)	Order by the weight.
		2	4	7				
Ethyl ether.	E.	—	++	++	4	0.5	13	
	R.	+	####	####	13	12.2	3	
Alcohol.	E.	+	++	##	6	2.1	10	
	R.	+	###	###	11	5.9	8	
Acetone.	E.	—	++	##	5	0.5	13	
	R.	+	###	####	12	11.2	4	
Chloroform.	E.	+	##	###	8	3.2	9	
	R.	++	##	###	10	9.5	5	
Benzene.	E.	—	##	##	6	1.5	11	
	R.	++	####	####	14	12.7	2	
Petroleum benzine.	E.	+	++	++	5	0.8	12	
	R.	+	##	##	9	8.1	6	
Nodule.	1%	+	####	####	13	13.6	1	

Notes: +—rate of growth, E—extract; R—residue.

The data given above are similar to those given in Table IV indicating that the residues were more effective than the extracts. In fact some of the extracts were worse than the control. The best growth was obtained in the nodules which was followed by the benzene residue.

Again Genge nodule bacteria, strain C was investigated in a similar manner as other strain, and the results are given in Table VI.

Table VI.
Growth Test on Genge Nodule Bacteria, Strain C. with Genge Nodules.

Solvents.	Condition of solvents.	Rate of growth by days.				Sum of +.	Wt. of bacterial cells, 7 days old. (mg.)	Order by the weight.
		2	4	7				
Control.	—	+	++	++	5	1.0	14	
Yeasts.	10%	+	###	###	11	7.2	7	

Table VI. (Continued.)

Growth Test on Genge Nodule Bacteria, Strain C. with Genge Nodules.

Solvents.	Condition of solvents.	Rate of growth by days.				Wt. of bacterial cells, 7 days old.	Order by the weight.
		2	4	7	Sum of +.		
Ethyl ether.	E.	—	‡	‡	6	(mg.) 3.8	12
	R.	+	‡‡‡	‡‡‡	11	8.0	5
Alcohol.	E.	+	‡	‡‡‡	8	5.2	8
	R.	+	‡‡‡	‡‡‡	11	9.1	3
Acetone.	E.	—	‡‡	‡	5	2.9	11
	R.	+	‡‡‡	‡‡‡	11	9.7	2
Chloroform.	E.	+	‡‡‡	‡‡‡	9	4.7	9
	R.	+	‡‡‡	‡‡‡	11	7.6	6
Benzene.	E.	—	‡‡‡	‡‡‡	8	4.1	10
	R.	+	‡‡‡	‡‡‡	11	8.4	4
Petroleum benzine.	E.	+	+	‡‡	4	2.1	13
	R.	+	‡‡‡	‡‡‡	9	9.1	3
Nodule.	1%	+	‡‡‡‡	‡‡‡‡	13	9.9	1

Notes: +—rate of growth; E—extract; R—residue.

As shown above, the results, as a whole, were similar to those of the preceding experiments although the extracts in this case were slightly better than those in the previous cases. The growth was best in the nodule followed by the acetone residue where the cellular weight was 9.7 mg. In the extracts 5.2 mg. was the highest cellular weight, followed by chloroform and benzene in the order.

The results, obtained with the bean culture are given Table VII.

Table VII.

Growth Test on Bean Nodule Bacteria.

Solvents.	Condition of solvents.	Rate of growth by days.				Wt. of bacterial cells, 7 days old.	Order by the weight.
		2	4	7	Sum of +.		
Control.	—	+	+	+	3	(mg.) 1.0	14
Yeasts.	10%	‡‡	‡‡‡	‡‡‡	10	8.5	4

Table VII. (Continued.)
Growth Test on Bean Nodule Bacteria.

Solvents.	Condition of solvents.	Rate of growth by days.				Wt. of bacterial cells, 7 days old.	Order by the weight.
		2	4	7	Sum of +.		
Ethyl ether.	E.	—	++	###	5	(mg.) 1.2	12
	R.	++	####	####	14	9.6	2
Alcohol.	E.	+	++	###	6	1.8	10
	R.	+	####	####	13	7.2	6
Acetone.	E.	—	+	++	3	1.1	13
	R.	++	###	###	10	5.8	7
Chloroform.	E.	++	###	###	8	2.5	9
	R.	+	###	###	11	4.7	8
Benzene.	E.	+	###	###	7	1.4	11
	R.	++	###	###	12	8.9	3
Petroleum benzine.	E.	+	++	++	5	1.1	13
	R.	++	###	###	10	7.3	5
Nodule.	1%	++	####	####	14	10.1	1

Notes: +—rate of growth; E—extract; R—residue.

The results in this case were also similar to those obtained in the previous cases. The nodule was the best followed by ethyl-ether. Among the extracts, the chloroform was best followed by that of alcohol.

Table VIII gives the results obtained with the clover culture as follows :

Table VIII.
Growth Test on Clover Nodule Bacteria.

