THE USE OF HEMICELLULOSIC WASTE MATTER FOR REDUCTION OF THE PHYTOTOXIC EFFECTS OF CHITIN AND CONTROL OF ROOT-KNOT NEMATODES

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Accepted:
16.IV.1985
Aceptado:

ABSTRACT

Culbreath, A. K., R. Rodríguez-Kábana, and G. Morgan-Jones. 1985. The use of hemicellulosic waste matter for reduction of the phytotoxic effects of chitin and control of root-knot nematodes. Nematropica 15:49-75.

In a greenhouse experiment, alkaline hemicellulosic waste material (HW) from the paper pulp industry was added to soil at six levels (0-2.0% w/w) alone and in combination with two levels (0 and 2.0% w/w) of crustacean chitin to control *Meloidogyne arenaria* (Neal) Chitwood. The treated soils were kept moist for one week before being planted with 'Yellow Crookneck' squash (*Cucurbita pepo* L.). Six weeks after planting, survival rate of the seedlings in chitin-amended soils was improved by the addition of HW; no plants survived in soils treated with chitin alone. HW amendments reduced galling of the roots by *M. arenaria,* but were not as effective as chitin. Galling was eliminated in plants from chitin-amended soils regardless of the level of HW added. Soils receiving chitin and HW had lower pH than those amended with chitin alone. Upon removal of the squash plants, 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) seedlings were planted in the same soils. Six weeks after planting, roots and shoots of plants grown in soil amended with chitin and HW were heavier than those of plants grown in soils treated with chitin alone. While HW alone had little effect on galling of tomato roots by *M. arenaria,* no galling occurred on any plant grown in chitin-amended soils. Nematode control with chitin amendments was not affected by addition of HW. Following removal of the tomato plants, soils receiving chitin and HW had higher pH than those soils receiving chitin alone. Reduction of the phytotoxic effects of chitin by HW is interpreted as being due to the immobilization of excess nitrogen through stimulation of microbial activity by the added carbon source, the buffering effect of the hemicellulose on soil pH, or a combination of the two.
Additional key words: biological control, organic amendments, use of industrial wastes, phytotoxicity, chitin.

RESUMEN

Culbreath, A. K., R. Rodríguez-Kábana, y G. Morgan-Jones. 1985. La utilización de desechos hemicelulósicos para reducir la fitotoxicidad de la quitina en el combate de los nematodos noduladores. Nematrópica 15:49-75.

En un experimento de invernadero se enmendó el suelo con seis niveles (0-2.0% w/w) de material hemicelulósico (MH), desecho de la industria papelera, en combinación con

dos niveles (0 y 2.0% p/p) de quitina de crustáceos, con el objetivo de combatir al *Meloidogyne arenaria* (Neal) Chitwood. Los suelos enmendados se mantuvieron humedecidos por una semana seguido lo cual, se plantaron con calabacín (*Cucurbita pepo* L.). Despues de seis semanas de desarrollo el número de plántulas supervivientes aumentó en suelos con MH y no hubieron supervivientes en suelos tratados con quitina sola. Las enmiendas con MH redujeron el índice de nodulación de las raíces por el nematodo pero no fueron tan efectivas como los tratamientos que incluían quitina. Los suelos tratados con MH y quitina resultaron con un pH más bajo que los tratados con quitina sola. Después del examen de las plantas de calabacín los mismos suelos se plantaron con plántulas de tomate 'Rutgers' (*Lycopersicon esculentum* Mill.). Después de 6 semanas de crecimiento, las raíces y tallos de los tomates provenientes de suelos tratados con quitina y MH resultaron más pesados que los de las plantas de suelos con quitina sola. Aunque MH solo no tuvo ningún efecto sobre la nodulación de las raíces no se observaron nódulos en las raíces de plantas de suelos enmendados con quitina sola o con MH. Los suelos con quitina y MH despues de los tomates tenían un pH más alto que los tratados con quitina sola. La disminución del efecto fitotóxico de la quitina por las enmiendas con MH se debe probablemente a la inmobilización del exceso de nitrógeno por las actividades microbiales del suelo debida al carbono adicional, al efecto amortiguador del MH sobre el pH del suelo, o bién a una combinación de los dos efectos.

Palabras claves adicionales: combate biológico, enmiendas orgánicas, ciclo del nitrógeno, manejo de desechos industriales, combate sin nematicidas.

INTRODUCTION

Since the recent banning of principal chemical nematicides, EDB and DBCP, biological control of plant-parasitic nematodes has been an area of renewed and increased interest. Many different forms of biological control are under investigation, of which one of the most feasible methods in many situations is the use of soil organic amendments. The amendments used are usually naturally occurring organic materials, often waste products, and generally effect their control by stimulation of microorganisms antagonistic to the target nematodes or by yielding materials toxic to the nematodes (17). Extensive studies of the effectiveness of organic amendments for control of plant-parasitic nematodes have been made, and many different materials have been utilized as amendments (17). Among the most effective are those materials with a low carbon:nitrogen ratio (15). Of these amendments chitin, an important component of tylenchoid nematode egg shells (3), a common material found in many organisms in nature (19), and a waste product of the seafood processing industry, has been reported very effective in reducing populations of plant-parasitic nematodes (9,13,14,16,26,27). The release of ammonia during microbial decomposition and stimulation of microorganisms antagonistic of nematodes may be credited for some of chitin's nematicidal properties. In addition to being weakly nematicidal, ammonia in high concentrations in the soil is very

phytotoxic (2,11,31). Use of high concentrations of chitin as a soil amendment for control of nematodes has resulted in problems with phytotoxic effects on plants grown in the amended soil (9,13,14). The addition of a carbon source has been reported to reduce the phytotoxic effects of urea, another ammonia-yielding compound (10,21). This experiment was conducted to determine the effectiveness of a carbon source, hemicellulosic waste (HW) (10), in reducing the phytotoxic effects of chitin amendments used for control of root-knot nematodes.

MATERIALS AND METHODS

A greenhouse experiment was conducted to study the effect of hemicellulose on the phytotoxicity of chitin, and on the nematicidal properties of both materials. The experiment was conducted using two levels of chitin, 0 and 2.0% (w/w), combined with six levels of hemicellulose, 0, 0.25, 0.50, 1.0, 1.5, and 2.0% (w/w). The treatments were disposed in a completely randomized design in factorial arrangement. Eight replications (pots) were used for each treatment.

Soil used in the experiment was a sandy loam with pH 6.2, collected from a peanut (*Arachis hypogea* L.) field near Headland, Alabama, infested with the peanut root-knot nematode, *Meloidogyne arenaria* (Neal) Chitwood. The soil was sifted through a 1-mm-mesh sieve, and was mixed 1:1 (v/v) with builders' sand (<1 mm mesh). This mixture will be referred to as soil in the remainder of the paper. The soil was apportioned in 1-kg quantities, and each portion was treated with the appropriate amendment(s). The chitin used was dry ground non-bleached chitin from crustacean exoskeletons (NBC, Cleveland, Ohio). The hemicellulose source was hemicellulosic waste (HW) products from the paper pulp industry. It was obtained from a paper mill near Pitts, Alabama. This material is composed mostly of lignins, xylans, and other hemicellulosic materials (10).

