INFLUENCE OF ANTIBACTERIAL SUBSTANCES FROM PLANTS ON SOIL MICROFLORA

by

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Bangalore, India 1968

HD - 6074

A THESIS

Submitted in partial fulfillment of the

requirement for the degree

MASTER OF SCIENCE

in

MICROBIOLOGY

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1972

Approved by:

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Introduction

From earliest times plants have been used in an attempt to cure disease. Claims have been made that certain plants have the ability to heal wounds and that others are effective in treatment of diseases such as dysentery and cholera. These plants are known to contain antibacterial substances. Successful attempts have been made to isolate, purify and to use these antibacterial substances for curative purposes.

Continued contact of microorganisms with the antibacterial substances brings out certain physiological changes in the microbes, such as the development of resistance to the antibacterial compounds or dependence on these for growth. This phenomenon is well understood by the scientists who work with pathogenic organisms and antibiotics which are established to be effective against certain diseases. Under the natural conditions of plant growth, antibacterial substances from plants come in contact with the soil microorganisms through the process of natural leaf fall and root exudation in the rhizosphere. This study was undertaken to investigate the influence of antibacterial substances from plants of two species, flame leaf sumac (Rhus glabra) and black walnut (Juglans nigra), on the soil microflora.

Literature Review

Osborn (1943) tested 2300 species of plants belonging to 166 families against Staphylococcus aureus and Escherichia coli. Sixty-three genera contained substances which inhibit the growth of one or the other test organisms. The plant material was ground with a pestle and mortar with sand and sufficient water to give a pulp. This was strained through fine silk and the resulting extract was tested against the test organisms. The test organisms were grown in a nutrient enriched agar (Difco) by seeding 0.5 ml of 18 to 24 hour broth cultures. The sensitivity tests were run by placing porcelain cylinders filled with the aqueous solutions of the plant extracts on the surface of the seeded agar. After incubation of the plates for 24 to 48 hours the sensitivity (areas of inhibition) was measured in millimeters. He also observed that the inhibitory substances in some cases are distributed throughout the plant and in some, restricted to some parts of the plant body. Drying of the plants caused the loss of antibacterial activity in some plants while in others it did not affect the activity.

Carlson et al (1948) devised an analytical method for the extraction of antibiotic substance from flame leaf sumac (Rhus hirta). They isolated antibiotic materials from three extracts of this plant using water, ethyl ether and acetone. The extracts were tested against 58 different species of bacteria and fungi. The test organisms were grown in nutrient enriched agar by seeding 0.5 ml of 18 to 24 hour broth cultures and several bacteria were grown on blood agar by smearing 1/10 ml of broth cultures on the surface of the agar. The sensitivity tests were run by placing porcelain cylinders

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fille h aqueous solutions of the plant extracts on the surface of the seeded agar. The areas of inhibition were measured in millimeters. The activity against these organisms showed that this plant contains two different antibiotic substances. Toxicity of the extracts were also tested on white swiss mice by injecting 0.5 ml and 1 ml amounts by intraperitonial route. Toxicity was observed at intervals of 15 and 45 minutes and at the end of 1 and 24 hours.

Gries (1943) showed that the oxidized form of hydroxyjuglone found in the members of the genus Juglans is toxic to certain higher plants growing in the proximity of walnut trees. Standard laboratory tests were done for fungicidal activity and this compound clearly approached the toxicity of copper in Bordeaux mixture. In field conditions juglone was found to be effective on the fungus which causes black spot of roses.

Westfall et al (1961) showed that a depressant agent is present in walnut hulls. Hulls extracted with ether yields an extract which sedates or depresses the movements of frogs and fishes. He purified the depressant compound 5-hydroxy 1-napthquinone (juglone) and tested it against the above animals. The effect of this compound on the microorganisms was not studied.

Daglish (1950) isolated an amorphous compound, hydrioxy juglone glycoside ($C_6H_{18}O_2$) from the ethanolic hydrochloric acid extracts of walnut by chromatography on alumina. Spectroscopic examination suggested that this compound existed as such in the plant. On hydrolysis it yielded glucose and -hydroxy juglone. Further chemical and spectroscopic evidence suggests that this compound is 5-glucoside of 1:4:5 trihydroxynapthalene.

Arkipov (1951) showed that the causative agent of anthrax, Bacillus

anthracis, reacts markedly to the action of certain plants. Cells of B. anthracis lose their capacity to form spores and become asporogenic and simultaneously avirulent. Degenerative forms, characterized by unusual structure and cell growths were obtained under the influence of the plant.

Timonin (1941) diffused the solution in which flax plants were growing, through the colloidal membrane in the soil. The microbial activity in the vicinity of the membrane were stimulated in a manner analogous to the natural rhizosphere of flax plant. Chemical analysis of the solution, after the growth of resistant varieties of flax, showed 25 to 37 mgm of HCN per plant grown. He suggested that a significant quantity of HCN from hydrolysis of linmarin, is excreted through the root system by the resistant varieties of flax plant, when grown under aseptic conditions. Further, from a comparison of the density of microbial accumulation in the rhizospheres of susceptible and resistant varieties, it can be assumed that some toxic matter is excreted by the resistant variety, creating unfavorable conditions for the growth of not only the pathogenic organisms but also of certain groups of flora of the soil.

