



Allelochemical activity of naturally occurring compounds from mungbean (*Vigna radiata* L.) plants and their surrounding soil

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(Received May 25, 1994; Accepted November 17, 1994)

Abstract. Continuous cropping of mungbean (*Vigna radiata* L.) can lead to plant growth inhibition. We have found that allelopathy may be the cause of as much as 10–25% of the inhibition in mungbean plants grown following mungbean plants. Mungbean plants are allelopathic, and the surrounding soil is often toxic. The phytotoxic activity is found primarily in the stems and the aerial parts (excluding the stems). We partitioned stem extracts with water and with organic solvents: the water extracts produced the greatest inhibition of mungbean and lettuce; the organic solvents caused both inhibition and stimulation. Bioassay of compounds present in the soil after harvesting mungbean (72-h incubation) plants showed inhibition of mungbean plants grown to maturity—*p*-hydroxycinnamic acid was identified as a major compound in mungbean plants and their rhizosphere soil. The discovery of enhancement of mungbean growth by crude mungbean saponins was serendipitous; the plants showed quicker germination and enhanced growth, but such treatment did not increase the yield.

Keywords: Allelochemical activity; Continuous cropping; Mungbeans; Phytotoxic activity; Saponins; Soils; *Vigna radiata* L.

Introduction

Allelopathic chemicals are secondary plant metabolites that have roles in plant-plant, plant-soil, plant-disease, plant-insect, and plant-predator interactions; they may be beneficial or detrimental to the plant. Mungbeans (*Vigna radiata* L.) planted in soil that has recently been used to grow mungbeans (plant-soil interaction) can encounter these metabolites. Mungbeans, an economic crop plant in Taiwan and many developing countries, were not known to have allelopathic activity until the work of Tang and Zhang (1986). They isolated the inhibitory compounds and found three *C*-glucosyl flavanoids; isovitexin was the most active. They introduced the concept of allelochemical spheres and extended their observation from the germinating seed to the plant root system. Many biologically active metabolites occur at the root-soil interface (Tang et al., 1989), thus the rhizosphere can be rich in allelochemicals. The *C*-glycosyl flavanoids that were identified and bioassayed are present predominantly in the seed coat, not in the growing tissue of the mungbean plants. They possess only a slight inhibitory effect on lettuce seedlings, and even less on mungbean seedlings; their role in lowering the production of mungbeans remains unknown.

In 1980, the Asian Vegetable Research and Development Center (AVRDC) of Taiwan (1980, 1981, 1983) noted that when five consecutive mungbean crops were grown without fallowing, there were non-uniform growth patterns and the plants were smaller and produced fewer pods per plant, fewer and lighter seeds, and poor yields (25 kg/ha). In comparison, where mungbeans had not been grown for at least three cropping seasons, the yield was 440 kg/ha. This led to the recommendation that a mungbean crop should not be followed by another such crop for at least three cropping seasons (AVRDC, 1980, 1981, 1983, and Wang, 1984). Among different crops (mungbean, soybean, tomato, Chinese cabbage, sweet potato, corn, crotalaria, sorghum, and buckwheat), mungbean was the most detrimental to a succeeding mungbean crop. In one instance, the mungbean yield following a previous mungbean crop was 65 kg/ha, compared to 346 kg/ha after a tomato crop.

A series of experiments was performed using a plant culture system designed to determine whether an allelopathic agent existed in the mungbean plant (C.C. Young, National Chung-Hsing University, Taiwan, pers. comm.). The results strongly indicated that the mungbean plant produces phytotoxic substances in its aerial parts and its root system, but he did not follow up this lead. A few years

later a mungbean root disease in the Philippines was described. It was reported that the primary cause was not fungi, but the researchers did not mention that allelochemicals from the mungbean plant might have been involved (Ventura et al., 1984).

Cheng (1989) suggested that the establishment of a specific cause/effect relationship should be sought via allelopathy experiments in which the following steps occur: a) a phytotoxic chemical is produced by a plant or from plant materials; b) the chemical is transported from its source to the target plant; c) the target plant is exposed to the chemical in sufficient quantity and for sufficient time to cause damage. In this paper we present data that records an attempt to establish this cause-and-effect relationship for allelopathy in the mungbean plant-soil system. A preliminary account of this work has been published (Waller et al., 1993).

Materials and Methods

Soil and Mungbean Information

Control soil was collected from the Asian Vegetable Research & Development Center (AVRDC) located at Tainan, Taiwan, in December, 1991 and February, 1992; the times of collection are shown in Table 1. The soil was type AS-2, sandstone shale, older alluvial, 3-noncaliferous soil (Wang and Shen, 1988). No mungbeans had been grown in the soil for at least three years. The December soil had been planted with a green manure crop of buckwheat (*Fagopyrum esculentum* Moench.) during the preceding year, and the February soil had lain fallow. The soils were stored at room temperature (16–40°C). The soil was air-dried, passed through a 1.5-cm² screen to remove large rock and plant debris, and pulverized. The small roots and stones were picked out, the soil was passed through a 20-mesh (0.46 mm²) sieve (Tokyo), and the soil was thoroughly mixed. Five hundred grams of soil was

placed in each pot, and 20–100 g was left to be divided among several petri dishes.

