ORGANIC AMENDMENTS WITH HIGH TANNIN AND PHENOLIC CONTENTS FOR CONTROL OF *MELOIDOGYNE ARENARIA* IN INFESTED SOIL.

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ABSTRACT

Mian, I.H. and R. Rodríguez-Kábana. 1982. Organic amendments with high tannin and phenolic contents for control of *Meloidogyne arenaria* in infested soil. Nematropica 12:221-234.

The nematicidal properties of 4 organic materials high in tannins and phenols were studied in greenhouse experiments with a silt loam soil infested with *Meloidogyne arenaria* (Neal) Chitwood. The materials were spent coffee grinds (*Coffea arabica* L), holly leaves (*Ilex opaca* Ait.), pecan shells [*Carya illinoensis* (Wang.) K. Koch] and tannic acid. The dry ground materials were added to soil at rates of 0-4% (w/w) and after 3 weeks the treated soil was planted with summer crookneck squash (*Cucurbita pepo* L) to assess degree of root galling caused by the nematode. Pecan shell amendments had no effect on *M. arenaria*. The most effective amendments for control of the nematode were those with tannic acid; however, these amendments caused severe phytotoxicity. Treatments with holly leaves at rates of 1.5% or higher reduced root galling and did not cause significant phytotoxicity. Coffee grind treatments were effective against the nematode at the 4% rate but caused phytotoxicity. Holly amendments resulted in increased activities of aryl phosphatase and urease in soil and increased pH and nitrate content of the soils.
Additional key words: biological control, waste management, soil enzymes, soil urease, aryl phosphatase activity, cultural practices, root-knot nematodes.

RESUMEN

Mian, I.H., y R. Rodríguez-Kábana. 1982. Enmiendas orgánicas con alto contenido de taninos y fenoles para combatir *Meloidogyne arenaria* en un suelo infestado. Nematrópica 12:221-234.

Se estudiaron las propiedades nematicidas de 4 enmiendas orgánicas con alto contenido de taninos y fenoles con experimentos de invernadero utilizando un suelo infestado con *Meloidogyne arenaria* (Neal) Chitwood. Las materias para el estudio fueron: borras de café (*Coffea arabica* L), hojas de acebo (*Ilex opaca* Ait.), cáscaras de pecanas [*Carya illinoensis* (Wang.) K. Koch] y ácido tánico. Estas materias se añadieron al suelo ya secas y en proporciones entre 0-4% (p/p) y 3 semanas después del tratamiento se plantó el suelo con calabacín (*Cucurbita pepo* L) para determinar el grado de agallamiento de las raíces causado por el nema-

todo. Las enmiendas con cáscaras de pecanas no afectaron el desarrollo de *M. arenaria*. Los tratamientos más eficaces contra el nematodo fueron aquellos con ácido tánico aunque este compuesto ocasionó fitotoxicidad aguda. Las enmiendas con hojas de acebo a niveles de 1.5% o más altos, redujeron el agallamiento de las raíces y no causaron fitotoxicidad de importancia. Las incorporaciones al suelo de borras de café sólo fueron efectivas al nivel de 4% aunque fueron fitotóxicas. Los tratamientos con hojas de acebo causaron aumentos en el pH y en las actividades de la ureasa y de la aril-fosfatasa del suelo así como en el contenido de nitrato.
Palabras claves adicionales: control biológico, manejo de desperdicios, enzimas del suelo, ureasa del suelo, aril-fosfatasa del suelo, prácticas de cultivo, nematodos noduladores.

INTRODUCTION

The use of organic amendments to soil to control plant parasitic nematodes is an old practice which has received considerable attention principally outside the U.S.A. Singh and Sitaramaiah (27) and Sitaramaraiah (29) reviewed the subject and listed a large number of organic additives that have been applied to soils in attempts to control various species of phytonematodes in various crops. The nature of these amendments is varied and include crop residues (5,8,20,21,24,25), oil cakes (2,3,4,5,8,9,12,16,17,18,19,21,23,28), and waste materials of animal and plant origins (5,6,8,11,20,21,22,23,24,25,26). Many of these materials, although available, have not been studied for control of nematodes in the U.S.A. Presently, in the U.S.A. there is a lack of effective nematicides for use by home gardeners. The use of organic amendments to control nematodes generally involves the addition of large quantities (10-50 MT/ha) of materials into soil. While these large amounts limit their use in row crops, they do not impair their use by home gardeners who treat relatively small areas and who can incorporate effectively large quantities of organic amendments into soil. In other studies we have shown that oil-cakes and chicken litter amendments can be used to control root-knot nematodes (22). These materials have relatively narrow C/N ratios and we believe they are effective against nematodes because they release ammoniacal nitrogen during their decomposition in soil. However, materials with high phenolic and tannin contents such as raspberry canes (22), tea wastes (24,25), and some oil cakes (2,3,4,5) have also been shown to be nematicidal when applied to soil. Information on the use of materials of this type available in southern U.S.A. for control of plant parasitic nematodes is lacking. This paper presents results of a preliminary investigation on the effectiveness of 4 common organic amendments with high phenolic and tannin content for control of *Meloidogyne arenaria* (Neal) Chitwood.