Solvents.	Condition of solvents.	Rate of growth by days.				Wt. of bacterial cells, 7 days old.	Order by the weight.
		2	4	7	Sum of +.		
Control.	—	+	+	+	3	(mg.) 0.2	15
Yeasts.	10%	###	###	###	12	11.0	8
Ethyl ether.	E.	++	###	###	8	1.8	12
	R.	###	####	####	15	16.0	1

Table VIII. (Continued.)
Growth Test on Clover Nodule Bacteria.

Solvents.	Condition of solvents.	Rate of growth by days.				Sum of +.	Wt. of bacterial cells, 7 days old.	Order by the weight.
		2	4	7				
Alcohol.	E.	+	##	###	8	2.9	9	
	R.	##	###	###	13	14.2	6	
Acetone.	E.	++	++	++	6	1.9	11	
	R.	##	###	###	13	14.6	5	
Chloroform.	E.	++	##	##	8	2.4	10	
	R.	##	###	####	14	15.2	3	
Benzene.	E.	+	+	+	3	0.4	14	
	R.	++	###	###	12	12.8	7	
Petroleum benzine.	E.	+	++	++	5	1.2	13	
	R.	++	###	###	12	14.7	4	
Nodule.	1%	##	####	####	15	15.8	2	

Notes: +—rate of growth; E—extract. R—residue.

As Table VIII indicates, the growth in the extracts was much worse than that in the residue. The ether residue was best and the cells weighed 16.0 mg. The alcohol, chloroform and ethyl-ether gave comparatively good growth, and the cellular weight in alcohol was 2.9 mg.

As the foregoing experiments with five strains of nodule bacteria indicate, the influence of various extracts on the growth was not marked although the alcoholic and chloroform extracts were better than the rest of extracts while the residues of these solvents were worse than the others. The results, as a whole, indicate that a large portion of so-called accessory substance in the nodules, is retained in the residue.

Next the alkaloids in the extracts were tested with various reagents, and obtained the results given in Table IX.

(See Table IX on next page.)

Table IX indicates that the presence of alkaloids was noted only in acetone and alcohol extracts and none in others.

Determination of nitrogen in the residue of different solvent:

The nitrogen contents in different residue was determined as shown in Table X.

Table IX.
Alkaloid Test applied to the Extract of Genge Nodules.

Names of reagents.	Ethyl ether.			Alcohol.			Acetone.			Chloroform.			Benzene.			Petroleum benzine.		
	C.	T.	P.	C.	T.	P.	C.	T.	P.	C.	T.	P.	C.	T.	P.	C.	T.	P.
Mercuric chloride.	M.W	+	-	M.W	+	+	M.W	##	##	M.W	+	+	N	-	-	M.W	+	-
Platinic chloride.	L.Y	-	-	Y	+	-	Y	##	##	M.W	+	+	L.Y	-	-	L.Y	-	-
Platinum-potassium cyanide.	M.W	-	-	O	-	-	Y	+	-	M.W	+	-	O	-	-	O	-	-
Tannic acid.	O	+	-	O	-	-	O	-	-	O	+	-	O	-	-	O	-	-
I: KI.	Y.B	-	-	R.B	-	-	R.B	-	-	R.B	##	+	R.B	-	-	R.B	-	-
KI: CdI.	M.W	+	-	M.W	##	+	M.W	##	+	M.W	+	-	N	-	-	N	-	-
HgI: KI.	L.Y	-	-	L.Y	##	-	L.Y	##	##	M.W	+	-	L.Y	-	-	L.Y	-	-
Picolonic acid.	Y	-	-	Y	-	-	Y	+	+	Y	##	-	M.W	+	-	Y	-	-
Picric acid.	Y	-	-	Y	-	-	Y	-	-	Y	-	-	Y	-	+	Y	-	-
Phosphotungstate.	M.W	+	-	M.W	+	+	M.W	##	##	M.W	##	-	M.W	-	-	M.W	+	-
Phosphomolybdate.	M.W	##	-	M.W	##	##	L.Y	##	+	M.W	+	-	M.W	-	-	M.W	+	-
Bromine water.	R.O	-	-	R.O	-	-	R.O	-	-	R.O	-	-	R	-	-	O	##	+
WENZELL'S	D.G	+	+	Bl	-	##	G	-	##	L.G	-	##	Br	-	-	O	-	-
LUCHINI'S	O	-	-	Y.P	-	-	Y.P	-	-	Br	-	-	P.B	-	-	P	-	-

Notes: † indicates the degree of reaction; C—Color; T—Turbidity; P—Precipitate.

(N)—colorless; (M.W)—milky white; (L.Y)—light yellow; (Y)—yellow; (Y.P)—yellowish purple; (R.O)—red orange; (O)—orange; (G)—green; (L.G)—light green; (D.G)—dark green; (L.O)—light orange; (Bl)—blue; (Br)—brown; (P.B)—purplish brown; (R.B)—red brown; (P)—purple; (R.P)—red purple; (Y.B)—yellowish brown; (L.R)—light brown; (R)—red.

Table X.
Nitrogen Contents in the Residue of Genge Nodules.