Vigna radiata L. (Wilzek) cv. Tainan No. 3 and Tainan No. 5 were grown to maturity (~90 days) in the plots designated.

Mungbean Pot Experiments to Demonstrate Allelopathy

These experiments were designed to measure the effect of growing mungbeans in soil that was previously used to grow mungbeans. The experiments were time-consuming (~90 days) and subject to considerably more variation than is the 72-h bioassay that is frequently employed. Control soil in which no mungbeans had been grown for the past three years was used in all experiments (Table 1, plots 40, 41, 74, and 75).

Allelopathic effect of mungbean soils used to grow mungbeans (81 days)—This experiment was designed to get significant data from 12 replications in a random arrangement. The plants were rotated every second day so that they received approximately the same exposure to light and temperature. Four seeds were planted per pot. After 1 week, the plants were culled to 2 per pot and grown for 85 days in a warm room with a 14L/10D photoperiod, a photon flux of 190 $\mu\text{E m}^{-2} \text{s}^{-1}$, and temperatures of 26–28°C (day) and 17–20°C (night). The experiment was started in early February, 1992. Soils (Table 1) used were: a) control soil from plots 40 and 41; b) 1-week soil (this terminology is used to refer to soil collected at the specified time after harvest) from plot number 38; c) 4-week soil from plot number 33; d) 7-week soil from plot number 38.

Allelopathic effect of mungbean soils (41 days)—This experiment repeated the pattern of the 81-day experiment using soils that had been exposed to atmospheric conditions at AVRDC for longer periods of time. It was not pos-

Table 1. Soils used for the allelopathic experiments for mungbean (MB) from AVRDC collected from plots during December 1991–June 1992.

Date collected	Treatment of soil	Soil plot numbers:							
		Weeks after harvest of mungbeans							
		Control	1	4	7	14	16	19	27
Dec. 11, 1991	3 yrs since MB were grown	40, 41							
	Plants left standing		38						
	MB plowed under			33					
Jan. 23, 1992	MB plants left standing			34					
	MB plants left standing				38				
Feb. 1992	Fallow since 1991	74, 75							
Mar. 10, 1992	MB plants plowed under					38			
Apr. 14, 1992	Planted in corn ^a and soybeans ^a								
	Plowed under						34		
	Planted in tomatoes ^b						38		
								33	
June 17, 1992	Plowed under								38

^a Soil sample collected beside the two crops.

^b Samples collected from the tomato bed row.

MB=mungbean.

sible, however, to use identical soil, because soils change over time. This experiment was begun in April, 1992. The soils (Table 1) used were: a) control soil collected from plots 74 and 75; b) 1-week soil collected from plots 33 and 34; c) 4-week soil collected from plots number 33 and 34; d) 7-week soil collected from plot number 38; e) 14-week soil collected from plot number 38; f) 19-week soil collected from plot number 33; g) 27-week soil collected from plot number 38.

There were 8 pots per treatment, each containing approximately 500 g soil and 4 seeds. After 1 week, the plants were culled to 2 per pot and grown for 41 days in a warm room with a 14L/10D photoperiod, a photon flux of $1190 \mu\text{E m}^{-2} \text{ s}^{-1}$, and temperatures of 28°C (day) and 26°C (night). The condition of the plants was observed and recorded, and the height was measured periodically. The experiment was terminated after 81 days.

Effect of mungbean plant parts mixed into the control soil—Four seeds were planted in each pot of control soil (plots numbers 40 and 41), 2 pots per treatment. Mungbean plant parts—large stems 0.5%, roots 0.2%, and tops 0.9% (small stems, leaves, hulls from mungbeans, and seeds)—were mixed thoroughly into 500 g soil. After 1 week, the plants were thinned to 2 per pot and grown in a plant growth chamber (Chang Kuang, Taipei, Taiwan) with a 14L/10D photoperiod and temperatures of 28°C (day) and 24°C (night). Plants were watered regularly. The condition of the plants was observed and recorded, and the height was measured periodically. The experiment was terminated after 90 days.

Bioassay Techniques

The bioassay experiments were designed to compare the early growth of mungbeans, lettuce, wheat, and tomato treated with water extracts and other solvent extracts of mungbean plants to a control treated with distilled water.

Modifications of the procedures of Chou and Young (1975) and Wyman-Simpson et al. (1991) were used with aqueous extracts of mungbean plants (1%, 2%, 3%, 4%, and 5%). Mungbean, lettuce, and wheat seed bioassays were performed three times using twenty seeds each.