Horsfall and Dimond (1959) made a reference to the interaction between microorganisms and higher plants. They suggested that the substances excreted from the shoot and roots of higher plants have more or less selective inhibitory action on the microorganisms. These toxic materials present in the tissues of the plants provide resistance against the pathogens.

Lucas (1949) reviewing the earlier works on the ecological adaptation, stated that through the leaves both organic and inorganic substances are excreted and are washed off into the soil in periods of rain and heavy dews.

These substances which become a part of the chemical environment, act as stimulants or deterents to the further development of the plant iself. These substances also contribute to the rise and fall of the microbial population or individuals of other species.

Outstanding work on the relationship between the soil microorganisms and higher plants has been done in the USSR. Krasilnkov (1958) in his book Soil Microorganisms and Higher Plants, stated that bacteria may be trained to antiseptics and boric acid. This training was achieved by steps, starting with small doses. Under the influence of an unusual substance in their environment the microbes elaborate suitable enzymes. Production of such adaptive enzymes is noted in many bacteria, fungi, protozoa and other microorganisms.

Materials and Methods

Collection and Source of Plant Material

Since antibacterial activity has been shown to be present in different parts of the plant, berries and leaves of flame leaf sumac (R. glabra) were collected from half mile south of Randolph, Kansas, west of highway, and the walnuts (J. nigra) were collected from the campus of Kansas State University near Ackert Hall in the month of October 1970. The samples were frozen in the deep freeze to maintain freshness in periodical extractions. A sample of dried fallen leaves from the sumac field was also collected to check if the antibiotic activity would be lost by drying of the leaves.

Extraction of Antibiotic Material

Twenty grams of each sample was blended with 100 ml of distilled water for 5 minutes and the extracts were filtered through Watman #42 filter paper. Water soluble fractions of all the above samples showed but slight antibacterial activity on organisms isolated from soil. Hence ethanol extracts of the samples were used in all the experiments. Extractions were made as mentioned above using 95% alcohol, instead of water. The volume of each extract was then reduced to 10 ml by transferring the solution to evaporating dishes and keeping it exposed in an exhaust hood at room temperature.

Preparation of Sensidiscs

Thick chromatography paper (Watman #1), cut into uniform circular discs of 6.5 mm diameter using a paper punch were used as sensidiscs. These discs were sterilized by autoclaving. The discs were then saturated with the

individual extracts and then allowed to dry in sterile petri plates, so that the alcohol was evaporated from the discs. These sensidiscs were used for sensitivity tests within 24 hours after preparation.

Isolation of the Test Organisms from the Soil

Surface (1-4 inches) soil samples collected from various sources were plated on large (6 inches) sterile petri plates by serial dilution plating technique up to 10^{-6} dilution using plate count agar. The plates were incubated for 24 to 48 hours at 30°C and the number of organisms per gram of original sample were counted. Pure cultures of the individual colonies were obtained by streaking the individual colonies on half strength nutrient agar plates, for isolation, using four flame streak method. These plates were incubated at 30°C for 24 hours and the isolated colonies, one from each plate, were transferred to nutrient agar slants and then stored. Twenty to thirty pure cultures were isolated for each soil sample. These formed the heterogeneous, pure cultures of soil bacteria, and were used as the test organisms under one particular set of conditions.

Sensitivity Test

Pure cultures isolated by the above method were smeared uniformly on the nutrient agar plates using a wet sterile cotton swab. Immediately after this, the discs, which contained the antibiotic substance from the plant material were placed on the agar, 5-6 per plate at equal distance from one another in a circular fashion using sterile tweezers. Plates were incubated at 30°C for 24 hours. The antimicrobial activity of the extracts, if present, was seen by the inhibition of growth of the test organisms on the agar plate only around the disc. The area of the zone of inhibition

depends on the rate of diffusion of the material into the agar, concentration of the material in the discs, and the sensitivity of the particular organism to that particular fraction of the plant material. In these experiments the concentration of the extracts was kept constant in all the discs and same media was used for all the organisms. The difference in the area of the zone of inhibition was attributed to the difference in the sensitivity of the different organisms to one particular fraction or fractions. The zone of inhibition was measured in millimeters from the rim of the disc to the rim of the inhibition zone.

Field Experimentation

In the beginning of fall 1970, soil samples were collected from the field where sumac and walnut plants were growing. Two samples in each case were collected. One from the plant base where it was presumed that considerable foliar material had accumulated. The second sample was collected further away from these plants, where there was little possibility of any plant part coming in contact with the soil. Soil samples were brought to the laboratory and plate counts were made as described earlier. Pure cultures obtained by the four flame streak method and 24 hour cultures were stored for further tests.

Organisms from the plant base and those away from the plant were tested separately, for sensitivity against the different extracts of sumac and walnut, to check the response of the organisms, to the presence of these antibiotic materials in their environment, under two different conditions. Extracts of walnut hulls, walnut leaves, sumac berries, sumac leaves and sumac dry leaves were tested.

Green House Studies

A similar experiment was conducted in the green house, on different soil types collected from different sources. The influence of these antibiotic materials was tested on the organisms isolated from the following soil samples.

