ANTIBIOTIC ACTIVITY OF SOME CRUDE PLANT JUICES

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It has been repeatedly demonstrated that some plant juices contain antibacterial and antifungal substances (Irving, Fontaine, and Doolittle, 1945; Osborn, 1943; Lucas and Lewis, 1944; Sanders, Weatherwax, and McClung, 1945; Huddleson, DuFrain, Barrons, and Giefel, 1944; Cavallito, Bailey, and Kirchner, 1945; Pederson and Fisher, 1944). Many of these studies have been directed toward uncovering compounds active against human pathogenic organisms. Recently, however, Irving, Fontaine, and Doolittle (1945) announced the discovery of a substance in the juice of the tomato that strongly inhibited the growth of *Fusarium oxysporum* f. *lycopersici*, the organism causing *Fusarium* wilt of tomatoes. This substance was named "lycopersicin," which was later changed to "tomatin." These investigators pointed out that the resistance of some plant varieties to phytopathogens might conceivably be related to the presence of more or less specific antibiotic substances in the juices of these plants.

The work in these laboratories was undertaken with a view to testing the validity of this postulate, and accordingly seeds of resistant and nonresistant varieties of common garden plants were secured. From these seeds plants were grown in the greenhouse, and juice was expressed from the leaves and stems by means of a screw press. The juices were centrifuged, and the supernatants were immediately sterilized by passage through a Seitz filter and stored in the refrigerator in sterile bottles. Approximately 75 ml of juice were sterilized in this manner, and the first filtrate to come through was not discarded, as is often done. The juices were tested within a few days against various phytopathogens, using the cylinder plate method described by Irving, Fontaine, and Doolittle (1945). The four pathogenic fusaria were grown on plates containing Czapek glucose agar, as suggested by these workers. The phytopathogenic bacteria were grown on Difco nutrient agar and the tests run in the same manner. These crude undiluted extracts were also assayed against *Staphylococcus aureus*, *Eberthella typhosa*, *Salmonella paratyphi*, and *Escherichia coli*. No attempt was made to make any of the assays quantitative. The test plates were prepared as follows: (1) A 24-hour culture of the various fusaria in "hormone" beef infusion broth containing 2 per cent glucose was shaken with glass beads to break up the mycelium; 1-ml portions of these suspensions were pipetted into sterile petri plates, and 10 ml of a modified Czapek glucose agar (Irving, Fontaine, and Doolittle, 1945) were added. (2) The plant and animal pathogenic bacteria were grown for 24 hours in nutrient broth (Difco), and 1-ml portions of these suspensions were plated in 1.5 per cent nutrient agar (Difco). Penicylinders were affixed to the surface of

TABLE 1

Effect of plant juices on human bacterial pathogens

PLANT	DESCRIPTION	HUMAN PATHOGENS			
		Staphylococcus aureus FDA 209	*Eberthella typhosa* FDA Hopkins	*Salmonella paratyphi* A	*Escherichia coli*
Bean, Great Northern	Resistant to halo blight caused by *Phytomonas medicaginis* var. *phaseolicola*	0	0	10	+
Bean, Hidatsa Red	Resistant to halo blight caused by *Phytomonas medicaginis* var. *phaseolicola*	0	15	10	+
Bean, White Kidney	Susceptible to halo blight	0	15	8	+
Corn, Iowa Inbred, 4473	Resistant to bacterial wilt caused by *Bacillus stewartii*	7	0	0	–
Corn, Iowa Inbred, 4528	Resistant to bacterial wilt caused by *Bacillus stewartii*	7	25	15	20
Corn, Iowa Inbred G.B. 134	Susceptible to bacterial wilt	0	20	22	20
Corn, Iowa Inbred G.B. 797	Susceptible to bacterial wilt	–	–	22	–
Cabbage, Wisconsin Jersey Queen	Resistant to cabbage yellows caused by *F. oxysporum* f. *conglutinans*	0	10	0	–
Cabbage, Wisconsin All Head	Resistant to cabbage yellows caused by *F. oxysporum* f. *conglutinans*	0	0	0	–
Cabbage, Penn State Ballhead	Susceptible to cabbage yellows	0	0	0	–
Cabbage, Danish Red	Susceptible to cabbage yellows	–	0	0	+
Mustard, Wild		0	0	0	0
Cucumber, Ohio 31	Resistant to bacterial wilt caused by *Erwinia tracheiphila*	0	0	0	–
Cucumber (Hybrid) W-4 Self	Resistant to bacterial wilt caused by *Erwinia tracheiphila*	0	0	0	–
Cucumber, Tokyo Long Green	Susceptible to bacterial wilt	0	0	0	–
Tomato, Pan-American	Resistant to fusarium wilt caused by *Fusarium oxysporum* f. *lycopersici*	0	0	0	–