Four milliliters of organic solvents containing the suspected phytotoxins and control organic solvents (hexane, ethyl ether, chloroform, 1-butanol, and ethyl acetate) were placed on one layer of Whatman No. 4 (12.5 cm) filter paper under a ventilation hood and left there until the organic solvent evaporated. The dried filter paper was placed in a polystyrene petri dish. After pre-wetting the seeds with the phytotoxic solutions for 10 h, they were placed in the petri dish, 4 ml of distilled water was added, the dish was covered, and the assembly incubated as above. For the lettuce bioassay, a 2-ml portion of each of the organic solvents containing the test compounds was placed on two layers of Whatman No. 3 MM 5×5 cm filter paper in a glass petri dish and allowed to dry under a ventilation hood, 2 ml distilled water was added, the dish was covered, and the assembly incubated as above.

Soil Bioassay Method

Soil (5 g) from AVRDC that had been used for growing mungbeans and was suspected to contain phytotoxins was uniformly distributed on one layer of Whatman No. 4 (9 cm) filter paper in a glass petri dish, seeds were arranged on the paper in a circle (mungbean, wheat, tomato, and lettuce), 4 ml of distilled water added, and the assembly incubated as above.

The lengths of the radicle of the seedlings of mungbean and lettuce were measured. The few plants showing signs of fungal activity, bacterial contamination, or changes in the appearance of the seedlings, such as root tip browning or necrosis, were discarded.

Statistical Analysis of Data

Mean lengths per dish per treatment were determined, and the Analysis of Variance (ANOVA) was calculated. The difference between samples and the corresponding controls was indicated by percent inhibition or stimulation and by Duncan's Multiple-Range Test (Steel and Torrie, 1985).

Extraction and Isolation Procedures to Obtain Material for Bioassays

Distilled water—Distilled water was used to extract mungbean powder (leaves, stems, and roots) at a concentration of 5% (w/v) in an erlenmeyer flask. The mixture was shaken for 2 h at room temperature with an orbital shaker (Model S-102, Firstek Scientific), successively filtered through Whatman No. 1 and 42 papers under the vacuum of a water aspirator, and diluted to 5, 4, 3, 2, and 1%. The osmotic pressure was measured with an osmometer (Model OS Osmometer Fiske Associates, Needham Heights, MA, USA), and pH values were measured.

Organic solvents—Extraction and isolation was a modification of the procedure of Weston et al. (1987) for possible mungbean phytotoxins (Figure 1). Aqueous extracts were made with distilled water at 1%, 2%, 3%, 4%, and 5%. The slurry was stirred at $4-7^\circ\text{C}$ for 24 h, strained through muslin to remove most of the solid materials, and sequentially filtered through Whatman No. 1 and 42 papers. The volume of the filtrate was reduced with a rotary evaporator, and with the use of a lyophilizer (Model No. 10-010, The Virtis Co., Gardiner, N. Y., USA), a brown dry powder was produced. Hexane, ethyl ether, chloroform, 1-butanol, and ethyl acetate were used (40 ml of each) to extract a portion (1 g) of the dry powder for 18 h; each extract was filtered through Whatman 42 filter paper and diluted to 0.1% and 1.5% in preparation for the bioassay.

Saponin Isolation and Purification

Saponins were isolated and purified (extraction with 1-butanol followed by 5-h dialysis) and analyzed according to the procedure of Waller et al. (1993).

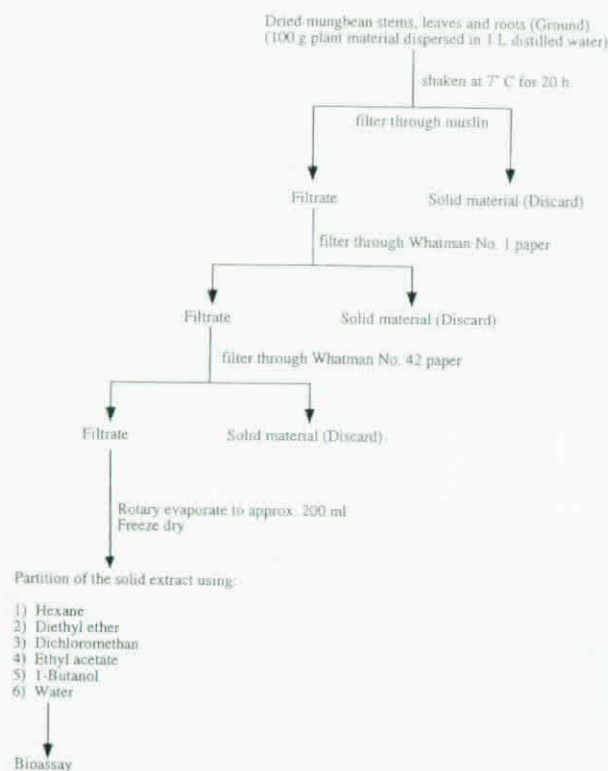


Figure 1. Extraction procedure used for isolation of the naturally occurring phytotoxins present in the mungbean plant part.

Plants for Allelopathy Tests

Lettuce (*Lactuca sativa* L., var. Great Lake) seeds were obtained from the Taiwan Agriculture Materials Co., Taipei, Taiwan; mungbean (*Vigna radiata* L. cultivars, Tainan 3, Tainan 5), and tomato (*Lycopersicon esculentum* Mill.) seeds were obtained from the Tainan District Agricultural Improvement Station, Tainan, Taiwan, and wheat (*Triticum aestivum*, CV Pioneer 2157) seeds were obtained in Oklahoma, USA in March 1985 and stored at 4°C.