Soil type	pH	Source
1. Kahola silt loam	6.0	Riley Co., Kansas
2. Ivan clay loam	7.0	"
3. Carr sarpy sand	8.4	"
4. Labette silty clay loam	6.1	Osage site*

(* Soil sample collected from the International Biological program's Osage site on the Adams ranch near Grainola, Oklahoma)

Prior to the incorporation of the plant material into the soil samples, plate counts were made from these samples and also pure cultures were isolated. All the five extracts were tested on these isolates for antibiotic activity.

The soil samples were mixed with walnut hulls and sumac dry leaves separately. These mixtures were kept in the green house in small earthen pots at 35°C for a period of 45 days with periodical watering to keep the soil moist.

At tne end of the 45 day period, plate counts were made separately from all the eight treatments and pure cultures were isolated. Antibiotic activity of the plant extracts was tested on these isolates, to see if there was any change in the sensitivity due to the incorporation of the plant material in the soil.

Immediately after winter, soil samples were collected from the sumac

field once again. Sensitivity tests were run on the isolates from the plant base soil samples and those away from the plants as mentioned earlier.

Five known organisms Bacillus subtilis, Staphylococcus aureus, Brevibacterium linins, Sarcina leutea and Proteus vulgaris were tested against the plant extracts for antibiotic sensitivity.

The efficiency of the antibiotic substance present in the two plants, walnut and sumac were compared with the known antibiotics which are in regular use. Selected pure cultures from Labette silty clay loamy soil were tested against the following eight antibiotics: 1, Penicillin; 2, Bacitracin; 3, Erythromycin; 4, Neomycin; 5, Chloromycitin; 6, Auromycin; 7, Magnamycin; and Streptomycin. Ready made sensidiscs of the above antibiotics from Difco Laboratories, Detroit, Michigan were used. The same cultures were tested against the plant extracts and the results were compared.

Results

Absorption spectra of the alcohol extracts of different samples of walnut and sumac were determined using a Beckman D U 2 Spectrophotometer. The absorption pattern is shown in Figures 1 and 2. In walnut hull and walnut leaf extracts two distinct peaks are seen at 455 mμ and 560 mμ and 400 and 410 mμ respectively, suggesting that two components are present in each extract. Sumac berry, leaf and dry leaf extracts showed one prominent peak at 520, 400 and 500 mμ respectively. The difference in the absorption pattern of dry leaf and green leaf extracts of sumac indicates that the drying of leaves does have some effect on the antibacterial property of the leaf component.

Plate counts from soil samples were made separately for soil from plant base and the soil away from the plant, using dilution plating technique. No significant difference was found in the total counts of the soil population. These results are given in Table 1.

Sensitivity tests were run on the bacterial isolates from the above six soil samples of walnut and sumac fields collected before and after winter. Average sensitivity of 20 to 28 isolates in each case for five extracts of plant parts are given in Tables 2, 3 and 4.

Data of these experiments were analyzed statistically to test the significance between the means of the two populations in comparison. The t-values were compared with t-table values given at 5% and 1% levels in Tables 2, 3, and 4. The t-values indicate that there is no significant difference in the sensitivity between the isolates from plant base and those away from the plant in all the three soil samples.

Figure 1. Absorption spectra of the alcohol extracts of walnut hulls and walnut leaves.

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FIGURE 1.

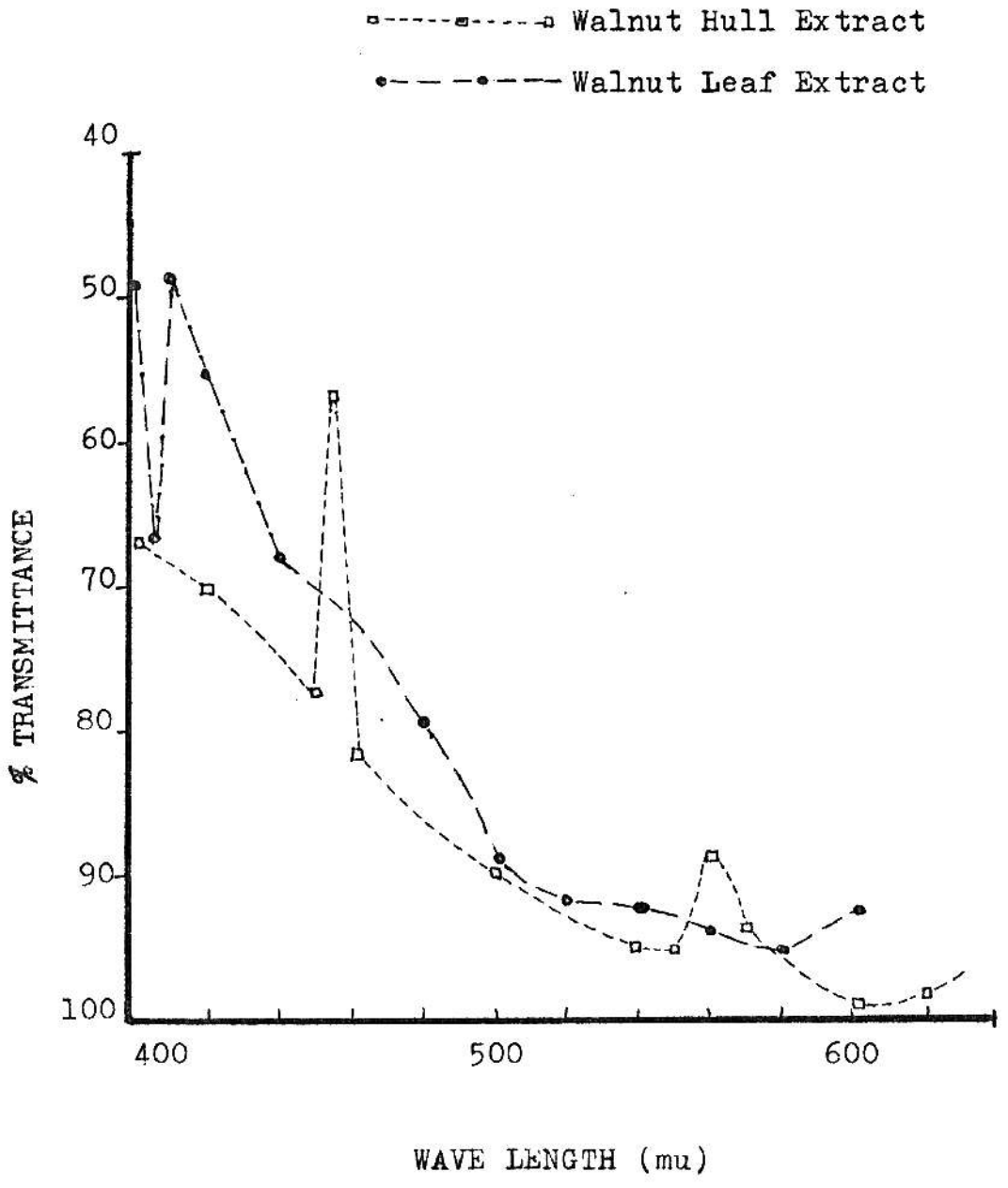
Walnut Hull Extract
Walnut Leaf Extract
40
50
60
70
80
90
100
% TRANSMITTANCE
400
500
600
WAVE LENGTH (mu)