The soils were treated by placing the appropriate amounts of each amendment in a polyethylene bag with 1-kg of the soil, and were mixed thoroughly by shaking. Soils receiving no amendments were handled similarly. The treated soils were placed in polyvinyl chloride (PVC) cylindrical pots (10-cm diam, 1-L vol), and the pots with soil were placed on a greenhouse table. The pots were watered daily, and were allowed to stand for 10 days to permit partial decomposition of the amendments by soil microorganisms.

After this period, five 'Yellow Crookneck' squash (*Cucurbita pepo* L.) seeds were planted in each pot. The squash plants were used as bioindicators for root-knot nematodes and to assess the effects of the amendments on the plants. The plants were watered daily, and were allowed

to develop for six weeks. After this time, the plants were removed from the soil and two 100 cm^3 samples of soil were taken from each pot. The seedlings were examined for plant variables, and soil samples were used for nematode, microbial, and chemical analysis.

Squash plants were washed, and the number of galls caused by *M. arenaria* in the roots were counted. The height of the shoot and the fresh weight of the shoots and roots were also determined. In addition, two subjective ratings of the roots were made. The first was a root-knot galling index based on a scale of 0 to 10, where 0 represented no galling, and 10 represented the maximum level of galling (32). The second subjective rating was an evaluation of the general appearance of the roots. This index was based on a scale of 1 to 5, where 1 represented the best root condition, and 5 the worst. In this index, root necrosis, rot, discoloration, and other indicators of the general health of the roots were considered. Mean values given were averages of measurements of surviving squash plants.

Nematode numbers were determined in 100 cm^3 soil samples using the "salad bowl" incubation technique described by Rodriguez-Kabana and Pope (23).

The other soil sample was used for soil analysis and to estimate microbial populations by dilution plating. The dilution plating method was a modified version of the method described by Rodriguez-Kabana (20). Ten grams of soil were taken from each sample and placed in a 500-ml Erlenmeyer flask containing 400 ml of sterilized water. The mixture was stirred using a magnetic stirrer to suspend the soil in the water. To estimate soil fungal populations, one drop of the suspension was placed into each of five sterile petri dishes. The drop was then covered with warm (32 C) Rose Bengal chitin agar (pH = 5.2) containing 150 µg/ml streptomycin sulfate (8). This process was repeated using Rose Bengal cellulose agar at identical pH. The cellulose agar was of similar composition and was prepared in the same manner as was the chitin agar (8,25). The cellulose agar differed from the chitin agar only in the substitution of cellulose for the chitin and in the addition of KNO_3 (2 g per L) as a nitrogen source. Colloidal cellulose suspension similar to the chitin suspension (25) was added to deliver cellulose in the same proportion as the chitin used in the chitin agar, 2.0 g (dry wt) per liter (8).

One milliliter (20 drops) of the soil suspension was transferred to a previously weighed aluminum weigh boat and was dried at 75 C to determine the amount of soil added to each petri dish.

Populations of bacteria and actinomycetes were assessed using the same soil suspension used for the fungi. Ten milliliters of the soil suspension was transferred to a 250-ml Erlenmeyer flask containing 200 ml of sterilized water. This mixture was stirred with a magnetic stirrer,

and one drop was placed in each of five sterile petri dishes, as described for the fungi. Both chitin and cellulose agars were used; however for bacteria, the pH was adjusted to 7.0 and contained neither Rose Bengal nor streptomycin sulfate.

In all cases, after the agar had hardened and cooled, the plates were incubated at 25 C. Fungal colonies were counted after 48 hr, and bacteria and actinomycete colonies were counted after 72 and 96 hr respectively.

The soil not used for microbial determination was air-dried, placed in a polyethylene bag, and stored in the dark at 4 C until analyzed. Soil pH was measured using 10 g of the dried soil in a plastic cup with 10 ml of demineralized water (12). The mixture was stirred and allowed to stand for 30 min, after which time the pH of the mixture was measured using a Corning® Model 12 pH Meter. Following pH determination, 10 additional ml of demineralized water were added to each cup. The contents of the cup were stirred and 10 ml of the mixture were centrifuged for 20 min at 5000 x g. Conductivity of the supernatant was then determined using a Wheatstone Bridge fitted with a conductivity cell (k = 1.0).

Following the removal of the squash plants and soil samples, the remaining soil in each pot was planted with a single 14-cm-high 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) seedling. These were maintained for 6 weeks as described for the squash. After this period, the tomato plants were removed, washed, measured and evaluated as described for the squash. Two 100 cm^3 soil samples were taken as before, one for nematode counts, and one for soil dilution plating and soil analysis. In the determination of microbial numbers, only the chitin agar was used. Procedures used and variables measured for the tomato were the same as those used for the squash. In addition, the roots of the tomato plants were incubated for 72 hrs using the "salad bowl" method (23) to obtain nematode counts from the roots of the plants.

All data were analyzed using standard factorial analysis procedures (29). Fisher's least significant differences were also calculated following standard procedures (29). Unless otherwise stated, differences referred to in the text were significant at the 5% or lower level of probability.

RESULTS

The addition of chitin at 2.0% (w/w) to the soil receiving no hemicellulose resulted in the death of all squash planted; however some plants survived in soils that received chitin and hemicellulose (Table 1). Chitin reduced heights of shoots and resulted in reduced weights of shoots and roots as compared to treatments without chitin (Tables 1,2). Root systems of plants from chitin-amended soils were stunted; the subjective

Table 1. Effects of chitin and hemicellulose amendments (HW) to soil infested with *Meloidogyne arenaria* on growth of squash plants.