	Nodules.	Solvents used.					
		Ethyl ether.	Alcohol.	Acetone.	Chloroform.	Benzene.	Petroleum benzine.
Nitrogen contents (%)	7.125	6.826	7.014	6.544	7.108	6.980	6.873
Nitrogen extracted (%)	—	0.299	0.111	0.581	0.017	0.145	0.252
Percent of extraction.†	—	4.196	1.558	8.154	0.239	2.035	3.537

Note: † is calculated by dividing the nitrogen extracted by the total nitrogen.

As the data in Table X indicate, the nitrogen content was highest in the chloroform residue and lowest in acetone. That is acetone extracted the largest amount of nitrogen which amounted to 8.154 percent while alcohol extracted only 1.558 percent of nitrogen.

The results with Genge nodules as a whole indicated that the accessory substance was not extracted to any amount by the solvents employed except little by acetone and alcohol. The alcohol extract influenced the growth best of all extracts tried although it contained a small quantity of nitrogen. This fact seems to indicate that the accessory factor or substance may not be related to nitrogenous compound such as the alkaloid.

Further experiment was carried out on the bean nodule in the same manner as Genge with an addition of water extract and microscopical examination for the change of cell morphology. The results are shown in Table XI.

Table XI.
Growth Test on Genge Nodule Bacteria, Strain A with Bean Nodules.

Solvents.	Condition of solvents.	Rate of growth by days.				7 days old culture.	
		2	4	7	Sum of +.	Forms.	Size. (μ)
Control.	—	+	+	+	3	Rod.	0.3 × 0.8—0.4 × 1.2
Yeasts.	10%	###	###	###	12	„	0.2 × 0.6—0.3 × 1.0
Ethyl ether.	E.	+	+	+	3	Rod.	0.3 × 0.5—0.5 × 1.5
	R.	++	###	###	12	„	0.3 × 0.8—0.5 × 2.2
Alcohol.	E.	+	##	##	8	Rod.	0.3 × 0.8—0.4 × 1.2
	R.	+	##	###	10	„	0.3 × 0.8—0.4 × 2.0
Acetone.	E.	+	++	++	5	Short rod.	0.2 × 0.8—0.3 × 1.0
	R.	##	##	###	12	Rod.	0.2 × 0.6—0.6 × 1.5
Chloroform.	E.	++	##	##	8	Short rod.	0.3 × 0.5—0.4 × 1.0
	R.	##	##	###	12	Rod.	0.3 × 0.5—0.5 × 1.2
Benzene.	E.	++	++	++	6	Short rod.	0.2 × 0.6—0.4 × 1.0
	R.	##	##	###	12	„	0.2 × 0.5—0.4 × 1.0
Petroleum benzine.	E.	++	++	++	6	Rod.	0.3 × 0.5—0.5 × 1.2
	R.	##	##	###	12	„	0.3 × 0.5—0.6 × 1.7
Water.	E.	++	##	##	10	„	0.2 × 0.5—0.5 × 2.0
	R.	++	##	##	8	„	0.3 × 0.5—0.5 × 1.2
Nodule.	1%	##	###	###	13	Short rod.	0.3 × 0.5—0.5 × 1.0

Notes: +—rate of growth; E—extract; R—residue.

As Table XI indicates, the best growth was obtained in the nodule and the growth in the residue was better than that in the extract although the water extract was better than the residue. Among the extracts, the good growth was

obtained in the water, alcohol and chloroform extracts while the ether and acetone extracts were not effective. The bad growth was obtained in the water and alcohol residue and the others were about the same. In a majority of cases, the cells were short rod or rod shape, and they were somewhat larger in alcohol and ether residue and some bacteroids were found.

The similar experiment as the previous case was undertaken with Genge nodule bacteria, strain B and the results are shown in Table XII.

Table XII.
Growth Test on Genge Nodule Bacteria, Strain B with Bean Nodules.

Solvents.	Condition of solvents.	Rate of growth by days.				7 days old culture.	
		2	4	7	Sum of +.	Forms.	Size. (μ)
Control.	—	+	+	+	3	Rod.	$0.2 \times 0.6 - 0.3 \times 1.2$
Yeasts.	10%	++	###	###	12	Short rod.	$0.2 \times 0.6 - 0.3 \times 1.0$
Ethyl ether.	E.	—	+	+	2	Rod.	$0.3 \times 0.6 - 0.5 \times 1.4$
	R.	++	###	###	12	„	$0.3 \times 0.5 - 0.4 \times 1.2$
Alcohol.	E.	—	##	###	8	Short rod & coccic.	$0.3 \times 0.4 - 0.5 \times 1.0$
	R.	##	##	##	11	Short rod.	$0.2 \times 0.6 - 0.4 \times 0.8$
Acetone.	E.	+	++	++	5	Rod.	$0.3 \times 0.6 - 0.4 \times 1.2$
	R.	##	###	###	13	Short rod.	$0.2 \times 0.6 - 0.3 \times 1.0$
Chloroform.	E.	++	##	##	8	Short rod.	$0.3 \times 0.5 - 0.4 \times 1.0$
	R.	##	##	###	12	Rod.	$0.2 \times 0.5 - 0.3 \times 1.2$
Benzene.	E.	+	++	##	6	Short rod.	$0.2 \times 0.5 - 0.4 \times 1.0$
	R.	##	###	###	13	Rod.	$0.2 \times 0.5 - 0.4 \times 1.2$
Petroleum benzine.	E.	+	++	++	5	Short rod.	$0.2 \times 0.4 - 0.3 \times 0.8$
	R.	++	###	###	12	Rod.	$0.3 \times 0.6 - 0.6 \times 1.8$
Water.	E.	—	##	###	9	Rod.	$0.3 \times 0.5 - 0.8 \times 1.8$
	R.	+	##	##	7	Short rod.	$0.3 \times 0.5 - 0.5 \times 1.0$
Nodule.	1%	##	###	###	13	Rod.	$0.2 \times 0.4 - 0.4 \times 1.2$

Notes: +—rate of growth; E—extract; R—residue.