Figure 2. Absorption spectra of the alcohol extracts of sumac berries, sumac leaves and sumac dry leaves.

FIGURE 2.

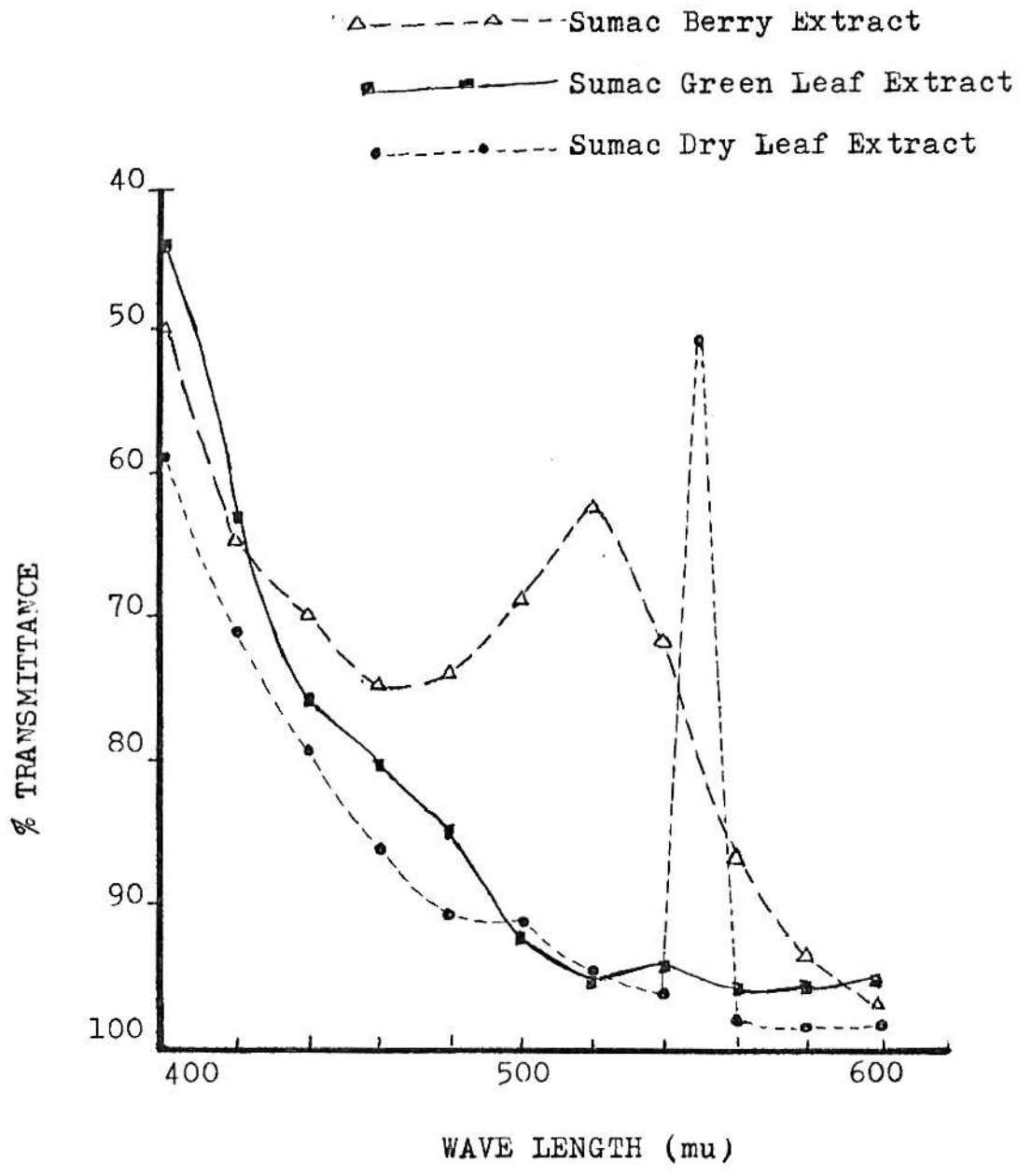
Sumac Berry Extract
Sumac Green Leaf Extract
Sumac Dry Leaf Extract
40
50
60
70
80
90
100
% TRANSMITTANCE
400
500
600
WAVE LENGTH (mu)