MATERIALS AND METHODS

The effectiveness of tannic acid (United States Biochemicals Corp., Cleveland, Ohio) and three other materials of plant origin as soil amendments for control of *Meloidogyne arenaria* (Neal) Chitwood was studied in greenhouse experiments. The soil was collected from a peanut (*Arachis hypogaea* L) field infested with the nematode and was a sandy loam with less than 1.0% (w/w) org. matter and pH of 6.0. The soil was screened (2 mm mesh) and apportioned in 1 Kg amounts into 4 L capacity polyethylene bags. The amendments were added to the soil and after thorough mixing the mixture was transferred to 1 L, 10-cm diam cylindrical plastic pots. The pots were kept moist (approx. 60% field capacity) in the greenhouse for 3 weeks to allow for decomposition of the amendments. The treated soil was then planted with 5 Summer Crookneck squash (*Cucurbita pepo* L) seeds and the resulting plants were maintained in good growing condition. After 6 weeks of growth the plants were carefully removed from the soil and shoot heights and fresh weights of roots and shoots were determined. The root systems were evaluated for numbers of galls caused by *M. arenaria* and were indexed for degree of galling and for general appearance. The gall index used was based on a scale of 0 to 10 where 0 represented root systems without galls and 10 roots with very severe galling (33). The root condition index measured general appearance of the roots and was based on a subjective scale ranging from 1-5; 1 was assigned to plants with very poor root growth and a 5 to those with excellent growth.

Each type of amendment was studied in separate experiments. Tannic acid was added to have 0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0% (w/w) of the chemical in the soil. The other amendments were dry, ground (1 mm mesh) coffee (*Coffea arabica* L) spent grinds, green leaves of holly (*Ilex opaca* Ait.), and pecan [*Carya illinoensis* (Wang.) K. Koch] shells. The elemental compositions of these materials were determined by standard methods of analysis (14) and are presented in Table 1. These three amendments were added to the soil at rates of 0.0, 0.5, 1.0, 1.5, 2.0, and 4.0% (w/w).

After removing the plants, the soil from the experiment with holly was spread on aluminum foil and was allowed to dry (25C). The dry soil was stored in the dark at 4C until analyzed. Conductivity of soil water extract, pH, nitrate N content, and activities of aryl phophatase and urease in the soil were determined as described elsewhere (15,22,23). Aryl phosphatase activity was expressed as μg of phenol released (substrate = phenyl disodium phosphate) per hr per gm soil. Urease activity was represented as μg of ammoniacal N released (substrate = urea) per hr per gm soil.

In each experiment each rate was represented by 8 replications (pots) arranged in a randomized complete block design. All data were analyzed following standard procedures for analysis of variance (31). Differences between means were evaluated for significance with a modified Duncan's multiple range test (31). Unless otherwise specified all differences referred to in the text were significant at the 5% or lower level of probability.

RESULTS

1. *Tannic acid.* Data from this experiment are presented in Table 2. Numbers of galls per gm of fresh root were reduced by applications of tannic

Table 1. Elemental composition of four organic materials used as soil amendments to control *Meloidogyne arenaria* in infested soil.