As Table XII indicates, the results obtained in this experiment agree with those of the previous test. The large cells were found in the petroleum benzine and benzene residue and water extract. The presence of bacteroids was noted also.

The similar experiment with Genge strain C was carried out and the results are given in Table XIII.

Table XIII.
Growth Test on Genge Nodule Bacteria, Strain C with Bean Nodules.

Solvents.	Condition of solvents.	Rate of growth by days.				7 days old culture.	
		2	4	7	Sum of +.	Forms.	Size. (μ)
Control.	—	+	+	+	3	Short rod.	$0.3 \times 0.5 - 0.4 \times 1.0$
Yeasts.	10%	++	###	###	12	„	$0.2 \times 0.6 - 0.3 \times 1.0$
Ethyl ether.	E.	—	+	+	2	Rod.	$0.3 \times 0.5 - 0.6 \times 1.5$
	R.	++	###	###	12	„	$0.3 \times 0.6 - 0.8 \times 1.8$
Alcohol.	E.	+	##	##	8	Short rod & coccic.	$0.3 \times 0.6 - 0.5 \times 1.0$
	R.	##	##	##	11	„ „	$0.2 \times 0.5 - 0.5 \times 1.0$
Acetone.	E.	+	++	++	5	Rod.	$0.3 \times 0.5 - 0.5 \times 1.2$
	R.	##	###	###	13	Short rod & coccic.	$0.2 \times 0.5 - 0.6 \times 0.8$
Chloroform.	E.	+	##	##	7	Short rod.	$0. \times 0.5 - 0.4 \times 1.0$
	R.	##	##	###	12	Rod.	$0.3 \times 0.5 - 0.5 \times 2.0$
Benzene.	E.	++	++	++	6	„	$0.2 \times 0.6 - 0.4 \times 1.2$
	R.	##	###	###	13	„	$0.4 \times 0.8 - 0.6 \times 1.5$
Petroleum benzine.	E.	+	++	++	5	Short rod.	$0.3 \times 0.6 - 0.4 \times 0.8$
	R.	++	###	###	12	Rod & coccic.	$0.3 \times 0.4 - 0.6 \times 1.5$
Water.	E.	—	##	###	9	Rod.	$0.3 \times 0.7 - 0.6 \times 2.0$
	R.	+	##	##	7	Short rod & coccic.	$0.2 \times 0.4 - 0.4 \times 0.7$
Nodule.	1%	##	###	###	13	„ „	$0.3 \times 0.3 - 0.4 \times 0.7$

Notes: +—rate of growth; E—extract; R—residue.

The above data indicate the same tendency as observed in the previous experiments, but more of the coccic forms were found and many bacteroids were

noted in the ether and chloroform residue as well as in the water extract. It is noteworthy that all three strains of Genge nodule bacteria presented the bacteroid form when the water extract was added.

The results obtained with the bean nodule bacteria are given in Table XIV.

Table XIV.
Growth Test on Bean Nodule Bacteria with Bean Nodules.

Solvents.	Condition of solvents.	Rate of growth by days.				7 days old culture.	
		2	4	7	Sum of +.	Forms.	Size. (μ)
Control.	—	+	+	+	3	Short rod.	$0.3 \times 0.5 - 0.5 \times 1.0$
Yeasts.	10%	≡≡	≡≡≡	≡≡≡	13	Rod.	$0.2 \times 0.5 - 0.4 \times 1.5$
Ethyl ether.	E.	—	+	+	2	Rod.	$0.3 \times 0.6 - 0.5 \times 1.5$
	R.	≡≡	≡≡	≡≡≡	11	„	$0.3 \times 0.5 - 0.5 \times 1.5$
Alcohol.	E.	+	≡≡	≡≡≡	9	„	$0.3 \times 0.7 - 0.6 \times 1.2$
	R.	≡≡	≡≡	≡≡≡	12	„	$0.2 \times 0.8 - 1.1 \times 2.5$
Acetone.	E.	+	≡≡	≡≡	5	Short rod.	$0.3 \times 0.6 - 0.4 \times 0.8$
	R.	≡≡	≡≡≡	≡≡≡	13	Rod.	$0.5 \times 0.8 - 0.8 \times 1.8$
Chloroform.	E.	+	≡≡	≡≡	7	Rod.	$0.3 \times 0.7 - 0.5 \times 1.5$
	R.	≡≡	≡≡	≡≡≡	12	„	$0.2 \times 0.5 - 0.6 \times 1.5$
Benzene.	E.	+	≡≡	≡≡	5	„	$0.3 \times 0.6 - 0.7 \times 1.2$
	R.	≡≡	≡≡≡	≡≡≡	13	„	$0.3 \times 0.7 - 0.6 \times 2.0$
Petroleum benzin.	E.	≡≡	≡≡	≡≡	6	„	$0.3 \times 0.7 - 0.7 \times 1.5$
	R.	≡≡	≡≡≡	≡≡≡	12	„	$0.3 \times 0.5 - 0.5 \times 1.5$
Water.	E.	+	≡≡	≡≡≡	10	„	$0.3 \times 0.7 - 0.7 \times 1.5$
	R.	≡≡	≡≡	≡≡	8	„	$0.3 \times 0.7 - 0.6 \times 1.5$
Nodule.	1%	≡≡	≡≡≡	≡≡≡	13	„	$0.3 \times 0.6 - 0.5 \times 1.5$