Table 5 gives the total counts of the soil population of four different soil types, before and after the incorporation of walnut hulls and sumac leaves into the soil. Comparing the total counts of soil population between treated and untreated soil samples, it is seen that the soils treated with walnut hulls have a higher soil population than the untreated soils. Treating the soils with sumac leaves did not increase the soil population.

Antibiotic sensitivity tests were run on the pure cultures of treated and untreated soils using five different extracts of sumac and walnut. The results are given separately for four different soil types in Tables 6, 7, 8 and 9. Results of the sensitivity tests showed significant differences between treated and untreated soil isolates. The general trend indicates a decrease in sensitivity due to the incorporation of the antibiotic material in the soil. However, the degree of sensitivity decrease varied with the soil type, the antibiotic material incorporated in the soil and the plant extract tested. Statistical analysis of the data shows that the Kahola silty loamy soil isolates showed a significant decrease in the sensitivity after the treatment with walnut and sumac against all the extracts, except sumac berries. Isolates from Ivan clay loamy soil showed a significant decrease in the sensitivity due to the incorporation of plant materials in the soil only against sumac berries. A highly significant decrease in the sensitivity was seen in the isolates from Carr sarpy sandy soil, due to the treatment of the soil with the plant materials against walnut hull and sumac leaf extracts. Labette silty clay soil isolates did not show considerable change in their response to the plant extracts due to the soil treatment.

About 40, 24 hours representative pure cultures used in these experiments

were stained to study the morphological characters of the test organisms. They were mostly Gram positive rods with very few Gram negative rods. No cocci were found. Most of the Gram positive rods were spore formers.

The antibiotic activity of the plant extracts was tested on some of the known organisms. It was found that some of the plant extracts have effective antibacterial activity on some of the pathogenic organisms. These results are given in Table 10.

The efficiency of the antibacterial substances present in these two plants was compared with known antibiotics which are in regular use. The comparative data, i.e. the sensitivity of each culture to different extracts and known antibiotics, are given in Tables 11 and 12.

Table 1. Total counts of the soil population of different soil samples

Soil sample	No. of organisms per gram of soil	
	Soil from plant base	Soil away from plant
1. Walnut field	73×10^5	52×10^5
2. Sumac field	56×10^5	59×10^5
3. Sumac field (after winter)	7.5×10^6	13×10^6

Table 2. Antibiotic sensitivity in mm of the isolates from the soil samples of sumac field. The t-table values given are at 5% and 1% points respectively.

Extracts	Ave. sensitivity in mms: Isolates from plant base	Isolates away from plant	t-value	t-table value
1. Walnut hulls	3.3	2.92	1.54	2.004 2.669
2. Walnut leaves	3.4	3.53	0.28	do
3. Sumac berry	3.4	3.1	0.74	do
4. Sumac leaves	4.0	3.6	0.90	do
5. Sumac dry leaves	3.1	2.8	0.64	do

Table 3. Antibiotic sensitivity in mm of the isolates from the soil samples of walnut field. The t-table values given are at 5% and 1% points respectively.

Extracts	Ave. sensitivity in mms: Isolates from plant base	Isolates away from plant	t-value	t-table value
1. Walnut hulls	2.7	3.0	0.54	2.017 2.697
2. Walnut leaves	3.1	2.6	0.88	do
3. Sumac berry	1.4	1.3	0.09	do
4. Sumac leaves	2.7	2.6	0.26	do
5. Sumac dry leaves	1.9	1.5	1.35	do

Table 4. Antibiotic sensitivity in mm of the isolates from soil samples of sumac field collected after winter. The t-table values given are at 5% and 1% points respectively.

Extracts	Ave. sensitivity in mms: Isolates from plant base	Isolates away from plant	t-value	t-table value
1. Walnut hulls	2.54	2.1	0.72	2.064 2.797
2. Walnut leaves	1.1	1.5	0.50	do
3. Sumac berry	3.2	2.9	0.39	do
4. Sumac leaves	1.5	2.5	1.20	do
5. Sumac dry leaves	0.9	1.4	0.65	do

Table 5. Total counts of soil population of treated and untreated soil types collected from four different sources

	Untreated soil samples	Total counts per gm of soil
1.	Kahola silty loam	34×10^5
2.	Ivan clay loam	34×10^5
3.	Carr sarpy sand	42×10^5
4.	Labette silty clay loam	18×10^5
	Treated soil samples	
1.	Kahola silty loam + sumac leaves	34×10^5
2.	Kahola silty loam + walnut hulls	45×10^5
3.	Ivan clay loam + sumac leaves	30×10^5
4.	Ivan clay loam + walnut hulls	55×10^5
5.	Carr sarpy sand + sumac leaves	34×10^5
6.	Carr sarpy sand + walnut hulls	48×10^5
7.	Labette silty clay loam + sumac leaves	26×10^5
8.	Labette silty clay loam + walnut hulls	18×10^5

Table 6. Comparative antibiotic sensitivity in mm of the isolates from soil samples of treated and untreated soil type Kahola silty loam. The t-table values given are at 5% and 1% points respectively.