	Survival rate[x] (plants/pot)		Shoot height[y] (cm)		Fresh shoot weight[y] (g)	
%NW	0% Chitin	2% Chitin	0% Chitin	2% Chitin	0% Chitin	2% Chitin
0	3.0	0	7.88	*	1.39	*
0.25	2.1	0.5	9.23	6.40	2.37	1.23
0.50	2.9	0.5	10.43	6.70	2.17	1.00
1.0	2.5	1.4	10.70	6.52	2.15	1.42
1.5	3.5	2.0	10.25	6.80	2.03	0.88
2.0	3.0	1.6	9.90	6.92	1.86	1.07
LSD (P=0.05)	1.30		0.90		0.35	

[x]Total number of plants per pot = 4.0.
[y]Values reflect mean of measurements of surviving plants only; *No plants survived.

root condition indicated that roots were in worse general condition than those of plants grown in soils with no chitin (Table 2). The addition of hemicellulose alone had quite the opposite effect on growth of the squash. Plants grown in hemicellulose-amended soils with no chitin showed increases in shoot and root growth compared to plants grown in untreated soil (Tables 1,2); however, greatest increases in growth were observed in response to 0.25 or 0.50% hemicellulose with higher rates resulting in little or no additional increase in growth. Similar beneficial effects of hemicellulose were evidenced by an improvement in the general condition of the roots of plants grown in soils with hemicellulose alone. Plants grown in soils amended with hemicellulose at rates of 1.0% or higher had the best overall root condition (Table 2). While no improvement in plant growth was evident with the addition of hemicellulose to chitin-amended soils, the addition of hemicellulose to soils treated with chitin allowed more plants to survive (Table 1). Survival rate of the plants grown in chitin-amended soils receiving the 1.5% hemicellulose was not different from that of plants grown in untreated soils (Table 1).

Addition of chitin to soils eliminated galling caused by root-knot nematode on the squash roots, regardless of the level of hemicellulose added (Table 3). No root-knot larvae were found in any soil to which chitin had been added (Table 4), and similar results were true for lesion nematodes *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans-Stekhoven in the soil (Table 4). In most cases only free-living nematodes were found in soils amended with chitin, and their numbers increased sharply in response to the chitin amendments when compared to soils that received no chitin, regardless of the level of hemicellulose (Table 4).

Hemicellulose alone had little effect on the gall rating although plants from soils treated with hemicellulose had fewer galls per gram of root tissue (Table 3). Also fewer root-knot nematode larvae were found in soils receiving hemicellulose alone than in untreated soils (Table 4). The addition of hemicellulose to chitin-amended soils did not affect the nematicidal properties of chitin.

Seven weeks after treatment, addition of chitin had little effect on numbers of fungi developing on chitin or cellulose agar (Table 5). Addition of hemicellulose alone had no effect on the numbers of fungi that grew on either medium (Table 5); the interaction of the effects of chitin and hemicellulose on the number of fungi present on either medium was not significant.

Addition of chitin to soil increased the number of bacteria that grew on both chitin and cellulose agar (Table 5). Addition of hemicellulose alone had no effect on the number of bacterial colonies on chitin agar but decreased the number of bacterial colonies that grew on cellulose

Table 2. Effects of chitin and hemicellulose amendments (HW) to soil infested with *Meloidogyne arenaria* on growth and condition of roots of squash plants.

	Fresh root weight (g)		Root condition[x]	
%HW	0% Chitin	2% Chitin	0% Chitin	2% Chitin
0	0.17	*	3.60	*
0.25	0.32	0.05	3.05	4.00
0.50	0.28	0.09	2.97	3.65
1.0	0.25	0.08	2.05	3.74
1.5	0.36	0.07	2.20	4.00
2.0	0.29	0.09	2.39	4.03
LSD (P=0.05)		0.06		0.30

[x]Subjective rating (1 = best condition, 5 = worst condition); *No plants survived.

Table 3. Effects of chitin and hemicellulose amendments (HW) to soil on squash root galling by *Meloidogyne arenaria.*

	Gall index[x]		Galls/g of root tissue	
%HW	0% Chitin	2% Chitin	0% Chitin	2% Chitin
0	3.27	*	124.0	*
0.25	2.78	0.17	53.2	1.40
0.50	3.07	0	67.1	0
1.0	2.52	0	45.0	0
1.5	2.96	0	39.4	0
2.0	2.70	0	52.5	0
LSD (P=0.05)		0.75		43.3

[x]Subjective rating (0 = no galls, 10 = maximum galling); *No plants survived.

Table 4. Effects of chitin and hemicellulose amendments (HW) to soil on soil populations of *Meloidogyne arenaria* larvae, *Pratylenchus brachyurus,* and free-living nematodes following squash.

	Number of nematodes per 100 cm^3 of soil					
	Meloidogyne arenaria		*Pratylenchus brachyurus*		free-living nematodes	
%HW	0% Chitin	2% Chitin	0% Chitin	2% Chitin	0% Chitin	2% Chitin
0	42.0	0	1.8	0	208	2880
0.25	16.9	0	2.4	0	49	1810
0.50	3.0	0	3.3	0	53	3000
1.0	8.0	0	5.6	0	332	2310
1.5	13.0	0	6.5	0	38	2500
2.0	2.1	0	3.0	0	40	2200
LSD (P=0.05)	8.6		2.1		545	

Table 5. Effects of chitin and hemicellulose amendments (HW) to soil on populations of fungi and bacteria following squash.

	Chitin agar				Cellulose agar			
	Fungi[x]		Bacteria[y]		Fungi[x]		Bacteria[y]	
%HW	0% Chitin	2% Chitin	0% Chitin	2% Chitin	0% Chitin	2% Chitin	0% Chitin	2% Chitin
0	7.36	6.67	1.81	31.7	8.40	6.54	12.1	19.4
0.25	7.99	6.42	1.02	36.5	7.42	4.87	2.53	19.3
0.50	7.59	5.34	2.98	10.6	7.66	5.55	1.91	13.1
1.0	6.36	7.11	3.28	7.4	8.95	9.25	3.55	13.7
1.5	6.75	7.06	1.97	11.3	9.81	4.92	3.03	3.96
2.0	6.87	6.39	5.14	4.7	10.0	7.19	3.13	3.61
LSD (P=0.05)	1.50		8.72		3.28		5.68	

[x]Fungal propagules x 10^{-4}/g of soil.
[y]Bacterial propagules x 10^{-7}/g of soil.

Table 6. Effects of chitin and hemicellulose amendments (HW) to soil on populations of actinomyetes.

	Chitin agar[x]		Cellulose agar[x]	
%HW	0% Chitin	2% Chitin	0% Chitin	2% Chitin
0	3.75	6.62	3.15	4.91
0.25	3.49	5.87	3.78	1.40
0.50	2.59	2.84	3.83	2.08
1.0	3.36	3.86	3.53	2.25
1.5	3.50	4.71	3.56	2.35
2.0	2.78	2.66	6.24	3.19
LSD (P=0.05)	1.48		1.58	

[x]Actinomycete populations x 10^{-6}/g of soil.

agar (Table 5). Addition of hemicellulose at levels above 0.25% to soils treated with chitin caused a decrease in the number of bacteria that were able to grow on chitin or cellulose agars compared to the number from soil treated with chitin alone (Table 5). Generally, higher numbers of bacteria from chitin-amended soils grew on chitin agar than on cellulose agar.

Numbers of actinomycetes on chitin and cellulose media were greater in soils receiving chitin alone than in soils receiving no chitin (Table 6). Numbers of actinomycetes on chitin agar from soils with no chitin were not affected by the addition of hemicellulose, although an increase was observed in the number of actinomycetes that grew on cellulose agar in response to the highest rate (2.0%) of hemicellulose (Table 6). Numbers of actinomycetes from soil amended with both chitin and hemicellulose were smaller on both media than those from soil treated with chitin alone (Table 6).