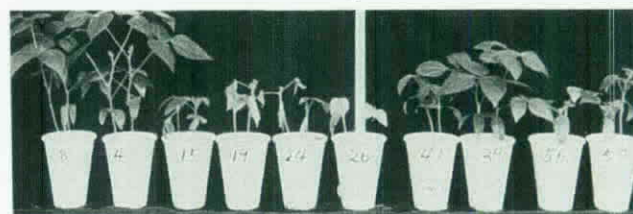
Results

Soils were used without adding fertilizers, insecticides, fumigants, or other agents, except the mungbean plant parts that were mixed with soil. The mungbean were allowed to grow naturally. Values of pH and overall inhibition results are shown in Table 2.

Growth of Mungbean Plants in Soil Taken at Different Intervals After a Previous Mungbean Harvest

Figures 2 and 3 provide evidence of allelopathy for plants grown for 40 and 81 days. The numbers on the pots (Figure 1) refer to one of the twelve plants grown in a particular plot of soil. The pH of plot number 33 was 7.8, and 65–70% inhibition occurred (Figure 3). There was no statistical difference between the 1-week, 4-week, and 7-week soils. This provides evidence that alkaline soil promotes allelopathic activity. In contrast there was a only a negligible amount of allelopathic activity in plot number 34, which had a pH of 6.7. There was no apparent fungal attack on the plants grown in plot 34 (acidic soil), which indicates that the phytotoxic activity in the acidic soil was considerably less than in the alkaline soil. This effect of pH on the allelopathic factors in the growth of mungbean is repeatable and represents a new observation.

40 days



Control 1WK 4W 4W 7W
Plot 33 Plot 34

81 days



Control 1WK 4W 4W 7W
Plot 33 Plot 34

Figure 2. Allelopathy of mungbeans growing in AVRDC soil for 40 and 81 days. Control soil: plots 40 and 41 (mixed), 1 week (plot 33): 15, 19, 14, and 20; 4 weeks (plot 33): 24, 26, 35, and 36; 4 weeks (plot 34): 41, 39, 45, and 38; 7 weeks (plot 33): 56, 57, 50, and 60.

Table 2. Determination of pH and summary of inhibition results from the pot experiments with AVRDC soils.

Soil plot	Age (after MB harvest)	pH	Summary of inhibition
33*	4 weeks	7.8	Yes
34	4 weeks	6.7	Very slight
34	16 weeks	6.6	Very slight
38	1 week	7.3	Yes
38	7 weeks	7.8	Yes
40, 41	Control	7.9	—
74, 75	Control	8.0	—

*Both control soils as well as those of plots 33 and 38 were alkaline whereas the soil of plot number 34 was acidic.

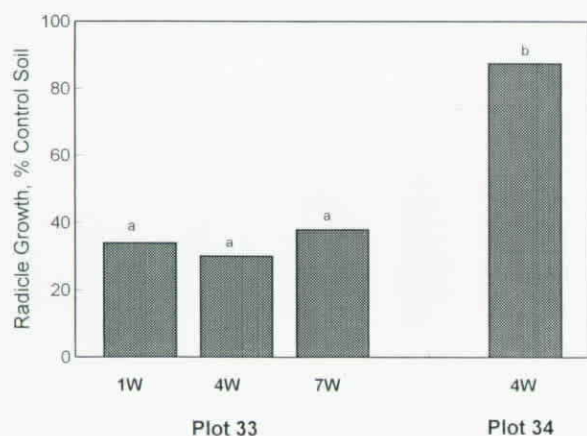


Figure 3. Allelopathy of mungbean plants grown in AVRDC soil following mungbeans. Duration of experiment (81 days); W = weeks following harvest of mungbeans; bars having different letters are significantly different, $P = 0.05$, ANOVA with Duncan's Multiple-Range Test.

A 40-day experiment was run (Figure 4), but the soils used had been stored at room temperature (15–40°C) from December 1991 until July 1992. This storage at elevated temperatures permitted microorganisms to grow using the naturally occurring phytotoxins as their substrates and thus becoming more active in the production of abnormal and normal metabolites. After 4 weeks, plot 33 (pH 7.8) showed the greatest inhibition. The soils of plot 38 (pH 7.3–7.8) (1, 7, 14, 19, and 27 weeks) were significantly different in their inhibitory activities.

Problems are always found in soils stored at room temperature—they are not exposed to similar conditions in the field. This makes the estimate of the interval required before the soil returns to its normal state a risky one. At 27 weeks, the soils still had about 25% inhibitory activity, so our best estimate is 1 year before the soil returns to non-allelopathic state. This is an improvement over the AVRDC recommendation of 3 years.