Notes: +—rate of growth; E—extract; R—residue.

The above data indicate that the results on the whole were similar to those obtained in case of Genge, and among the extracts, water, alcohol and chloroform gave good growth especially the better growth was obtained with the water extract than that in the residue. A majority of cells were rod and a few bacteroids

were found in the alcoholic residue.

The clover nodule bacteria were investigated and obtained the following results as shown in Table XV.

Table XV.
Growth Test on Clover Nodule Bacteria with Bean Nodules.

Solvents.	Condition of solvents.	Rate of growth by days.				7 days old culture.	
		2	4	7	Sum of +.	Forms.	Size. (μ)
Control.	—	+	+	++	4	Rod.	0.3×0.8—0.8×1.5
Yeasts.	10%	+++	++++	++++	13	„	0.5×1.0—0.7×2.0
Ethyl ether.	E.	+	++	++	5	„	0.3×0.7—0.7×1.2
	R.	+++	++++	++++	13	„	0.3×0.6—0.6×1.3
Alcohol.	E.	++	+++	+++	8	„	0.3×0.6—0.6×1.5
	R.	+++	++++	++++	15	„	0.5×1.2—1.0×2.0
Acetone.	E.	++	+++	+++	8	„	0.3×1.0—0.7×2.0
	R.	+++	++++	++++	15	„	0.5×1.0—1.0×1.5
Chloroform.	E.	++	+++	+++	8	„	0.3×0.6—0.6×1.5
	R.	+++	++++	++++	15	„	0.5×1.2—1.0×2.0
Benzene.	E.	+	++	++	5	„	0.3×0.6—0.5×2.0
	R.	+++	++++	++++	15	„	0.5×0.8—1.0×2.5
Petroleum benzine.	E.	+	++	++	5	„	0.5×1.0—0.8×1.5
	R.	+++	++++	++++	15	„	0.5×0.7—1.0×1.2
Water.	E.	++	+++	+++	11	„	0.3×0.8—0.6×1.5
	R.	++	+++	+++	8	„	0.5×0.6—1.0×2.5
Nodule.	1%	+++	++++	++++	16	„	0.4×0.5—0.8×1.8

Notes: +—rate of growth; E—extract; R—residue.

As shown above, the good growth was obtained in the water and alcohol while some other extracts were less effective than the control. Almost all the cells were rod and the bacteroids were observed in benzene, chloroform and water residue.

From the foregoing results it was found that the best solvent to extract the most effective constituent from the bean nodule was warm water, followed by alcohol and chloroform while the others were not effective. However the warm water did not extract the accessory substance completely from the nodule since the residue showed better influence than the control. As to the formation of bacteroids, no definite relation to the kind of extract was found.

The results of examination for the presence of alkaloids in the extracts are given in Table XVI.

Table XVI.
Alkaloid Test applied to the Extract of Bean Nodules.

Names of reagents.	Ethyl ether.		Alcohol.		Acetone.		Chloroform.		Benzene.		Petroleum benzene.		Water.	
	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.
Mercuric chloride.	N	--	W	+-	N	--	N	--	N	--	W	+-	L.Y	+++
Platinic chloride.	Y	--	Y	+-	Y	--	Y	--	Y	--	Y	--	Y	+++
Platinum-potassium cyanide.	O	--	O	++	O	--	O	--	O	--	O	--	O	##-
Tannic acid.	O	--	O	+-	O	--	O	--	O	--	O	+-	L.Y	+++
I : KI.	Br	--	Br	+-	Br	--	Br	--	Br	--	Br	--	Br	++-
KI : CdI.	Br	--	W	+-	N	--	N	--	N	--	N	--	L.Y	--
HgI : KI.	O	--	W	+-	N	--	N	--	N	--	W	+-	L.Y	++-
Picrolonic acid.	Y	+-	Y	+-	Y	--	Y	--	Y	--	Y	--	Y	+++
Picric acid.	Y	+-	Y	++	Y	--	Y	--	Y	--	Y	+-	Y	##-
Phosphotungstate.	W	+-	W	++	W	+-	W	+-	N	--	W	+-	W	+++
Phosphomolybdate.	W	+-	W	++	W	+-	W	+-	M.W	--	W	+-	W	+++
Bromine water.	Br	--	Br	++	Br	+-	Br	--	Br	--	L.B	+-	O	+++
WENZEL'S.	G	--	G	--	O	--	O	--	O	--	O	--	D.G	++-
LUCHINI'S.	N	--	Y.B	+-	N	--	P	--	P	--	N	--	L.Y	+-