Soil pH seven weeks after amendment of the soil increased in response to the addition of chitin alone. The addition of hemicellulose alone at rates greater than 0.25% also increased pH although the effect of even the highest amount of hemicellulose was not as great as the increase resulting from the chitin amendments (Fig. 1). The pH of soils amended with hemicellulose and chitin was lower than that of soil treated with chitin only. Little difference in pH was observed between soils treated with chitin and hemicellulose above 0.25% (Fig. 1).

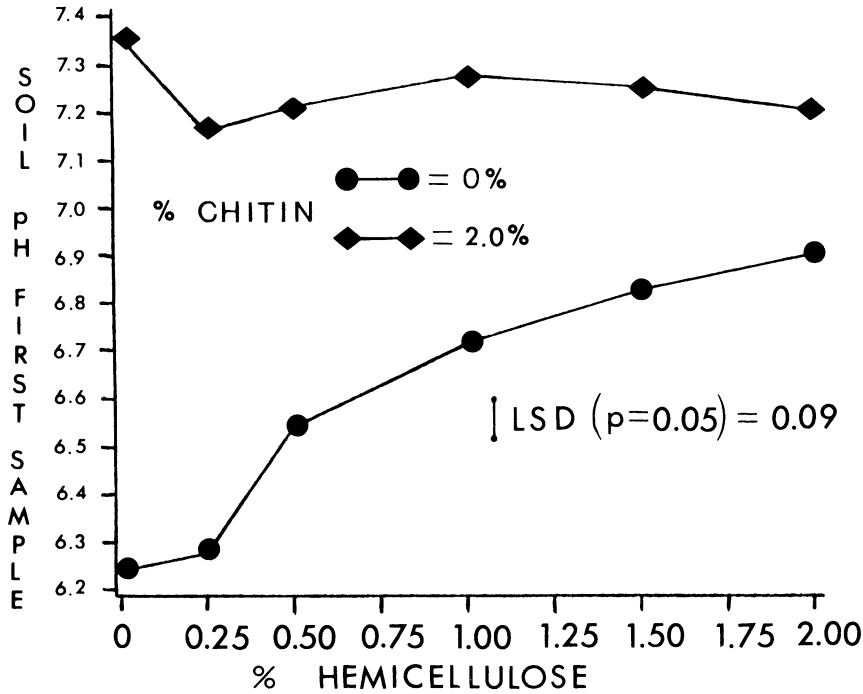

Fig. 1. Effect of chitin and hemicellulose soil amendments on pH of amended soil 7 weeks after treatment.

Soil conductivity increased in response to amendments with chitin and with all rates of hemicellulose (Fig. 2). Conductivity of soils treated with chitin was increased further by the addition of hemicellulose (Fig. 2).

Tomato plants grown in the same pots of soil as the squash indicated less acute phytotoxic effects from chitin than did the squash. All plants survived in all treatments, and growth of plants in soils amended with chitin alone was comparable to that of plants grown in untreated soil (Figs. 3,4,5). Improvement was also noted in the root condition of plants grown in chitin-amended soils in comparison to that of plants grown in untreated soil (Table 7). The addition of hemicellulose alone was detrimental to growth of the tomato plants, as indicated by decreases in shoot height and root weight (Figs. 3,5). Tomato plants in soils treated with chitin showed a sharp positive growth response to the addition of hemicellulose. This response was evidenced by the increases in shoot heights and weights of shoots and roots. Root weight best reflected the effects of chitin and hemicellulose soil amendments on plant growth

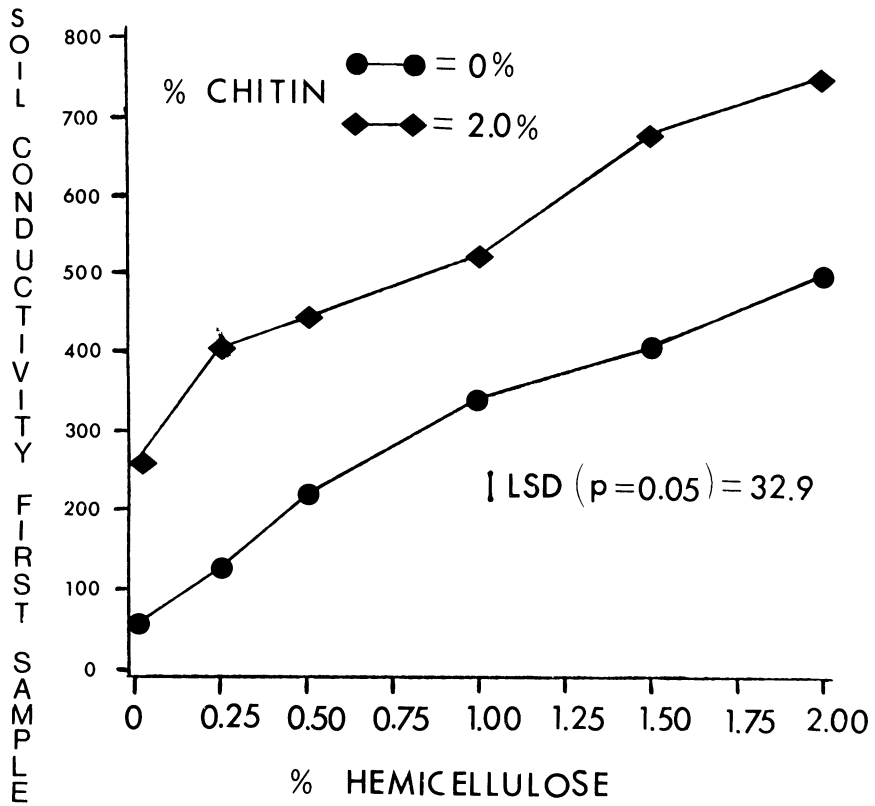

Fig. 2. Effect of chitin and hemicellulose soil amendments on conductivity (micromohs) of amended soil 7 weeks after treatment.

(Fig. 5). Plants grown in soil with 2.0% chitin plus 2.0% hemicellulose had root weights over twice as heavy as those of plants grown in soils receiving chitin alone, and were much heavier than the roots of plants grown in soils with no chitin (Fig. 5). Root condition indices of plants from soils treated with chitin plus the upper levels of hemicellulose (> 1.0%) were the best and were either equal to 1.0 or very near that value (Table 7).