Control Soil Mixed with Mungbean Plant Parts

Table 3 shows the overall result of comparing the height and weight of plants grown for 81 days in the modified soil to those grown in the control soil. The stems inhibited the height at a 0.5% concentration and the roots caused almost the same degree of inhibition at a concentration of 0.2%, but leaf or other aerial parts at a concentration of 0.9% caused about a 15% increase in plant height. The stems caused the greatest inhibition of dry weight, the roots showed somewhat less, but the leaf/aerial soil mixture showed there was a 24% increase. It is clear that stems of the mungbean plant contain the predominant chemicals that exert their allelopathic activity toward mungbean plants. The plant material added was estimated, based on weights obtained from plants grown in the field just prior to harvest, to be about the normal amount plowed under in the field.

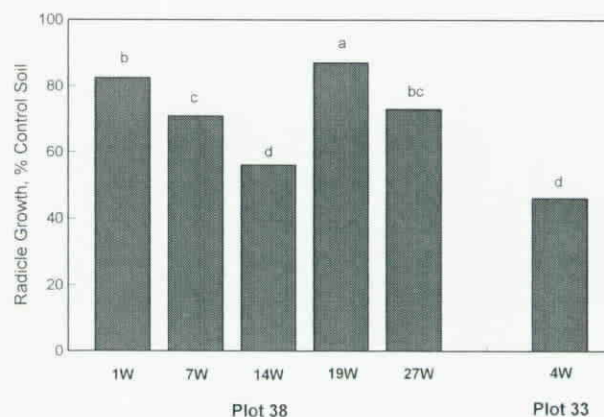


Figure 4. Allelopathy of mungbean plants grown in AVRDC soil following mungbeans. Duration of experiment (41 days); W = weeks; bars having different letters are significantly different, $P = 0.05$, ANOVA with Duncan's Multiple-Range Test.

Table 3. The effects of mixing mungbean residue powder with control soil on the growth of mungbean at 81 days.

Treatment	Growth and weight, % of control	
	Height	Dry weight
mixing rate (g/g)		
Root powder/soil (0.2%)	98ab	76
Stem powder/soil (0.5%)	87b	33
Leaf/aerial powder/soil (0.9%)	115a	124

Values are the means of 12 replicates. The letters in a column not followed by the same letter are different, $P = 0.05$, ANOVA with Duncan's Multiple-Range Test.

These data suggest that the complete mungbean plant when plowed under might have a negligible allelopathic effect on the new crop of mungbeans, since the effect of chemical compounds that can inhibit plant growth can be overcome by those which have stimulatory activity. As the plant material decays in the soil, residual compounds, sometimes in high concentrations do occur. Such compounds if allelopathic, could also stimulate or inhibit the growth of a crop of mungbeans planted soon after the harvest.

Bioassay of Mungbean Plant Parts

Aqueous extracts of roots (Figure 5) showed a significant inhibition of lettuce and wheat seedlings and a small but typical inhibition of mungbean seedlings. The assay was run at the same pH (7.0 ± 0.3) as that of the plant parts extract. The osmotic concentration ranged from 16 to 82 milliosmol kg^{-1} in the 1–5% concentrations. A bioassay was run on mungbean and lettuce at 25 milliosmol kg^{-1} . It showed 12% and 27% inhibition, respectively (Cheng, 1993).

The bioassay of extracts of mungbeans leaves (Figure 6) demonstrated a significant of inhibition of mungbean, lettuce, and wheat. The pH of the extracts were 6.0 ± 0.2

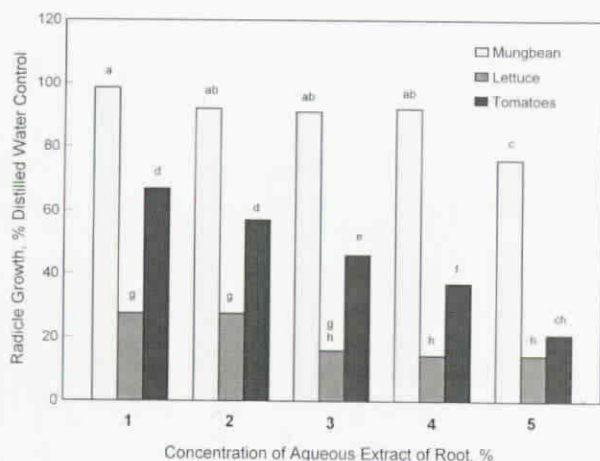


Figure 5. Inhibition of mungbeans, lettuce, and tomato by application of aqueous extractions of mungbean roots for 72 h. Legend: Bars having different letters are significantly different, $P = 0.05$, ANOVA, with Duncan's Multiple-Range Test.

and remained constant throughout the assays. The osmotic concentration ranged from 20 to 87 milliosmol kg^{-1} , which was nearly the same value as for the roots. The inhibition by stem extracts (not shown) was similar to that of the leaf extracts. The stem extracts had pH values of 6.3 ± 0.3 and an osmotic concentration that was almost identical to that of the leaf fraction.

The results of the root extract bioassay agree with the findings on the whole plants grown for 81 days (Figure 3).