TABLE 1—*Concluded*

PLANT	DESCRIPTION	HUMAN PATHOGENS			
		Staphylococcus aureus FDA 209	*Eberthella typhosa* FDA Hopkins	*Salmonella paratyphi* A	*Escherichia coli*
Tomato, Break O'Day	Resistant to fusarium wilt caused by *Fusarium oxysporum* f. *lycopersici*	0	0	15	—
Tomato, Bonny Best	Susceptible to fusarium wilt	8	0	0	—
Cauliflower W. S. 300	Resistant to black rot caused by *Phytomonas campestris*	0	10	0	0
Cauliflower, Improved Holland Erfurt	Susceptible to black rot	0	10	0	0

\+ = growth stimulation.
0 = no inhibition.
— = no assay.
Numerals indicate size of inhibition zone in millimeters.

these plates immediately after hardening of the agar, and filled with the juices to be tested. Preliminary incubation, for a period of 18 hours, of the plates containing the four species of fusaria was necessary before placing and filling the penicylinders, but was not necessary in the case of the plates containing the bacterial species. The plates containing bacteria parasitic for plants were incubated at room temperature (18 to 25 C), and those containing zoopathogens were incubated at 37 C. The juices were adjusted to the optimum pH before use. The diameters of the inhibition zones were measured and recorded in millimeters.

In some instances there was no visible zone, but removal of the penicylinder revealed a clear area. When this occurred, the zone was listed as 7 mm.

The results obtained when these juices were tested against some human bacterial pathogens are shown in table 1.

The same juices were all tested against seven phytopathogenic bacteria, *Phytomonas medicaginis* var. *phaseolicola*, *Bacillus stewartii*, *Actinomyces scabies*, *Erwinia amylovora*, *Erwinia carotovora*, *Phytomonas campestris*, and *Phytomonas solanacearum*, with the following results: The juices of all three bean varieties were stimulatory to both *E. carotovora* and *E. amylovora*. Corn, Iowa Inbred no. 4528 (*B. stewartii*—18 mm; *E. carotovora*—10 mm); cabbage, Wisconsin Jersey Queen (*P. campestris*—8 mm); wild mustard (*P. campestris*—10 mm); cucumber, Ohio 31 (*E. carotovora*—10 mm). Juices of the three tomato varieties were all inactive against these oganisms.

When the same juices were used against four phytopathogenic fusaria, the following positive results were obtained: Corn, Iowa Inbred no. 4473 (*F. oxysporum* f. *niveum*, Snyder & Hansen—7 mm; *F. oxysporum* f. *melonis*, Snyder & Hansen—7 mm); corn, Iowa Inbred no. 4528 (*F. oxysporum* f. *melonis*—7 mm);

cabbage, Penn State Ballhead (*F. oxysporum* f. *conglutinans*, Snyder & Hansen—+); tomato, Pan-American (*F. oxysporum* f. *conglutinans*—16 mm; *F. oxysporum* f. *lycopersici*, Snyder & Hansen—7 mm; *F. oxysporum* f. *niveum*—+); tomato, Break O'Day (*F. oxysporum* f. *conglutinans*—7 mm; *F. oxysporum* f. *lycopersici*—7 mm; *F. oxysporum* f. *niveum*—+; *F. oxysporum* f. *melonis*—7 mm); tomato, Bonny Best (*F. oxysporum* f. *conglutinans*—16 mm; *F. oxysporum* f. *niveum*—+); cauliflower, W. S. 300 (*F. oxysporum* f. *conglutinans*—+). All other juices were inactive.

Although some antibiotic substances have been shown to be active against plant pathogenic organisms (Brown and Boyle, 1945; Waksman, Bugie, and Reilly, 1944), it appears that such compounds are not widespread in the juices of common garden plants. Of the plants tested, only corn, cucumber, wild mustard, and cabbage demonstrated any activity against the bacterial phytopathogens, whereas the juice of corn and tomatoes was detrimental to the growth of the plant pathogenic fusaria. A rather pronounced variation in susceptibility of the four fusaria to these juices is demonstrated. Also, it is interesting that although the different tomato varieties yield juices more active against the fusaria than do the corn varieties, the latter are inhibitory to the bacterial phytopathogens which are completely resistant to the action of the tomato plant juices.

The data indicate that these juices are much more active against animal pathogens than against those causing diseases of plants. Also, the inhibition seems to be more pronounced in the case of the three gram-negative organisms than against the gram-positive organism, *Staphylococcus aureus*. Beans, corn, cabbage, cauliflower, and tomatoes are active, with the juice of corn being the most generally effective.

The many cases of growth stimulation may be due to specific stimulatory substances, or they may perhaps be examples of border-line inhibition. Further concentration and purification of these juices should yield information on this point.

It is obvious that no clear distinction may be drawn between the activities of the juices of resistant and nonresistant varieties, except perhaps in the case of the action of corn juices against the bacterial phytopathogens. However, the fact that these differences have not been demonstrated does not mean that they are nonexistent. Indeed, it is probable that in at least some of the cases our methods were not accurate enough to be useful in bringing out differences which are probably quantitative, rather than qualitative, in nature.

Work is continuing at this laboratory on the purification and characterization of the most promising of these factors.

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