Notes: + indicates the degree of reaction; C—Color; T—Turbidity; P—Precipitate;

(N)—colorless; (M.W)—milky white; (L.Y)—light yellow; (Y)—yellow; (Y.P)—yellowish purple; (R.O)—red orange; (O)—orange; (G)—green; (L.G)—light green; (D.G)—dark green; (L.O)—light orange; (Bl)—blue; (Br)—brown; (P.B)—purplish brown; (R.B)—red brown; (P)—purple; (R.P)—red purple; (Y.B)—yellowish brown; (L.B)—light brown; (R)—red.

Table XVI indicates that the alkaloid was present only in the water extract and doubtful in the alcoholic extract.

Next the nitrogen content in various residues from the bean nodules was determined and the results are shown in Table XVII.

Table XVII.
Nitrogen Contents in the Residue of Bean Nodules.

	Nodules.	Solvents used.						
		Ethy ether.	Alcohol.	Acetone.	Chloroform.	Benzene.	Petroleum benzine.	Water.
Nitrogen contents. (%)	5.335	4.949	5.264	4.724	5.170	5.264	5.076	4.385
Nitrogen extracted. (%)	—	0.386	0.071	0.611	0.165	0.071	0.259	0.950
Percent of extraction.†	—	7.235	0.133	11.453	3.093	0.133	4.855	17.807

Note: † is calculated by dividing the nitrogen extracted by the total nitrogen.

As Table XVII indicates, a large amount of nitrogen was found in the alcohol and benzene residue and least in water. The water extracted 17.80 and acetone, 11.45 percent of nitrogen, and the other solvents extracted only a small amount.

The foregoing results obtained with the bean nodule bacteria seem to indicate that the water is the best solvent for the extraction of accessory substance. Alcohol and chloroform were fairly effective while all the others were ineffective. The presence of alkaloids was detected markedly only in the water and a trace in the alcoholic extract. Although the acetone extract contained a large amount of nitrogen, no alkaloid was found. Considering these facts, the alkaloids and nitrogenous compounds have no important connection with the accessory substance.

Extraction of accessory substance by using the different solvents successively:

Thus far the extraction was made by using a single solvent at a time, and further experiments are carried out by using various solvents successively to see if a large portion of the accessory substance could be extracted. The solvents used and the duration for extraction, are given as follows:

Names of solvents.	Days of extraction.	Names of solvents.	Days of extraction.
Ethyl ether.	3	Benzene.	2.5
Alcohol.	7	Petroleum Benzine.	2.0
Acetone.	3	Water.	30 min. after boiling.
Chloroform.	3		

Ten grams of bean nodules were taken and the extraction was made successively one after the other by using the solvents named and also by the order indicated above, and after each extraction, one gram of residue was taken and added to the medium in which the growth test was carried out, and the results are given in Tables XVIII, XIX, XX, XXI and XXII.

Table XVIII.
Influence of Fractional Extraction on Genge Nodule Bacteria,
Strain A.

Order of extraction.	1. Rate of growth by days.				2. Rate of growth by days.			
	2	4	7	Sum of +.	2	4	7	Sum of +.
Control.	+	+	+	3	+	+	+	3
Yeasts.	+	###	###	9	+	###	###	9
Nodules (1%).	+	###	####	11	+	###	####	10
Ethyl ether.	+	###	###	10	+	###	###	10
Alcohol.	+	##	##	7	+	##	###	8
Acetone.	+	++	##	6	+	##	##	7
Chloroform.	+	++	##	6	+	##	##	7
Benzene.	+	++	##	6	+	##	##	7
Petroleum benzine.	+	++	##	6	+	##	##	7
Water.	+	++	++	5	+	++	++	5

Note: +—indicates the degree of growth.

Table XIX.
Influence of Fractional Extraction on Genge Nodule Bacteria,
Strain B.

Order of extraction.	1. Rate of growth by days.				2. Rate of growth by days.			
	2	4	7	Sum of +.	2	4	7	Sum of +.
Control.	+	+	+	3	+	+	+	3
Yeasts.	+	##	###	8	+	##	###	8
Nodules (1%).	+	###	####	11	+	###	####	11
Ethyl ether.	+	###	###	11	+	###	####	12
Alcohol.	++	##	##	8	+	##	###	8
Acetone.	+	##	##	7	+	++	###	7
Chloroform.	+	++	##	6	+	##	##	7
Benzene.	+	##	##	7	+	##	##	7
Petroleum benzine.	+	##	##	7	+	##	##	7
Water.	+	++	++	5	+	++	++	5

Note: +—indicates the degree of growth.

Table XX.
Influence of Fractional Extraction on Genge Nodule Bacteria,
Strain C.