Bioassay of Compounds from Mungbean Stems Extracted into Water and Partitioned into Organic Solvents

The effect of extracts made with distilled water and then partitioned with organic solvents is shown in Figure 7 (top and bottom) at 1% and 15% of the original plant weight. There was mixed inhibition and stimulation of mungbean and lettuce. Chloroform and hexane extracts showed slight stimulation of growth, whereas the water, ether, 1-butanol, and ethyl acetate extracts inhibited mungbean growth. At a concentration of 1% the extracts of lettuce stems were somewhat less inhibitory than were those of mungbeans, but showed greater inhibitory activity at 15%.

It appears that this partitioning system provides information on the compounds responsible for the effects described. This is not discussed here. The water extract was the most inhibitory at both concentrations; it contains phenolic acids and other soluble chemical compounds from the partitioning by organic solvents.

Bioassay of Soils After Mungbean Harvest

The bioassays of mungbean soils (Figure 8) support the experiments with mungbean plants grown to maturity (Figure 4)—with the exception of the lack of allelopathic activity shown by the 1 week soil. The soil samples taken after 4 weeks from plots 33 (pH 7.8) and 34 (pH 6.7) showed approximately 8% and 18% inhibition respec-

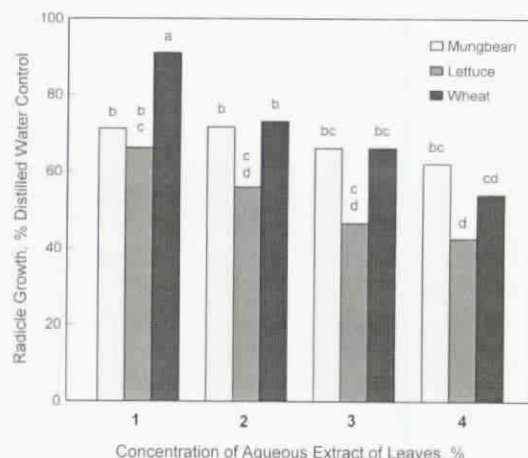


Figure 6. Inhibition of mungbeans, lettuce, and wheat by application aqueous extracts of mungbean leaves for 72 h. Bars having different letters are significantly different, $P = 0.05$, ANOVA, with Duncan's Multiple-Range Test.

tively, where as at 7 weeks the inhibition of mungbean was much greater (~32%).

The effect on lettuce was surprising in that the compounds present in the 1-week and 4-week alkaline soil were most inhibitory, but the 4-week acidic soil and 7-

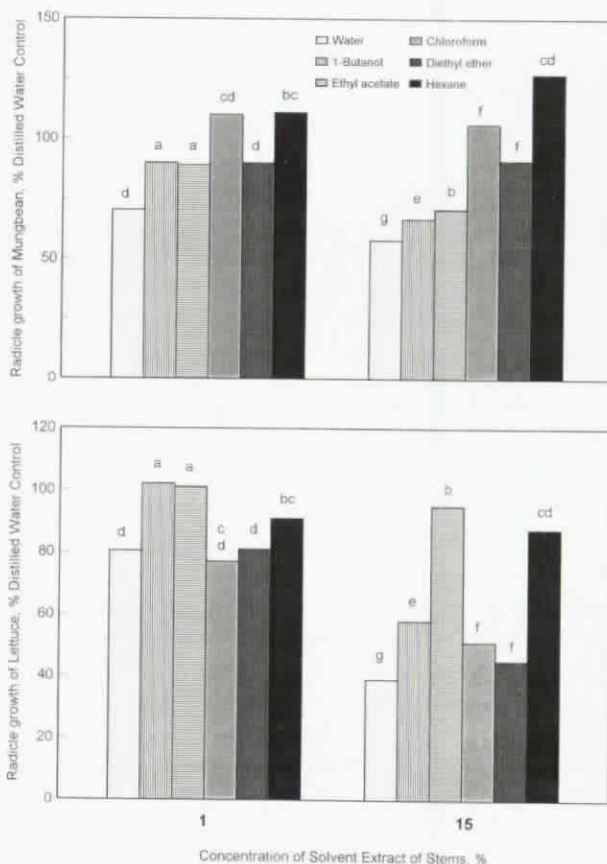


Figure 7. Allelopathic effect of application of water- and organic solvent- extracts of mungbean stems on mungbeans (top) and lettuce (bottom) for 72 h. Bars having different letters are significantly different, $P = 0.05$, ANOVA, with Duncan's Multiple-Range Test.

week alkaline soil were slightly stimulatory. Clearly, the compounds extracted from each soil differed markedly in amount and type.