	Percent (w/w) of dry weight								μg/gm dry weight			
	C	N	P	K	Ca	Mg	S		Cu	Fe	Mn	Zn
Coffee grinds	52.90	2.13	0.10	0.40	0.13	0.14	0.11		10	48	36	10
Holly leaves	47.28	0.83	0.06	0.36	0.64	0.33	0.12		6	34	880	48
Pecan shells	47.18	0.37	0.03	0.15	0.38	0.06	0.04		4	12	54	54
Tannic acid	53.65	--	--	--	--	--	--		--	--	--	--

Table 2. Effect of tannic acid as a soil amendment on growth of squash (*Cucurbita pepo* L.) plants and development of *Meloidogyne arenaria*.[x]

Amount Added (% w/w)	No. Plants per Pot	Shoot Height (cm)	Fresh Shoot Weight (gm)	Fresh Root Weight (gm)	Root Condition Index[y]	Galls per gm Root	Gall Index[z]
0.0	5.0 A	17.5 A	1.60 A	0.52 A	2.95 A	77.1 A	5.4 A
0.1	4.6 A	13.9 B	1.34 B	0,60 A	2.47 B	65.7 A	5.1 A
0.2	4.4 A	10.4 C	0.89 C	0.33 CD	2.00 C	74.5 A	4.0 B
0.4	4.4 A	10.1 C	0.76 CD	0.44 BC	1.68 CD	22.8 B	3.0 C
0.6	4.4 A	9.1 C	0.55 D	0.33 D	1.57 CD	30.3 B	1.8 D
0.8	4.6 A	9.1 C	0.61 D	0.30 D	1.48 DE	0.6 C	0.1 E
1.0	4.0 A	6.2 D	0.67 D	0.25 D	1.08 E	0.1 C	0.0 E

[x] Values are the averages of 8 replications; those within the same column with a common letter are not statistically different ($P = 0.05$).

[y] Based on a scale of 1-5, where 1 represented plants with very poor root growth and 5 those with excellent growth.

[z] Based on a scale of 0-10 where a value of 0 represented roots with no galls and 10 roots with the greatest number of galls (33).

acid of 0.4% or higher and a similar response was observed for gall index values. The relation between numbers of galls and the amount of tannic acid could also be described by $Yg = 76.42 - 84.85X$, where Yg was the number of galls and X the percent of tannic acid added to the soil. The corresponding equation for galling index was $Yi = 5.41 - 5.95X$, where Yi represented gall index values. Numbers of galls were positively and linearly correlated ($r = 0.94$) with galling index values.

All applications of tannic acid at rates of 0.2% or higher resulted in shorter shoots, lower shoot and root weights, and lower values for the root condition index than the values corresponding to plants from untreated soils. Tannic acid had no effect on the number of seedlings that emerged in each pot.

2. *Pecan shells.* Results obtained from the experiment with pecan shell amendments are presented in Table 3. These amendments did not reduce the number of galls, galling index, root weights, root condition, or number of seedlings per pot. Plants from untreated soil had heavier shoots than those from soils with pecan shells; the lightest shoots were those of plants from pots with the 3 highest rates of the amendment. The addition of pecan shells to soil at the 4% rate resulted in shorter shoots than those of plants from unamended soil.

3. *Coffee grinds.* Table 4 contains data obtained from the experiment with coffee grinds. The 4% rate of this material was the only treatment that reduced the number of galls and gall index values, root weight and root condition index. Coffee grinds had no effect on shoot height or on the number of seedlings per pot; however, shoot weight was reduced markedly.

4. *Holly.* Data from the experiment with the holly amendments are presented in Tables 5 and 6. Additions of this material to soil at rates of 1.5% or higher resulted in lower numbers of galls in squash roots and lower gall index values than for plants from untreated soil or from soils that received lower amounts of the material. The amendments had no effect on root weights, root condition index or in the number of surviving plants per pot. The amendments however, resulted in plants with lighter and shorter shoots than those from untreated soils.

The relation between number of galls per gm of roots (Yg) and the percent of holly amendment added to soil (X) was inverse and could be described by: $Yg = 23.95 - 6.89X$. Similarly, a linear function could also be used to describe the relation between gall index values (Yi) and X; the equation was $Yi = 3.55 - 0.937X$. Yg was positively and linearly correlated with Yi ($r = 0.989$).

Soils that received holly amendments of 1.5% or higher exhibited higher aryl phosphatase activity than those with lower amounts of the material or untreated soils. Aryl phosphatase activity (Xp) was inversely and linearly correlated ($r = -0.961$) with Yg; the equation relating the two variables was $Yg = 77.21 - 6.11Xp$.