Order of extraction.	1. Rate of growth by days.				2. Rate of growth by days.			
	2	4	7	Sum of +.	2	4	7	Sum of +.
Control.	+	+	+	3	+	+	+	3
Yeasts.	+	###	###	9	+	###	###	9
Nodules (1%).	+	##	####	10	+	###	###	10
Ethyl ether.	+	##	###	9	+	###	###	10
Alcohol.	+	++	##	6	+	++	##	6
Acetone.	+	++	##	6	+	++	##	6
Chloroform.	+	++	++	5	+	++	##	6
Benzene.	-	++	##	5	+	++	##	6
Petroleum benzine.	+	++	++	5	+	++	##	6
Water.	-	+	+	2	+	+	+	3

Note: +—indicates the degree of growth.

Table XXI.
Influence of Fractional Extraction on Bean Nodule Bacteria.

Order of extraction.	1. Rate of growth by days.				2. Rate of growth by days.			
	2	4	7	Sum of +.	2	4	7	Sum of +.
Control.	+	++	++	5	+	++	++	5
Yeasts.	+	###	###	10	+	###	###	10
Nodules (1%).	+	###	####	11	+	###	###	10
Ethyl ether.	+	###	###	10	+	###	###	10
Alcohol.	+	++	##	6	+	++	##	6
Acetone.	+	++	##	6	+	++	##	6
Chloroform.	+	++	##	6	+	++	##	6
Benzene.	+	++	##	6	+	++	##	6
Petroleum benzine.	+	++	##	6	+	++	##	6
Water.	+	++	++	5	+	++	++	5

Note: +—indicates the degree of growth.

Table XXII.
Influence of Fractional Extraction on Clover Nodule Bacteria.

Order of extraction.	1. Rate of growth by days.				2. Rate of growth by days.			
	2	4	7	Sum of +.	2	4	7	Sum of +.
Control.	+	++	++	5	+	++	++	5
Yeasts.	++	###	###	11	++	###	###	11
Nodules (1%).	++	###	####	13	++	###	####	13
Ethyl ether.	++	###	####	13	++	###	####	13
Alcohol.	++	##	###	9	+	##	###	8
Acetone.	++	##	###	9	+	##	###	8
Chloroform.	+	##	##	7	+	++	###	7
Benzene.	+	++	###	7	+	++	###	7
Petroleum benzene.	++	++	##	7	+	++	###	7
Water.	+	++	++	5	+	++	##	6

Note: +—indicates the degree of growth.

As the foregoing five tables indicate that the results obtained with five different nodule bacteria were about the same indicating the residue from the ethyl ether retained a large portion of so-called accessory substance giving equally good growth as the nodule added directly. The residue from alcohol gave poor growth although it was better than the control. Acetone, chloroform, benzene and petroleum benzene showed very slight difference while the water extracted a large portion of accessory substance so that the poor growth was obtained on an addition of the residue. By the process of extraction applied, about 80—90 percent of accessory substance was extracted, especially alcohol and water were very effective.

The results of tests for the presence of alkaloids in the various extracts are given in Table XXIII.

(See Table XXIII on next page.)

As the above results indicate that the presence of alkaloid was noted in the water extract markedly and a trace in the alcohol extract, and none in the others which agree with those results obtained in an individual extraction. From the results, it seems that the alkaloids may have some accessory influence on the growth of nodule bacteria.

Nitrogen content in the residue :

The nitrogen content in the residue was determined and the results are given in Table XXIV.

Table XXIII.
Alkaloid Test applied to each Extract.

Names of reagents.	Ethyl ether.		Alcohol.		Acetone.		Chloroform.		Benzene.		Petroleum benzine.		Water.	
	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.	C.	T.P.
Mercuric chloride.	N	--	L.Y	--	N	--	N	--	N	--	N	--	M.W	##
Platinic chloride.	Y	--	Y	##	Y	--	Y	--	Y	--	Y	--	Y	##
Platinum-potassium cyanide.	O	--	Y	##	O	--	O	--	O	--	O	--	O	##
Tannic acid.	O	--	Y	+	O	--	O	--	O	--	Y	--	O	##
I:KI.	Y.B	--	R.B	+	R.B	--	R.B	--	R.P	--	R.P	--	R.B	--
KI:CdI.	Y	--	Y	+	Y	--	N	--	N	--	L.R	--	L.R	--
HgI:KI.	L.Y	--	L.Y	##	L.Y	--	N	--	N	--	L.Y	--	N	--
Picrolic acid.	Y	--	Y	+	Y	--	Y	##	Y	--	Y	--	Y	###
Picric acid.	Y	--	Y	+	Y	--	Y	--	Y	--	Y	--	Y	##+
Phosphotungstate.	M.W	+	M.W	+	N	--	N	--	N	--	N	--	M.W	##+
Phosphomolybdate.	L.Y	--	L.Y	##	W	--	M.W	+	N	--	N	--	M.W	###
Bromine water.	O	--	O	--	R.O	--	R.B	--	R.O	##	R.B	--	O	##
WENZEL'S.	G	--	G	--	R	--	R	--	O.B	--	R.B	--	O.B	--
LUCHINI'S	Br	##	R.B	--	P	--	P	--	P	--	O.P	--	P	--

Notes: +—indicates the degree of reaction; C—Color; T—Turbidity;

P—Precipitate;

(N)—colorless; (M.W)—milky white; (L.Y)—light yellow; (Y)—yellow
(Y.P)—yellowish purple; (R.O)—red orange; (G)—green; (O)—orange;
(L.G)—light green; (D.G)—dark green; (L.O)—light orange; (Bl)—Blue;
(Br)—brown; (P.B)—purplish brown; (R.B)—red brown; (P)—purple;
(R.P)—red purple; (L.R)—light red; (O.B)—orange brown;
(Y.B)—yellowish brown; (L.B)—light brown; (R)—red.