Enhancement of Mungbean Growth by Crude Mungbean Saponins

Crude mungbean saponins (Waller et al., 1993) were applied to the soil at concentrations of 15, 150, and 450 ppm. Mungbean seeds were germinated in pots and allowed to grow until maturity. The height of the plants was recorded for several experiments, but only the pertinent results are included in Table 4. The experimental plants clearly showed elongation of stems and other growth-enhancement effects when the 1-butanol-extracted and 5-h-dialysis-treated saponins were added, but we cannot be certain that all the active compounds are saponins. Mungbean plants showed quicker germination and other enhancement effects throughout their growth. The plants had larger, darker green leaves, which is an indication of enhanced photosynthesis, but the number of seed pods was about the same; the increased growth was not accompanied by an increased yield. After 40 days, all concentrations caused about 10% enhancement of growth, but after 67 days the two higher concentrations showed 10% and 15% enhancement respectively, while the effect of 15 ppm of saponin continued to drop to nearly zero. At the end of the experiment (84 days) the lowest concentration had no effect, and 150 and 450 ppm saponins showed 20–25% enhancement (measured by height of the plant). The preliminary indications are that 150 ppm of crude saponins (5-h dialysis) is required to enhance mungbean growth.

The crude saponins added to the soil may have provided a source of nutrients. Little effort was made to remove inorganic elements, other than dialysis for 5 h (a common technique to reduce the inorganic nutrients and small organic molecules by a factor of 30–50). Our results should be considered preliminary.

Table 4. Enhancement of the growth of mungbeans by soyasaponin I and other saponins produced by mungbean plants added to AVRDC soil.

Date (1992)	Day	Observations of pots ^{a,b,c,d} (Compared to control without saponins)
Feb. 13	0	Experiment started
Feb. 21	8	Primary leaves, no trifoliolate, growth enhancement with 88% germination as compared with 58% of the control
Feb. 25	12	First trifoliolate, growth enhancement
Feb. 28	15	Second trifoliolate, growth enhancement
Mar. 4	20	Third trifoliolate, growth enhancement
Mar. 7	23	27% higher than control; more third trifoliolate
Mar. 14	30	Less growth enhancement
Mar. 24	40	Plants average 10% higher than control; some flowering
Apr. 20	67	Plants average 13% higher than control; pods appear; maturity attained in a shorter time than for control
May 6	83	Plants 20–25% higher than control

^aSaponins were extracted with 1-butanol and purified by 5 h dialysis with 3000 Mw tubing.

^bConcentrations of added saponins were 15, 150, and 450 ppm.

^cSoils from Asian Vegetable Research and Development Center where no mungbeans were grown during the previous three years; plots 41 and 42.

^dSmall differences of enhancement may be seen at: flowering, pod maturity, not pod number.

Attempts to recover the saponins immediately following their addition to the soil were unsuccessful using several extraction techniques, but no humic or fulvic acid was isolated. This was interpreted to mean that the saponins added might become bound to the humus fraction of the soil.

Roots and their rhizosphere have been the subject of research for more than a hundred years, but their action on biological membranes and sites of enzyme action have not been thoroughly treated, although Tang et al. (1989) alluded to the reactions that occur at the molecular level. Soil moisture, temperature, gases, humus, and inorganic (mineral) and organic compounds have important effect on the development of the root system, and interact with each other in establishing the pattern of root development and function. Mungbean roots can take part in symbiotic

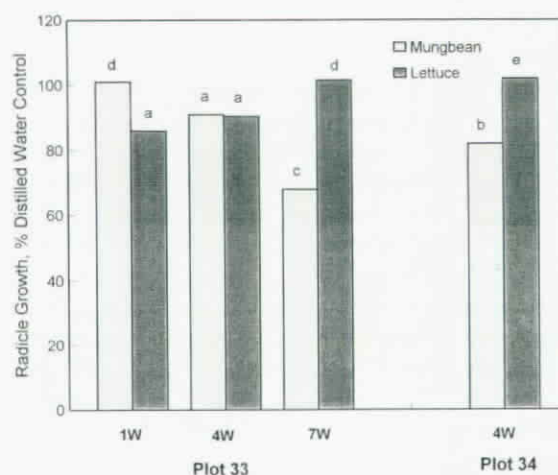


Figure 8. Allelopathic effect of soil on mungbean and lettuce seedlings after 72 h. Soil (5 g) was placed on the filter paper, distilled water and seeds were added, and the culture was set to incubate. Bars having different letters are significantly different, $P = 0.05$, ANOVA, with Duncan's Multiple-Range Test.

associations with mycorrhizae and bacterial nodules where fungi and bacteria bring in mineral constituents in exchange for some of the plants vitamins, carbohydrates, etc. We suggest that saponins, such as soyasaponin I (Figure 9), which is the predominant saponin present in 7-day old mungbeans (Waller et al., 1993) can become attached to the root hair and facilitate the transport of water and nutrients. The sugar portion of the saponin is responsible for the primary enhancement of mungbean growth; but it could be hydrolyzed off by enzymatic cleavage and produce another saponin molecule.

The saponins occurring in the mungbean rhizosphere prior to plant maturity may undergo several changes in the microstructure of cell membranes (Potter and Kimmerer, 1989). The rhamnose-galactose-glucuronic acid-soyasapogenol B (soyasaponin I) (Figure 9) is the dominant structure during the early germination period. We suggest that as the plant develops, that hydrolysis catalyzed by enzymes produced by microorganisms in the soil cleaves the sugars from the saponin in the same manner as for medicagenic acid glycosides (Oleszek et al., 1992; Waller, 1989) to give the aglycone (soyasapogenol B). Although the structural integrity of soyasapogenol B is not understood, this compound can be further broken down by microorganisms to serve as a carbon source. Hydrolysis may progress through the sequential cleavage of sugars (which we think most likely) (Figure 9), or cleavage could release the rhamnose-galactose-glucuronic acid as 2- or 3-piece fragments, leaving soyasapogenol B. Because plant root hairs have a short lifetime, the process would be repeated many times, continually exposing the root hairs to saponin molecules.