Extracts	Ave. sensitivity in mms: Kahola silty loam	Kahola silty loam + sumac	Kahola silty loam + walnut	t-value	t-table value
1. Walnut hulls	4.4	2.68		3.27	2.008 2.678
			1.86	3.95	2.021 2.704
2. Walnut leaves	2.59	1.24		2.32	2.008 2.678
			1.20	2.14	2.021 2.704
3. Sumac berry	5.04	3.84		1.95	2.008 2.678
			3.66	1.79	2.021 2.704
4. Sumac leaves	3.15	1.60		3.20	2.008 2.678
			0.80	4.31	2.021 2.704
5. Sumac dry leaves	1.85	0.72		2.62	2.008 2.678
			0.13	3.47	2.021 2.704

Table 7. Comparative antibiotic sensitivity in mm of the isolates from soil samples of treated and untreated soil type Evan clay loam. The t-table values given are at 5% and 1% points respectively.

Extracts	Ave. sensitivity in mms: Ivan clay loam	Ivan clay loan + sumac	Ivan clay loam + walnut	t-value	t-table value
1. Walnut hulls	3.44	3.40		0.05	2.021 2.704
			3.5	0.17	2.011 2.684
2. Walnut leaves	2.7	1.5		1.58	2.021 2.704
			2.33	0.59	2.011 2.684
3. Sumac berry	4.83	2.66		3.46	2.021 2.704
			3.62	2.22	2.011 2.684
4. Sumac leaves	3.10	2.97		0.14	2.021 2.704
			2.70	0.64	2.011 2.684
5. Sumac dry leaves	1.6	1.7		0.20	2.021 2.704
			1.90	0.85	2.011 2.684

Table 8. Comparative antibiotic sensitivity in mm of the isolates from soil samples of treated and untreated soil type, Carr sarpy sand. The t-table values given are at 5% and 1% points respectively.

Extracts	Ave. sensitivity in mms				
	Carr sarpy sand	Carr sarpy sand + sumac	Carr sarpy sand + walnut	t-value	t-table value
1. Walnut hulls	5.46	2.58		6.29	2.004 2.669
			2.26	8.00	do
2. Walnut leaves	1.38	1.76		1.04	do
			1.75	0.89	do
3. Sumac berry	4.46	4.13		0.83	do
			4.15	0.71	do
4. Sumac leaves	3.00	1.69		3.25	do
			1.36	4.40	do
5. Sumac dry leaves	1.70	1.41		0.82	do
			1.18	1.33	do

Table 9. Comparative antibiotic sensitivity in mm of the isolates from soil samples of treated and untreated soil type, Labette silty clay loam. The t-table values given are at 5% and 1% points respectively.

Extracts	Ave. sensitivity in mms: Labette clay loam	Labette clay loam + sumac	Labette clay loam + walnut	t-value	t-table value
1. Walnut hulls	4.70	3.25		1.85	2.025 2.714
			4.00	0.88	2.043 2.787
2. Walnut leaves	2.80	1.87		1.48	2.025 2.714
			1.84	1.82	2.043 2.787
3. Sumac berry	4.48	4.56		0.17	2.025 2.714
			4.9	0.76	2.043 2.787
4. Sumac leaves	2.3	2.75		0.84	2.025 2.714
			3.61	2.07	2.043 2.787
5. Sumac dry leaves	1.2	1.81		1.39	2.025 2.714
			2.00	1.79	2.043 2.787

Table 10. Antibiotic sensitivity of known pathogenic organisms against five different plant extracts.

Organisms	Walnut hulls	Walnut leaves	Sumac berries	Sumac leaves	Sumac dry leaves
1. Brevi linins	2	1	5	3	0
2. Bacillus subtillis	3	2	3	0	0
3. Staph aureus	2	2	1	0	0
4. Proteus vulgaris	0	0	0	0	0
5. Sarcina leutea	2	0	7	5	0

Table 11. Antibiotic sensitivity in mm. of the isolates from Labette silty clay loamy soil tested against plant extracts. Same cultures were tested against the known antibiotics and the results are given in Table 12.

Culture #	Sensitivity in mm.					
	Walnut hulls	Walnut leaves	Sumac berries	Sumac leaves	Sumac dry leaves	Sumac fresh leaves
1	6	2	4	0	2	2
2	1	0	4	0	0	0
3	3	2	4	0	2	2
4	4	0	4	1	0	0
5	6	3	8	4	3	3
6	4	2	4	0	0	1
7	3	5	4	6	5	3
8	5	6	5	6	5	4
9	3	6	4	7	6	4
10	5	5	3	6	4	5
11	2	3	4	4	3	3
12	3	3	2	3	3	2
13	4	4	2	4	3	4
14	4	3	3	2	2	2
15	7	6	8	8	4	8
16	4	0	4	0	0	1
17	2	2	4	2	0	2

Table 12. Antibiotic sensitivity in mm. of the isolates Labette clay loamy soil, tested against known antibiotics. Same cultures were tested against the plant extracts and the results are given in Table 11.