Table XXIV.
Nitrogen Contents in the Residue of each Extract.

	Nodules.	Solvents used.						
		Ethyl ether.	Alcohol.	Acetone.	Chloroform.	Benzene.	Petroleum benzine.	Water.
Nitrogen contents. (%)	5.335	5.170	5.107	4.654	4.586	4.502	4.374	4.128
Nitrogen extracted. (%)	—	0.165	0.228	0.681	0.749	0.833	0.961	1.207
Percent of extraction.†	—	2.093	4.274	12.765	14.040	15.614	18.013	22.624
Percent nitrogen extracted by each solvent.††	—	0.165	0.063	0.453	0.068	0.084	0.128	0.246

Notes: † is calculated by dividing the nitrogen extracted by the total nitrogen.

†† Shows the nitrogen extracted by a single solvent alone.

As Table XXIV indicates, the nitrogen content decreased as the extraction progressed and finally 23 percent of nitrogen was extracted which is 5 percent larger than the previous case. Acetone extract contained a large amount of nitrogen as before while alcohol extracted a very small amount of nitrogen as in the previous case.

Since alcohol and water have been proven to be very effective in extracting the accessory substance, the fractional extraction was tried by using only two of these solvents, and the results are noted in Table XXV.

Table XXV.
Influence of Alcohol and Water in Extraction of Accessory Substance from the Nodules.

	Genge A.		Genge B.		Genge C.		Bean.		Clover.	
	Growth on 4th day.	Wt. of cells on 7th day.	Growth on 4th day.	Wt. of cells on 7th day.	Growth on 4th day.	Wt. of cells on 7th day.	Growth on 4th day.	Wt. of cells on 7th day.	Growth on 4th day.	Wt. of cells on 7th day.
Control.	++	(mg.) 2.3	+	(mg.) 1.1	++	(mg.) 3.4	++	(mg.) 5.1	+	(mg.) 2.9
Yeasts.	###	10.7	###	9.3	###	12.5	###	11.6	###	13.7
Nodule (1%)	###	15.1	###	17.4	###	14.4	###	16.1	###	17.7
Residue from alcohol.	###	8.1	###	11.1	###	10.2	###	12.7	###	11.9
Residue from alcohol and water.	+	2.4	++	2.1	++	4.1	++	5.8	++	4.7

Note: +—indicates the degree of growth.

As Table XXV indicates, the residue from the alcoholic extract contains a fairly large quantity of the accessory substance, but by extracting with water, practically all the accessory substance was extracted to an extent of 80—90 percent. The efficiency of alcohol as a solvent for such substance has been demonstrated by ALLISON⁶⁾ in extracting co-enzyme R from cane sugar, honey, egg-albumin and yeasts, and also by OTANI⁷⁾ in connection with his investigation with accessory substance.

Summary and Conclusions.

This investigation was undertaken to ascertain as to an agent in the nodule which stimulates the growth of nodule bacteria by extracting the constituents from the nodule by means of different solvents. The nodule bacteria used in this investigation were three strains of Genge, one of bean and clover. The nodules from Genge and bean were used. The tests for the presence of alkaloids in the extract and the determination of nitrogen content in the residue were made. The results are summarized as follows:

1.) The accessory substance in the nodules of leguminous plants is best extracted by water, followed by alcohol and chloroform in the order while ethyl-ether, acetone, benzene and petroleum benzine were not effective.

2.) The extraction by a single solvent except water did not remove the accessory substance sufficiently so that an addition of the residue always gave better growth than that in the solution. Even in case of water, the residue contained enough of the accessory substance to support better growth than the control.

3.) By the fractional extraction applying ethyl-ether, alcohol, acetone, chloroform, benzene and petroleum benzine in the order, 80—90 percent of the accessory substance was removed from the nodule while alcohol and water alone were equally effective.

4.) The presence of alkaloids was noted in the acetone and alcoholic extracts of Genge, and in the water and alcoholic extracts of bean.

5.) The largest amount of nitrogen was found in the water extract followed by acetone and ether in the order, and a comparatively small quantity in alcohol.

6.) No definite relation was found among the accessory substance, alkaloids and nitrogen contents. But judging from the fact that the water and alcohol which were effective in extracting the accessory substance contained the alkaloids in the extracts, it seems to suggest that the presence of alkaloids has some action on the growth.

7.) As to the influence of extracts on the morphology of nodule bacteria, no definite result was obtained except Genge nodule bacteria, strain A, B and C produced bacteroids in the water extract.

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