Chitin amendments provided complete control of root-knot nematodes in tomatoes. No root-knot galls were found on any plants grown in soils treated with chitin (Table 8). There was a notable decrease in galling compared to plants from soils with no chitin. Significant galling was observed on roots of all plants grown in soils without chitin (Table 8). No root-knot larvae were obtained from the roots of plants grown in any soil to which chitin had been added (Table 9). Addition

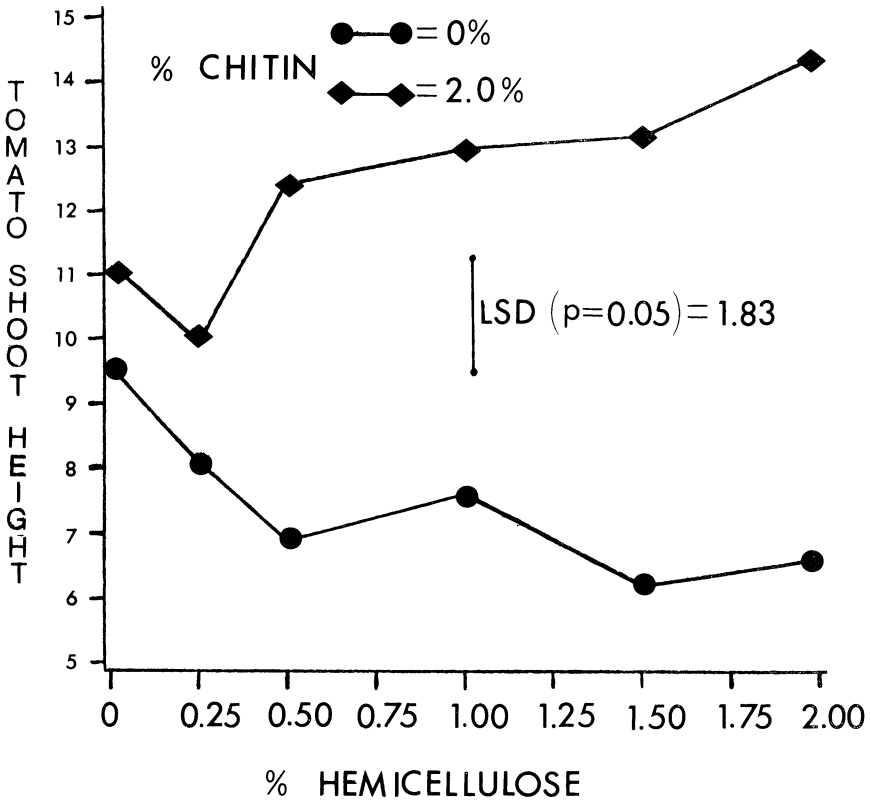

Fig. 3. Effect of chitin and hemicellulose soil amendments on shoot height (cm) of tomato plants.

of hemicellulose alone at all levels except 1.5% decreased the galling rate slightly, though hemicellulose had no effect on the number of galls found per gram of root tissue (Table 8). The number of root-knot larvae extracted from roots of plants from soils amended with hemicellulose was lower than that from roots of plants from unamended soil and were comparable to numbers found in chitin-amended soil (Table 9).

Thirteen weeks after treatment, numbers of fungi that developed on chitin agar from soil amended with chitin alone were not different than those from soils with no chitin (Table 10). Amendments of hemicellulose alone had no effect on fungal counts (Table 10). Addition of hemicellulose at rates of 2.0% to chitin-amended soils resulted in the development of the greatest numbers of fungal colonies (Table 10). Fungal populations of all soils were greater after tomato growth than at the first sampling.

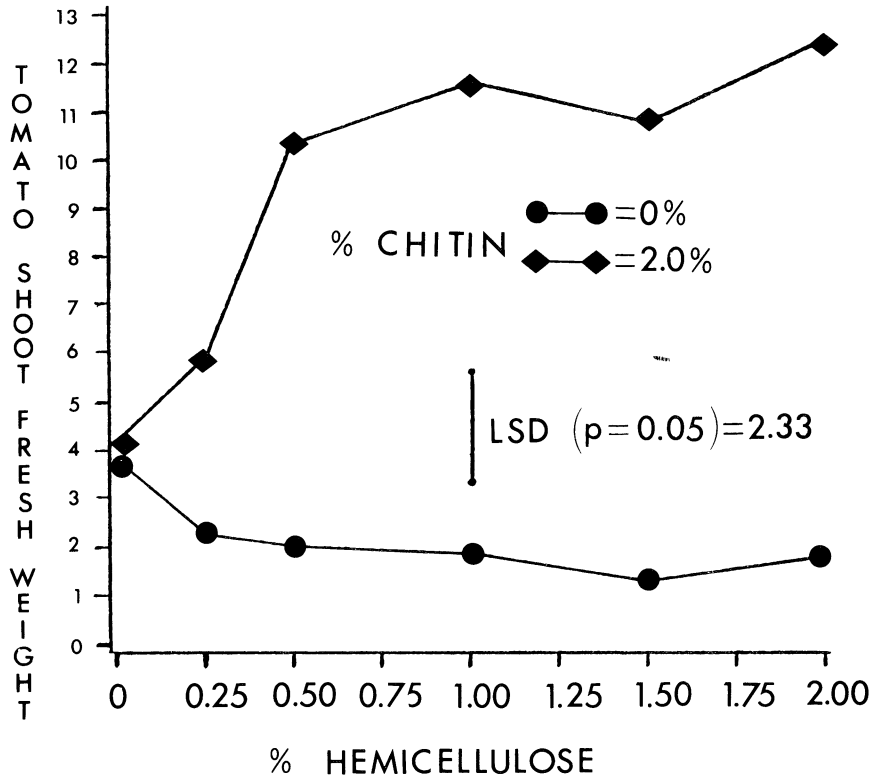

Fig. 4. Effect of chitin and hemicellulose soil amendments on shoot fresh weight (g) of tomato plants.

Addition of chitin to soil increased the number of bacteria colonizing chitin agar, in comparison to numbers that grew from control soils (Table 10). Amendments of hemicellulose alone had little effect on bacterial numbers (Table 10). Addition of hemicellulose to chitin-amended soils caused no further increase in bacterial populations above that of the soils receiving chitin alone (Table 10). In general, bacterial populations of chitin-amended soils were smaller than those seen in the same soils following squash growth (Tables 5,10).