	Antibiotics							
Culture #	1	2	3	4	5	6	7	8
	Sensitivity in mm.							
1	0	8	7	3	8	7	--	--
2	0	1	--	6	5	5	13	--
3	1	7	8	3	7	5	--	--
4	0	8	10	4	9	9	--	--
5	1	10	10	2	10	6	--	--
6	0	6	5	4	7	7	--	--
7	16	10	10	--	12	12	13	--
8	24	21	--	8	13	18	18	--
9	0	3	7	5	3	5	--	--
10	15	7	--	5	7	13	13	--
11	7	2	7	4	9	10	--	--
12	0	1	11	3	9	8	--	--
13	15	6	--	3	14	12	13	--
14	0	6	5	3	7	6	--	--
15	20	20	--	7	14	12	17	--
16	12	3	--	3	7	9	8	--
17	0	0	--	0	8	4	--	0

Antibiotics used:
1. Penicillin
2. Bacitracin
3. Magnamycin
4. Neomycin
5. Chloromycitin
6. Auromycin
7. Erythromycin
8. Streptomycin

-- in the table indicates that the organism was not tested against that antibiotic

Discussion

The response of microorganisms to the presence of an antibiotic in their environment can take three different forms: a) sensitivity; b) resistance; and, c) dependence. In this investigation those organisms which exhibited inhibition of growth around the antibiotic disc were termed sensitive to that particular fraction of the plant material. Resistant organisms very few in number were also found in the population, among the isolates from soil collected from the plant base as well as the isolates away from the plant. No significant difference was found in the sensitivity of the isolates to the plant extracts between the isolates from plant base and isolates away from the plant.

It was presumed that the green house studies provided a more favorable situation for microbial activity than that of the natural field conditions. However, the concentration of the plant material incorporated into the soil per unit area was higher in the case of green house experiments than the natural field conditions.

It was seen that the isolates from the soil that were treated with the plant material for a period of time, responded differently to the action of antibacterial substances from plants, compared to the isolates from natural field conditions. Isolates from different soil types showed decreased sensitivity against plant extracts compared to the isolates from untreated soil types. This phenomenon can also be referred to as the development of resistance to the antibiotic substance.

Watanabe (1963) observed the overall sensitivity of Shigella strains from the cases of epidemic dysenteria, turned into 60 to 80% resistance to four important drugs, sulphanamide, streptomycin, chloromycitin and tetra-

cycline. He attributed the cause of antibiotic resistance to a class of extrachromosomal genetic elements, usually referred to as "R factors". These additional genetic elements which are also capable of governing hereditary properties, independent of the chromosomal genes, are classified as bacterial plasmids. It has been shown that the antibiotic resistance is due to the spread of the resistance factor among the bacteria and also due to the infection of the sensitive cells with the genes of resistance by means of transduction or bacterial conjugation.

Nisioka et al (1969) suggest that the acceptance of a plasmid from external sources gives the cell a new property. The size of the DNA molecule of some R-factors enables coding for various functions like immunity, compatability etc., in addition to the gene which codes for individual resistance.

Watanabe (1963) described the phenomenon of infectious drug resistance transferred from one strain to the other by a plasmid that carries the gene for multiple resistance. If an antibacterial drug is added to the liquid culture of bacteria that are sensitive to the drug, after a while the cells in the culture are found to be resistant to the drug. The earlier notion was that the drug must have somehow induced the resistance to the drug. However, Watanabe (1963) suggested that a few cells in the original culture were already resistant to the action of the drug; these cells survive and their daughter cells multiply when the sensitive majority of the bacteria succumb to the drug. This resistance is due to a spontaneous mutation which occurs once in 10 million or a billion cell divisions and when it occurs it alters the cell's sensitivity to one particular drug or perhaps two related drugs.

Watanabe (1969) also suggests that these drug resistant genes (R determinants) are derived from the chromosomal genes of some as yet unknown bacteria.

However, Fredrieqe supposes that R-factors existed in very few varients of enteric bacteria or staphylococci far before the antibiotics were discovered and perhaps might develop in the process of frequent contact of these bacteria with fungi, actinomycetes etc. which produce antibiotics in nature.

Watanabe (1967) described the mechanism of infectious drug resistance. When the bacteria with the R-factor come in contact with a population of drug sensitive bacteria, a few R-factors are transmitted by conjugation from donor cells bearing pili into the cytoplasm of recipient cells. Immediately these cells become resistant. The transfer process is repeated from cell to cell. The normal process of cell division also contributes to the rapid proliferation of the multiple resistance in the recipient population.

A similar situation can be seen in the present investigation. It is noted that in addition to the sensitive cells to the antibacterial substances tested, there were also a few cells which were completely resistant to the action of antibiotic substances. Incorporation of a concentrated dose of these substances in the soil must have decreased the population of sensitive bacteria in the soil and the resistant bacteria multiplied and increased in number. When this resistant population came in contact with the sensitive bacteria, they could have transferred the resistance (R-factor) into the sensitive cells through the process of conjugation. These recipient cells in turn became resistant or less sensitive to the action of the antibacterial substances.