All soils treated with the holly material showed higher urease activity than those without it. The relation between soil urease activity (Yu) and the percent of holly in the soil was described by $Yu = 1.269 + 2.209X$. The linear correla-

Table 3. Effect of dry ground pecan (*Carya illinoensis*) shells on growth of squash (*Cucurbita pepo* L.) plants and development of *Meloidogyne arenaria*[x]

Amount Added (% w/w)	No. Plants per Pot	Shoot Height (cm)	Fresh Shoot Weight (gm)	Fresh Root Weight (gm)	Root Condition Index[y]	Galls per gm Root	Gall Index[z]
0.0	4.9 AB	14.3 AB	1.92 A	0.61 A	3.05 A	20.9 B	2.8 C
0.5	5.0 A	15.4 A	1.39 B	0.52 A	3.00 A	29.5 B	3.3 BC
1.0	5.0 A	15.0 A	1.21 BC	0.70 A	2.97 A	30.6 AB	4.2 AB
1.5	4.7 AB	13.5 ABC	1.02 C	0.65 A	2.92 A	48.0 A	4.7 A
2.0	4.7 AB	12.8 BC	1.05 C	0.61 A	3.00 A	16.7 B	2.1 C
4.0	4.0 B	11.8 C	1.13 C	0.69 A	3.33 A	15.4 B	2.6 C

[x] Values are the averages of 8 replications; those within the same column with a common letter are not statistically different ($P = 0.05$).

[y] Based on a scale of 1-5, where 1 represented plants with very poor root growth and 5 those with excellent growth.

[z] Based on a scale of 0-10 where a value of 0 represented roots with no galls and 10 roots with the greatest number of galls (33).

Table 1. Elemental composition of 2 oil cakes and chicken litter tested as amendments to control *Meloidogyne arenaria* in infested soil.

	Percent (w/w) of dry weight							µg/gm of dry weight			
	C	N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
Chicken litter	31.90	3.059	1.60	3.31	2.47	0.50	0.49	32	558	364	188
Cotton oil cake	44.72	6.331	0.99	1.56	0.11	0.50	0.45	8	20	22	28
Peanut oil cake	43.06	6.121	0.66	1.48	0.09	0.31	0.24	10	62	42	22

tion coefficient (r = -0.824) between Yg and urease activity was significant; the equation relating the two variables was Yg = 27.17 - 2.958Xu, where Xu represents soil unrease activity.

Soil treated with 1.5% or higher of the material contained more nitrate N than soil with other treatments or the control. All water extracts of soils with amendments exhibited higher conductivity than the extracts from control soils. The relation between conductivity values (Yc) and the amount of holly was best described by Yc = 42.43 + 24.04X. Conductivity values were linearly correlated with soil urease activity as described by Yc = 30.49 + 10.47 Xu. Conductivity values were also correlated (r = 0.875) with the amount of nitrate N in the soils (Xn), the relation being according to Yc = 50.38 + 25.71Xn. The relation between conductivity values and aryl phosphatase activity was best (r = 0.945) expressed as Yc = -119.06 + 19.00Xp.

A gradual increase in soil pH value in response to increasing amounts of the amendment was observed; this response in pH (YpH) could be described by YpH = 6.06 + 0.306X.

DISCUSSION

Results from this study indicated that all materials but pecan shells were effective in reducing root-knot in squash when applied at some level. The most effective treatments for depressing galling of the roots were those with tannic acid. Results also showed that considerable phytotoxicity resulted from effective treatments with tannic acid and coffee grinds as evidenced by reductions in the weights of shoots and roots, low values for the root condition index, and reductions in shoot height in plants from soils amended with tannic acid. While some of these phytotoxic effects were also observed with the holly amendments, they were not as pronounced. Thus, while tannic acid at the 1% rate resulted in reductions of more than 50% in shoot height, weights of shoots and roots, and in values for the root condition index, holly amendments of 4% did not affect the root condition index or the weight of roots, and reduction in shoot height and weight was less. Similar comparisons between the effects of holly amendments and those of the coffee grind treatments on plant development also indicated that the holly material is not only less phytotoxic but also more effective against *M. arenaria* than the coffee grinds.