Numbers of actinomycetes that grew from chitin-amended soils were not different than those from untreated soil (Table 10).Combination of hemicellulose with chitin promoted an increase in the number of actinomycetes (Table 10). Numbers of actinomycetes that grew from soil treated with chitin and hemicellulose at rates of 0.25% or 0.50% were greater than the number that grew from soils that received chitin only,

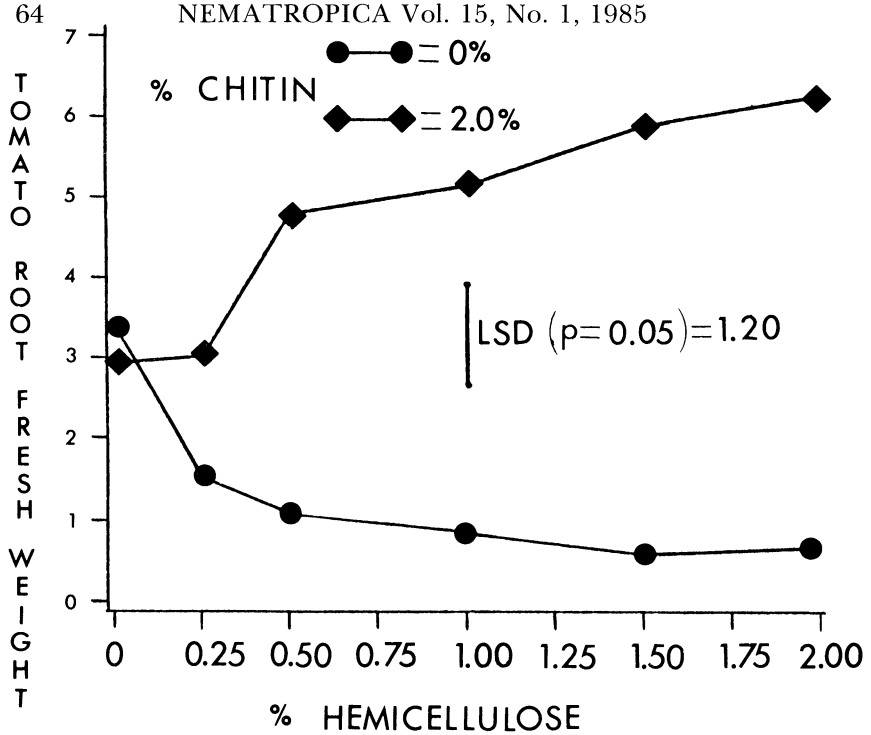

Fig. 5. Effect of chitin and hemicellulose soil amendments on root fresh weight (g) of tomato plants.

though no further increase occurred with addition of hemicellulose at rates above 0.25% (Table 10).

Soil pH 13 weeks after treatment was lower in soil amended with chitin alone than in soils receiving no chitin; soil pH of chitin-amended soils was lower than that observed in the same soils seven weeks after treatment (Figs. 1,6). Soil pH increased in response to the addition of hemicellulose alone (Fig. 6) in a manner similar to that observed 6 weeks after treatment (Fig. 1). In soils treated with chitin, the addition of hemicellulose prevented the drastic drop in soil pH observed in soils treated with chitin alone (Figs. 1,6). Chitin-amended soils receiving hemicellulose at rates of 1.0% (w/w) and 1.5% had pH values that were not different from that of the control soil (Fig. 6).

Soil conductivity of chitin-amended soils was higher than that of unamended soils (Fig. 7). Amendments of hemicellulose alone also increased soil conductivity although addition of hemicellulose at rates greater that 1.0% resulted in no further increase (Fig. 7). The addition

Table 7. Effects of chitin and hemicellulose amendments (HW) to soil infested with *Meloidogyne arenaria* on root condition of tomato plants.

%HW	Root condition[x]	
	0% Chitin	2% Chitin
0	3.63	2.06
0.25	2.56	2.63
0.50	3.19	1.31
1.0	3.0	1.13
1.5	3.19	1.00
2.0	2.56	1.00
LSD (P=0.05	0.56	

[x]Subjective rating (1 = best, 5 = worst).

Table 8. Effects of chitin and hemicellulose amendments (HW) to soil on tomato root galling by *Meloidogyne arenaria.*

%HW	Gall index[x]		Galls/g of root tissue	
	0% Chitin	2% Chitin	0% Chitin	2% Chitin
0	5.63	0	66.4	0
0.25	3.38	0	44.0	0
0.50	4.25	0	59.8	0
1.0	4.50	0	59.6	0
1.5	4.88	0	58.5	0
2.0	3.75	0	45.3	0
LSD (P=0.05)	0.92		21.1	

[x]Subjective rating (0 = no galls, 10 = maximum galling).

of hemicellulose to chitin-amended soils resulted in further increase in soil conductivity over that of soils amended with chitin alone, with chitin-amended soils with 1.5% hemicellulose exhibiting the highest conductivity values (Fig. 7).

Table 9. Effects of chitin and hemicellulose amendments (HW) to soil on populations of *Meloidogyne arenaria* following tomatoes.

	Soil counts[y]		Larvae/g of Root tissue	
%HW	0% Chitin	2% Chitin	0% Chitin	2% Chitin
0	13.8	0	27.80	0
0.25	0	0	8.44	0
0.50	0	0	6.71	0
1.0	0	0	2.54	0
1.5	0	0	7.30	0
2.0	0	0	2.90	0
LSD (P=0.05)	1.3		9.9	

[y]Counts expressed as larvae per 100 cm^3 of soil.

DISCUSSION

Chitin, poly-β-(1-4)-N-acetyl-D-glucosamine, when added to the soil, is converted through chitinase-mediated microbial activity to ammonia, acetate, glucosamine, and sugars according to the following equations:

$$\equiv C\text{-}NHCOCH_3 + H_2O \rightarrow \equiv C\text{-}OH + CH_3CONH_2$$

$$CH_3CONH_2 + H_2O \rightarrow CH_3COOH + NH_3$$

(18). Free ammonia in the soil exists in a pH-determined equilibrium with ammonium ions:

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

Increase in pH caused by a release of OH^- ions through the lysis of water molecules by the ammonia shifts the equilibrium to the left, promoting accumulation of free ammonia. Free ammonia permeates cellular membranes much more readily than ionized ammonia. Since toxicity is dependent on the amount of ammonia entering living cells, the ammonia in the free state is much more toxic (31). While ammonia is an important source of nitrogen for living plants, high concentrations of ammonia in the soil have been shown to be phytotoxic, inhibiting both seed germination (11) and growth (2,5,31). Also, high concentrations of ammonia have been shown to be weakly nematicidal (7,22,24), illustrating another beneficial effect of ammonia in the soil. High concentrations of ammonia in the soil may also promote the temporary accumulation

Table 10. Effects of chitin and hemicellulose waste amendments (HW) to soil on soil microbial populations following tomatoes.

	Fungi[x]		Bacteria[y]		Actinomycetes[z]	
%HW	0% Chitin	2% Chitin	0% Chitin	2% Chitin	0% Chitin	2% Chitin
0	2.13	2.24	2.09	4.81	1.52	2.51
0.25	2.51	2.07	3.53	3.65	2.53	6.36
0.50	1.82	2.42	2.55	2.47	2.52	3.30
1.0	2.70	3.24	3.00	3.49	2.90	6.39
1.5	2.22	3.05	2.72	3.51	2.63	5.15
2.0	1.57	3.67	2.81	3.78	2.22	4.06
LSD (P=0.05)	0.07		1.53		2.44	

[x]Fungal propagules x 10^{-5}/g of soil.
[y]Bacterial propagules x 10^{-7}/g of soil.
[z]Actinomycete propagules x 10^{-6}/g of soil.