Microbial adaptations and variability

It has been shown that when bacteria are grown in a medium which is unusual for them and has unassimilated sources of nutrients, they begin to assimilate the latter and the medium becomes ordinary one and even indispensable. It is also known (Krasilnkov 1959) that the bacteria can be conditioned to

tolerate antiseptics, boric acid, borax and other toxic materials. He found that the anthrax bacillus and other bacteria may be conditioned to high concentrations of disinfectants. This conditioning is achieved by steps starting with small amounts and gradually increasing the concentration. This allows the microbes to develop enzymes that break down these toxic materials. Production of such adaptive enzymes is noted in many bacteria, fungi, protozoa and other microorganisms.

Krasilnkov (1959) suggests that the enzyme penicillinase produced in defense against penicillin by widely known microbes is strictly an adaptive phenomenon. Such enzymes which are produced in defense against a particular antibiotic substance are called adaptive enzymes and their action is specific to the substances which induce their production. However, in many cases the specificity of resistance of the variant organism is not an absolute one. In addition, when acquiring resistance to one antibiotic, bacteria often become less sensitive to other antibiotics. The acquired resistance to an antibiotic is stable and is transmitted hereditarily through long series of generations. It is not the entire culture, but the individual cells which become adapted to antibiotics.

The above theory of microbial adaptation to antibiotics could be the other explanation for the development of resistance in bacteria against the antibacterial substances used in these studies. Incorporation of lower doses of the antibacterial substances in the soil may have induced the formation of resistant variants by inducing the synthesis of specific adaptive enzymes. These enzymes then were capable of degrading the particular antibacterial substance which induced their synthesis. Isolates from soils treated with walnut also developed resistance against extracts of sumac which conforms with the fact that, in

acquiring resistance to one antibiotic, bacteria often become less sensitive to other antibiotics. As a result of prolonged adaptation of these organisms to the antibacterial substances in the soil, variants were also formed which require the given antibacterial substance for growth. This was seen by the increased growth of bacteria around the antibiotic discs, indicating the formation of dependent variants.

The sensitivity and the development of resistance of the isolates varied with the four different soil types. Krasilnkov (1959) observed that the formation of adaptive enzymes is greatly influenced by the environmental factors like temperature, pH etc. The four soil types used in the green house experiments were from four different sources with different chemical composition, pH, structure and texture. These differences in the soils used could be responsible for the difference in the response of the organisms against the plant extracts before and after soil treatments.

No correlation was found between the results of the green house experiments and field studies, even though it was thought that a similar situation existed in both the cases. The exact reason for this disagreement of results was not investigated. However, one possible explanation could be that the concentration of the antibacterial substances incorporated in the soil per unit area was much higher in green house experiments. Nevertheless, it is evident that such a possibility of developing resistant or dependent varients of microorganisms due to the exposure to the naturally occurring antibacterial substances in nature does exist.

Summary

Antibacterial properties of two plants flame leaf sumac (Rhus glabra) and black walnut (Juglans nigra) were tested against soil microflora. It was seen that the incorporation of the plant materials in the soil resulted in a change in the response of the soil bacteria to the action of antibacterial substances from plants. Studies conducted on isolates of different soil types from different sources showed a development of physiological resistance or a decreased sensitivity against the antibacterial substances extracted from different plant parts. This change in the response of soil bacteria to the action of antibacterial substances was attributed to the following two phenomena which are known to occur in pathogenic bacteria.

1. Transferable infectious drug resistance from a resistant to susceptible strain of bacteria through the extrachromosomal genetic elements named plasmids, which occur through the process of bacterial conjugation.

2. Adaptation of microorganisms to unusual substances in their environment by the production of adaptive enzymes, specific for the substances which induce their synthesis.

Antibacterial properties of the plant extracts tested on known pathogenic organisms show that, some of the components of plant extracts are effective against certain pathogenic organisms. In their efficiency as antimicrobial agents these plant extracts compare favorably with the known antibiotics which are very effective as therapeutic agents.

Acknowledgements

The author expresses his deep appreciation to Professor John O. Harris for his invaluable guidance and help in the completion of this work and preparation of this manuscript.

The assistance obtained from Drs. C. L. Kramer and R. E. Clegg in the preparation of this manuscript is gratefully acknowledged.

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INFLUENCE OF ANTIBACTERIAL SUBSTANCES FROM PLANTS ON SOIL MICROFLORA

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1968

AN ABSTRACT OF A MASTER'S THESIS

Submitted in partial fulfillment of the

requirement for the degree

MASTER OF SCIENCE

in

MICROBIOLOGY

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1972

Abstract

Antibacterial properties of two plants, flame leaf sumac (Rhus glabra) and black walnut (Juglans nigra) and their influence on soil microflora were investigated. Ethanolic extracts of different plant parts showed varying degrees of sensitivity against soil isolates. Sensitivity was tested using antibiotic sensidiscs saturated in plant extracts. Field experiments were conducted to test the effect of incorporation of antibacterial substances in the soil. Similar experiments conducted in the green house on different soil types showed a development of physiological resistance in the soil bacteria against the antibacterial substances from plants. This change in the response of the soil bacteria to the action of antibacterial substances was attributed to the following two phenomena which are known to occur in pathogenic microorganisms.

1. Transferable infectious drug resistance from a resistant to susceptible strain of bacteria through the extrachromosomal genetic elements named plasmids, which occur through the process of bacterial conjugation.

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