The four materials chosen for this study ranged in nitrogen content from 0 for tannic acid to 2.1% for the coffee grinds. These materials are known to contain high levels of tannins and phenolic compounds (1,13), although no determinations of these compounds were performed. Results from the tannic acid experiment demonstrated that it is possible to add materials with no nitrogen to soil and obtain control of *M. arenaria*. Phenolic compounds have been reported to be effective soil amendments against plant parasitic nematodes (2,4,5,7,8,10,30). Our results with tannic acid also demonstrated a dual action from this material resulting in phytotoxicity against squash and control of *M. arenaria*. It may be possible that the inclusion of a source of

Table 5. Effect of dry ground green holly (*Ilex opaca*) leaves on growth of squash (*Cucurbita pepo* L.) plants and development of *Meloidogyne arenaria*.[x]

Amount Added (% w/w)	No. Plants per Pot	Shoot Height (cm)	Fresh Shoot Weight (gm)	Fresh Root Weight (gm)	Root Condition Index[y]	Galls per gm Root	Gall Index[z]
0.0	4.9 A	18.7 A	2.29 A	0.60 A	3.37 A	23.4 A	3.2 A
0.5	4.9 A	16.8 B	1.61 B	0.53 A	3.12 A	28.6 A	4.0 A
1.0	5.0 A	16.8 B	1.36 C	0.53 A	3.10 A	20.1 A	3.3 A
1.5	5.0 A	15.3 C	1.44 C	0.55 A	2.97 A	5.B A	1.0 B
2.0	5.0 A	15.4 C	1.45 C	0.59 A	3.32 A	2.8 B	1.0 B
4.0	4.2 A	15.0 C	1.45 C	0.46 A	3.36 A	1.4 B	0.3 B

[x] Values are the averages of 8 replications; those within the same column with a common letter are not statistically different (P = 0.05).

[y] Based on a scale of 1-5, where 1 represented plants with very poor root growth and 5 those with excellent growth.

[z] Based on a scale of 0-10 where a value of 0 represented roots with no galls and 10 roots with the greatest number of galls (33).

Table 6. Effect of dry ground green holly (*Ilex opaca*) leaves on pH and other variables related to its decomposition in soil.[x]

Amount Added (% w/w)	Soil pH	Conductivity (micromohs)	Nitrate Nitrogen (μg/gm soil)	Urease Activity[y]	Aryl Phosphatase Activity[z]
0.0	5.75 E	29.12 E	0.00 D	0.00 D	8.31 B
0.5	6.15 D	42.81 D	0.29 D	3.40 C	8.44 B
1.0	6.45 C	71.18 C	0.00 D	2.93 C	9.55 B
1.5	6.84 B	100.06 B	1.09 C	4.80 BC	12.44 A
2.0	6.92 A	103.88 B	2.03 B	7.00 AB	11.74 A
4.0	7.04 A	124.12 A	3.14 A	9.38 A	11.91 A

[x] Values are the averages of 8 replications; those within the same column with a common letter are not statistically different (P = 0.05).

[y] Expressed as μg of ammoniacal nitrogen released per hour per gm of soil.

[z] Expressed as μg of phenol released (substrate = phenyl disodium phosphate) per hour per gm of soil.

nitrogen with the tannic acid amendments could reduce its phytotoxicity and retain its nematicidal activity. The addition of nitrogen would facilitate decomposition of tannic acid by soil microrganisms prior to planting of squash. Results from the holly study support this view. The holly amendments contained sufficient nitrogen to stimulate soil microflora as evidenced by increased urease and aryl phosphatase activities of the soil, and accumulations of nitrate and possibly other forms of nitrogen (higher conductivity) in amended soils.

Results from experiments with pecan shells and coffee grinds suggest that the type and availability of phenols and tannin may determine the relative efficacy of amendments against *M. arenaria.* Pecan shells contain large amounts of tannis, digallic acid and gallo tannins (Harry Amling, personal communication). However, our results suggest that these materials were not available in the amended soil in a form that was nematicidal or that they were released into soil too slowly to affect development of *M. arenaria.* Similarly, coffee grinds contain ortho, di-, and and trihydroxyphenols (1) which are either not nematicidal or were not released in sufficient quantity (treatments with less 4%) to affect nematode development. Clearly, the relation between the specific composition of the materials used in our study and their nematicidal properties will require further research.

The amendments used in the present study may be expected to stimulate a specific microflora capable of decomposing phenolic materials. It is possible that species of such microflora may be capable of parasitizing eggs or other developmental stages of *M. arenaria* as has been shown for some fungal species (11,22).

CONCLUSIONS

The least phytotoxic, and effective material for control of *M. arenaria* in this study was the holly leaves amendment. The minimal effective dosage against the nematode was 1.5% equivalent (broadcast basis) to 34 MT/ha. Since holly leaves can be obtained from forests or ornamental trees, this material may be of use to home gardeners to treat small areas infested with nematodes.

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