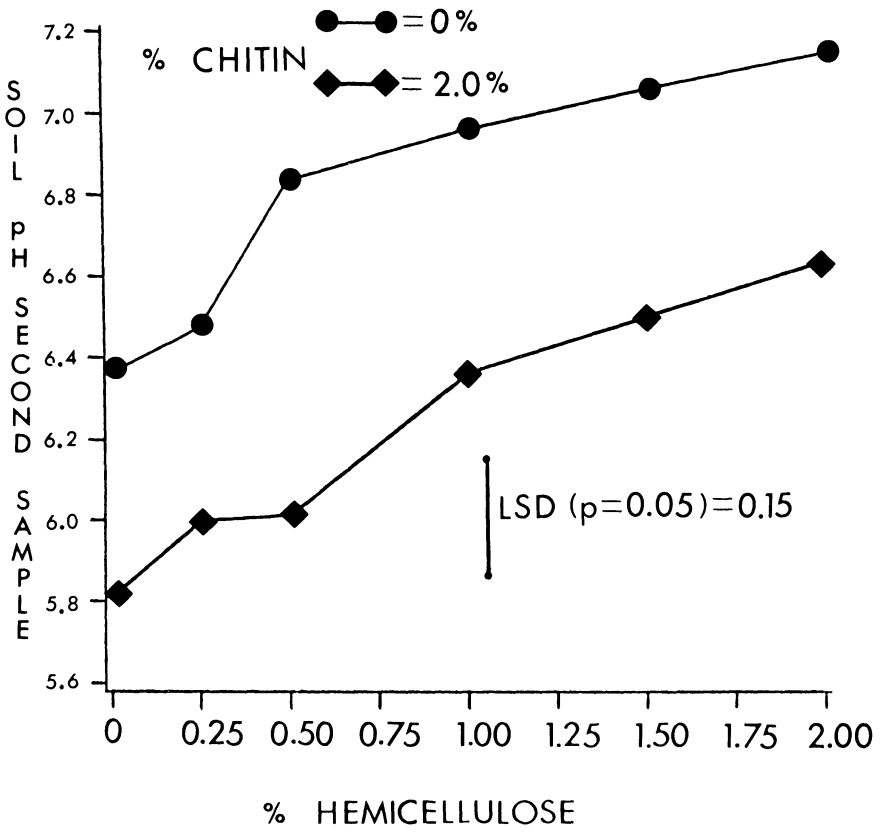

Fig. 6. Effect of chitin and hemicellulose soil amendments on pH of amended soil 13 weeks after treatment.

of nitrites in the soil (4). Addition of urea, which is also decomposed to yield ammonia, has been shown to promote accumulation of nitrites, followed by accumulation of nitrates (6). This may be due to the slight difference in pH optima for the different organisms involved in the steps of nitrogen oxidation. Bacteria, primarily *Nitrosomonas* spp., oxidizing NH_4^+ to NO_2^- have a slightly higher optimum pH range than do bacteria oxidizing NO_2^- to NO_3^-, primarily *Nitrobacter* spp. (1). High pH caused by high concentrations of ammonia may inhibit *Nitrobacter* spp. allowing accumulation of nitrites before *Nitrobacter* spp. could recover. The lag in nitrate formation might also be due to lack of a sufficient population of nitrite oxidizers to convert to nitrates the large amounts of nitrites produced following the addition of ammonia-yielding compounds to the soil (6) or combination of the different factors.

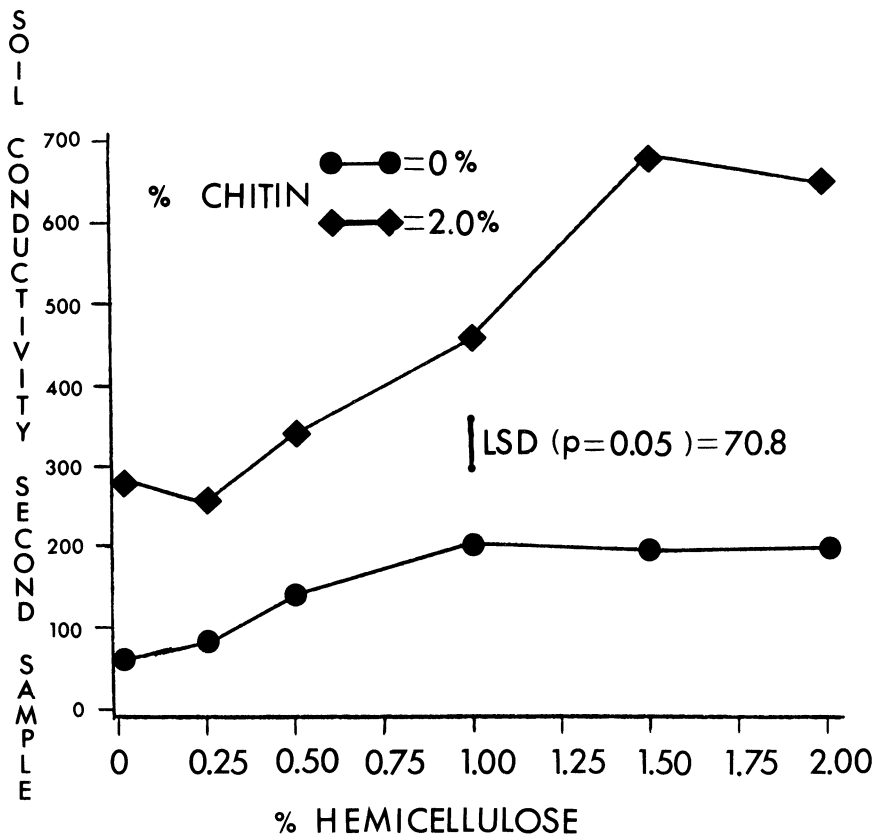

Fig. 7. Effect of chitin and hemicellulose soil amendments on conductivity (micromohs) of amended soil 13 weeks after treatment.

In any case, addition of chitin and thus ammonia, could promote such an accumulation of levels of nitrites that would be toxic to plants as well as plant-parasitic nematodes. Hence, this factor must be considered in evaluating the deleterious as well as beneficial effects of chitin amendments on plant growth.

