

Variations in Inhibition of Growth of Five *Chlorella* Strains by Mycotoxins and Other Toxic Substances

Wide variations in the inhibition of growth of four strains of *Chlorella pyrenoidosa* and one strain of *Chlorella vulgaris* by acrylic acid, aflatoxin B₁,

diacetoxyscirpenol, digitonin, gramicidin J, and zearalenone are found.

In a previous report (Ikawa *et al.*, 1969) it was shown that *Chlorella pyrenoidosa* is sensitive to certain toxic substances of fungal and algal origin. In subsequent work with other strains of *Chlorella*, it has become evident that strains vary, sometimes very dramatically, in their sensitivity to the same compound. The types of response obtained with five strains of *Chlorella* under identical conditions to several toxic agents is illustrated below.

The strains of *Chlorella* used in this work were *Chlorella pyrenoidosa* (UNH strain maintained at the University of New Hampshire), *Chlorella pyrenoidosa* [strains 395, 251, and 252, from the Culture Collection of Algae, Indiana University,

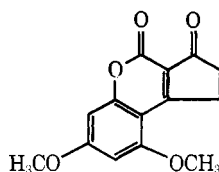
Bloomington, Ind. (Starr, 1964)], and *Chlorella vulgaris* (strain 15-2075 from the Carolina Biological Supply Co., Burlington, N.C.). The *Chlorella*-seeded agar plate technique previously described (Ikawa *et al.*, 1969) was used. The buffered medium was used, but, instead of 1.3-cm sterile paper disks, 0.6-cm disks (Difco Laboratories, Detroit, Mich.) were used, and 20 μ l of solution was applied to each disk.

As shown in Table I, marked differences in response were observed with acrylic acid, aflatoxin B₁, diacetoxyscirpenol, digitonin, gramicidin J, and zearalenone. A weak response was observed with emodin by all five strains and none of the strains was inhibited by kainic acid, rubratoxin B, or aflatoxins

Table I. Growth Inhibitory Activity of Some Toxins and Inhibitors on *Chlorella* Strains^a

Compound	Solvent	Concn, mg/ml	Diameter of net zone of inhibition, mm ^b				
			<i>C. pyrenoidosa</i>				<i>C. vulgaris</i>
			UNH	395	251	252	
Acrylic acid	Ethanol	1	36	35	0	0	0
	Ethanol	0.1	15 ^c	0			
	Ethanol	0.01	0				
Aflatoxin B ₁	DMSO ^d	1	25	18	21	21	0
	DMSO	0.1	0	0	0	0	
Aflatoxin B ₂	DMSO	1	0	0	0	0	0
Aflatoxin G ₁	DMSO	1	0	0	0	0	0
Aflatoxin G ₂	DMSO	1	0	0	0	0	0
Aflatoxin B ₁ A ^e	DMSO	1	0	0	0	0	0
Diacetoxyscirpenol	Ethanol	1	40 ^c	39 ^c	8 ^c	8 ^c	4
	Ethanol	0.1	19 ^c	26 ^c			
	Ethanol	0.01	Tr ^f	Tr			
Digitonin	Ethanol	1	17	25	0	0	20 ^c
	Ethanol	0.1	Tr	0			0
Emodin	Ethanol	1	4	6	6	6	Tr
Gramicidin J	Water	1	10	13	0	0	28
	Water	0.1	Tr	Tr			14
	Water	0.01					Tr
Kainic acid	Ethanol	1	0	0	0	0	0
Rubratoxin B	Ethanol	1	0	0	0	0	0
Zearalenone (F-2) ^g	Ethanol	1	15 ^c	Tr	14 ^c	13 ^c	0
	Ethanol	0.1	0	0	0	0	

^a Sterile disks (Difco Laboratories) of 0.6-cm diameter were used on buffered agar plates. Values of inhibition represent an average of at least three separate determinations each run in duplicate with two disks per plate. To illustrate the variability of response, the mean and standard deviation from the mean in the case of *C. pyrenoidosa* (UNH strain), on which the most assays were run, were 36 ± 3 for acrylic acid, 25 ± 2 for aflatoxin B₁, 40 ± 4 for diacetoxyscirpenol, 17 ± 3 for digitonin, 3.8 ± 0.5 for emodin, 10 ± 1 for gramicidin J, and 15 ± 1 for zearalenone, when all compounds were tested at 1 mg/ml. ^b Diameter of disk subtracted from total diameter of inhibition zone. ^c Weak growth of *Chlorella* was observed within the inhibition zone which was surrounded by a background of denser growth. This often made reading the zones difficult. In the case of diacetoxyscirpenol, small zones at complete inhibition were also observed within the zone where partial growth had occurred. ^d DMSO = dimethylsulfoxide. ^e A zone of weak *Chlorella* growth extending 3.5 mm beyond the disk was observed with DMSO blanks. This value has been subtracted from the inhibition zones observed with DMSO as solvent. This zone had been ignored in previous work (Ikawa *et al.*, 1969). ^f A synthetic analog of aflatoxin B₁ was kindly supplied by J. V. Rodricks,



Food and Drug Administration, Washington, D.C. ^g Trace indicates a net zone diameter of 3 mm or less. ^h F-2 crystals were kindly supplied by C. J. Mirocha, University of Minnesota, St. Paul, Minn.

B₂, G₁, G₂, and B₁A under the conditions of the experiment. Differences in the behavior of strains of *C. pyrenoidosa* and *C. vulgaris* to the action of various herbicides can be and have been attributed to strain differences (Vance and Smith, 1969; Thomas *et al.*, 1971) and to differences in growth conditions. By changing from autotrophic conditions to heterotrophic conditions by the addition of a reduced carbon source such as glucose, it has been shown that the inhibitory effects of some herbicides which affect the photosynthetic process on strains of *C. pyrenoidosa* and *C. vulgaris* can be reversed (Geoghagen, 1957; Ashton *et al.*, 1966; Sikka and Pramer, 1968). In order to reduce the time of the assay and in order to avoid special provisions for a carbon dioxide source, glucose has been used as a carbon source under our conditions.

The results presented here emphasize the importance of strain differences when *Chlorella* is used as a test organism.

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Correction

DETERMINATION OF CARBETAMIDE RESIDUES
AND ITS ANILINE METABOLITE

In this article by Alvaro Guardigli, William Chow, and Morton S. Lefar [*J. Agr. Food Chem.* **20**(2), 348 (1972)], on page 348 the first sentence, last paragraph of the second column, should read: "To the acidified hydrolyzed aqueous extract was added 25 ml of benzene followed by 10 ml of a 0.01% solution of 4-bromobenzoyl chloride in benzene."

Correction

AMINO ACID COMPOSITION OF BUCKWHEAT

In this article by Yeshajahu Pomeranz and George S. Robbins [*J. Agr. Food Chem.* **20**(2), 270 (1972)], on page 272 the footnote to Table III should read: "All *absolute* figures above 0.632 are in italics and all figures above 0.765 are in bold face to indicate statistical significance."