This discussion assumes that the 1-butanol-extracted saponins, purified by dialysis for 5 h, were pure. Other compounds (e.g. steroidal glycosides), however, may be present. It is important to recognize that the saponin has a strong detergent action, regardless of whether it is a triterpenoid or a steroidal glycoside.

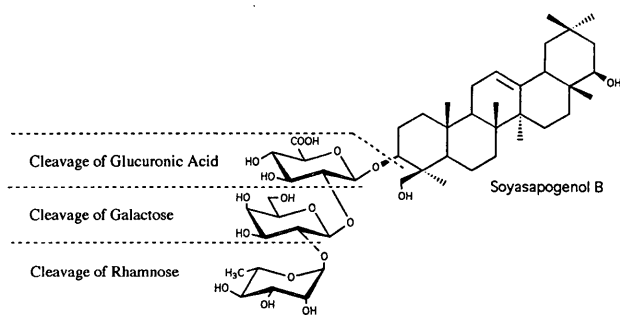


Figure 9. Suggested structural changes in soyasaponin I (3-O-[α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-galactopyranosyl-(1 \rightarrow 2)- β -D-glucuronopyranosyl] soyasapogenol B) that occur on the mungbean root surface and in the surrounding rhizosphere.

Discussion

The inhibition of growth of mungbeans in pots, was found to be 20–25%, and was often less, depending upon temperature, water, and soil characteristics. As the plants developed for 30–45 days, the effect of phytotoxins from the mungbean plant almost disappeared, and the root pathogens grew with pronounced deleterious effect on the mature plants. There is evidence in the literature that Taiwan, the Philippines, and Kenya all have a problem with continuous cropping of mungbeans (Poehlman, 1991) that is associated with root pathogens. The present paper recognizes for the first time the role that allelopathy or allelochemicals have in causing damage when mungbeans are planted in the soil in which mungbeans were previously grown.

The inhibitory effect of partitioned extracts of mungbean stems show that the naturally occurring phytotoxins were distributed throughout the water, 1-butanol, ethyl ether, and ethyl acetate fractions, which indicates that several types of compounds are represented.

We find that under certain conditions, mungbean plants grown under continuous cropping conditions in subtropical or tropical regions suffer reduction in height and yield from allelochemicals. There are allelopathic phytotoxins from the mungbean plant present at all stages of growth. Soil acidity is an important factor: the more alkaline the soil, the more active the metabolism of the microorganism population. Each of these factors, and perhaps others, should be considered when growing mungbeans.

Mungbean saponins might interact with dioxins in a manner similar to the brassinosteroids (Mandava, 1988). Early references (Heftman, 1965 and references cited therein; Rosenthal and Berenbaum, 1992) to the role of various saponins produced by plants indicate that low concentrations promote germination, high concentrations inhibit the growth, and treating the seed has a lasting growth-promoting effect. Bisset (1991) states that one reason saponins are advantageous to the plant producing them is that they function as growth regulators. This research provides the first definitive evidence that when saponins produced by mungbean plants are added to the soil, they enhance the growth of new mungbean plants as an allelochemical plant growth regulator.

Acknowledgments. We appreciate the assistance of C. Y. Chiu for advice and critical discussion on the soils, O. C. Dermer for critically reviewing this manuscript, and J.M. Mertz for the computer drawings of the bioassay data. This is published with the approval of Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, OK. G. R. Waller expresses appreciation to Academia Sinica, Institute of Botany, Taipei, Taiwan, and the National Science Council, Republic of China on Taiwan, for financial support for the sabbatical year, 1991–92 under Grant No. NSC 82-0211-13-001-04 to C.H. Chou.

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在綠豆植物及其周邊土壤中具天然植物 相生相剋之活性物質

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在世界許多地方如台灣，連作綠豆後會產生相當多的問題。在台灣，栽植綠豆一次後，再植綠豆則會有 10-25% 之生長抑制作用。這個抑制作用源自綠豆植物本身及其周圍土壤。其中植物毒性存在於綠豆莖及地上部植株，而根部則不顯著。以不同有機溶劑分離綠豆植物體之水溶萃取液之植物毒物質，而發現在水層之植物毒性最高。在綠豆收割後，其土壤中所存在的植物毒物質會抑制綠豆植物之生長至成熟的階段。然而，在土壤中可萃得粗萃取的皂苷物質，此粗萃取物對綠豆發芽及生長有促進作用，但對綠豆生產量卻無助益。

關鍵詞：植物相生相剋作用；連作；綠豆；植物毒物作用；皂苷；土壤；*Vigna radiata* L.°