Nitrogen is an important factor in microbial decomposition of any organic material in the soil. Heterotrophic organisms of decay require a source of nitrogen for their various metabolic activities. As more organic material is added to the soil, more nitrogen is required to allow the microorganisms to decompose the material. If the amount of nitrogen supplied by the organic matter itself is not sufficient to meet the needs of the microorganisms, they will utilize NH_4^+ or NO_3^- from other sources in the soil, thus incorporating them into components of living

systems. Generally, decomposition of materials having a carbon to nitrogen (C:N) ratio greater than 30 require additional nitrogen during the initial stages of decomposition (30). The addition to the soil of the hemicellulosic waste with C:N ratio of 33.96 (10) should facilitate the temporary immobilization of a portion of the nitrogen supplied by the decomposition of chitin. This would reduce the amount of free ammonia and nitrites in the soil and thus reduce the phytotoxic effects of chitin amendments. This hypothesis is supported by the results of the experiment. The increase in number of squash plants surviving in soils treated with chitin plus the three highest levels of hemicellulose, ($\geq$ 1.0%), compared to those in soils treated with chitin alone indicates that the hemicellulose reduced at least some of the phytotoxic effects of chitin and its decomposition products. Cucumber (*Cucumis sativus* L.) plants have been reported to be more sensitive to ammonia than other tested plant species (28). Growth of squash plants, also in the family Cucurbitaceae, in other tests using ammonia-yielding compounds has indicated that squash plants are likewise highly sensitive to ammonia, though the minimum level required for toxicity has not been determined. Considering the apparent high susceptibility of squash seedlings to damage by high concentrations of ammonia, the survival of any plants in soils treated with chitin indicates a positive effect of the additional organic matter supplied by the hemicellulosic waste.

Control of root-knot nematodes was maintained in all soils treated with chitin regardless of the amount of hemicellulose added. This indicates that factors involved in the nematicidal activities of chitin were not affected by the addition of hemicellulose.

The lack of effect of chitin amendments on number of fungi and general increase in numbers of bacteria and actinomycetes found in soils treated with chitin, in comparison to soils receiving no amendment, is not surprising considering the effects that the breakdown products of chitin have on soil chemistry and on organisms directly. Addition of chitin resulted in increased soil pH, an effect selecting for bacteria and actinomycetes but detrimental to fungi. Eno *et al.* (7) reported a drop in number of all soil microorganisms immediately after field application of anhydrous ammonia. A similar reduction soon after the addition of chitin is not unlikely, though in this experiment, samples were not taken at time intervals that would allow documentation of such effects. The possibility exists that differences in microbial populations observed may reflect differences in recovery rates between bacteria and actinomycetes, and fungi. Fungi would be expected to be slower in reestablishing themselves due to unfavorable soil pH soon after the addition of chitin.

While both chitin and hemicellulose applied alone increased soil pH, the effect of chitin alone was greater than that of hemicellulose at any

level. Soil pH values indicate that hemicellulose had a buffering effect on the soil; increases in pH of soils treated with both chitin and hemicellulose were not as great as those in soil treated with chitin alone. Also, effects of the hemicellulose on pH of chitin-amended soils may have been even greater at earlier times. Eno *et al.* (7) reported a sharp increase in soil pH immediately after addition of anhydrous ammonia, and a similar increase in pH was reported by Court *et al.* (6) after the addition or urea. After the initial increase, the pH gradually dropped to a level lower than that of the soil prior to addition of the ammonia or urea. In both of these cases, as with chitin amendments, the pH effect is due to the ammonia added to or released into the soil. It is likely that pH measured 6 weeks after the addition of chitin is not the maximum pH attained in the soil. The pH of chitin-amended soils may have followed a trend similar to that seen with other ammonia-yielding nitrogen sources, a sharp increase in pH initially, followed by a gradual decrease in pH. Though with only one sampling it can only be speculative, buffering the soil with hemicellulose may have had even greater effect on pH of chitin-amended soils at earlier times than is evident at the time the soil was sampled. Further study should elucidate this question.

Increases in soil conductivity with the addition of chitin or hemicellulose and further additive increases with the combination of the two is interpreted as reflection of increases in microbial activity in response to addition of organic amendments resulting in accumulation of nitrates and other salts in the soil. Free ammonia may be oxidized more rapidly in soils with the greatest microbial activity. Increased soil conductivity in chitin-amended soils with the addition of hemicellulose may reflect less toxicity of the chitin amendments on microorganisms themselves due to the addition of the hemicellulose.

Chitin did not affect survival of the tomato plants, and shoots of plants grown in soils receiving chitin alone were as large and as heavy as plants grown in control soils. This suggests that the phytotoxic effects of chitin diminished with time. Still, growth of tomato plants in soil receiving chitin alone did not reflect the addition of an abundant source of nitrogen, suggesting phytotoxic effects of chitin were limiting factors in the growth of the tomato plants in spite of time elapsed and increased tolerance to ammonia. Addition of hemicellulose to chitin-amended soils promoted an increase in the shoot weight and height as well as the root weight of the plants grown in the amended soil, suggesting that the addition of hemicellulose allowed the plants to utilize nitrogen provided by the amendments rather than be adversely affected by its excess. This occurred in spite of the phytotoxic effects that were seen with hemicellulose alone.

The combination of the two amendments to reduce the phytotoxic effects of each individual material did not reduce control of root-knot nematodes. Extended control observed with amendments in this test suggests that in addition to nematicidal activity of ammonia, other microbial or chemical antagonism of the nematodes is promoted by the addition of chitin.

Selection for fungal species capable of parasitizing nematode eggs by the addition of chitin amendments may be responsible for the extended control of the nematodes in soils treated with chitin. The development of a special soil mycoflora in response to the addition of chitin has been reported, and may species in this particular group of fungi can parasitize nematode eggs (8,9,14,26). An increase in number and activity of a specialized mycoflora rather than an increase in general fungal activity is likely responsible for extended control of plant-parasitic nematodes observed in soil amended with chitin.

Stimulation of numbers of bacteria and actinomycetes by addition of the materials may also contribute to control of the nematodes. Mankau and Das (13) reported increases in the number of bacteria and actinomycetes in soil amended with chitin. Direct and indirect antagonism of the nematodes by these organisms may be partially responsible for reduction of plant-parasitic nematode populations in soil treated with chitin.

The pH of soils treated with chitin alone dropped sharply during the 6 weeks the tomato plants were present while pH of soils receiving hemicellulose was more stable, and remained at a higher level than that of the control soil. Such decreases in pH over short periods of time subject the plants to great variations in nutrient availability and availability of toxic elements in the soil (30), adding yet another source of stress to the growing plants. Effects of the hemicellulose on pH were evident even after removal of the tomato plants. While hemicellulose alone raised the pH of the treated soil, hemicellulose provided a continued buffer in the chitin-amended soils preventing the sharp drop in pH seen in soils amended only with chitin. While pH readings in this experiment give only an indication of the general, relatively long-term effects of the amendments, the maintenance of a stable pH, a stable environment more favorable for plant growth, may be a major factor in the explanation of the increase in plant growth seen with the addition of hemicellulose to chitin-amended soils.

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Received for publication:
19.III.1985
Recibido para publicar: