JOURNAL OF NEMATOLOGY

VOLUME 31 SEPTEMBER 1999 NUMBER 3

Journal of Nematology 31(3):241–263. 1999.

Plant Sources of Chinese Herbal Remedies: Effects on *Pratylenchus vulnus* and *Meloidogyne javanica*

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Abstract: More than 500 plant species, used alone or in combination, are documented in Chinese traditional medicine to have activity against helminth and micro-invertebrate pests of humans. We subjected 153 candidate medicines or their plant sources to multilevel screening for effectiveness against plant-parasitic nematodes. For extracts effective in preliminary screens, we determined time-course and concentration-response relationships. Seventy-three of the aqueous extracts of medicines or their plant sources killed either *Meloidogyne javanica* juveniles or *Pratylenchus vulnus* (mixed stages), or both, within a 24-hour exposure period. Of 64 remedies reported as antihelminthics, 36 were effective; of 21 classified as purgatives, 13 killed the nematodes; of 29 indicated as generally effective against pests, 13 killed the nematodes. Sources of extracts effective against one or both species of plant-parasitic nematodes are either the whole plant or vegetative, storage or reproductive components of the plants. Effective plants include both annuals and perennials, range from grasses and herbs to woody trees, and represent 46 plant families.

Key words: botanicals, China, herbal remedies, *Meloidogyne javanica,* narcotic effects, natural products, plant extracts, plant-parasitic nematodes, *Pratylenchus vulnus,* suppression, toxic effects.

Over the past five decades, the components and metabolites of many plants have been tested for their potential in the management of plant-parasitic nematodes. Plants have been selected for local convenience or on the basis of reported efficacy against nematodes or other pests. Some successes have been documented but the knowledge base, in general, is fragmented. Water extracts from many plants may reduce levels of several nematode species (e.g., Grewal, 1989; McGawley et al., 1990; Siddiqui and Alam, 1990; Verma et al., 1989; Whapham et al., 1994). Incorporation of certain plants as green manures also may lower nematode numbers (Germani et al., 1983; Hendro et al., 1992; Mojtahedi et al., 1991, 1993; Nicolay and Sikora, 1989; Prot et al., 1992), while results of other studies are inconclusive (e.g., Johnson et al., 1992; MacGuidwin and Layne, 1995).

In the present study we drew on a 5,000-year history documented in ancient through modern literature on Chinese herbal medicine (e.g., Chen, 1965; Huang, 1993; Jian Shu New Medical College, 1986; Li, 1975; Lu, 1972; Miao, 1993; Shih, 1972; Shu, 1992; Zhang and Xia, 1996). Two important recent compilations are the *Zhong Hua Yao Hai* (Miao, 1993) and the *Zhong Hua Yao Diang* (Ministry of Public Health, 1985), which are standard reference works in Chinese medicine. These texts contain detailed descriptions of the common and botanical names of medicinal plants as well as methods of identification, collection, and cultivation. Both books also include descriptions of the physical characteristics, uses, chemical composition, pharmacological action, dosage, and possible side effects of herbal medicines. Some 8,500 parts, products, and metabolites of plants have been identified which, alone or in combination, result in thousands of ‘‘prescriptions’’ for ailments of animals and humans (Huang, 1993; Huang, 1994; Li and Lai, 1994; Lu, 1972). Active constituents in many of the plants are well characterized (e.g., Hsu et al., 1982, 1985; Huang, 1993).

Received for publication 26 August 1998.
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The authors thank Robert C. Venette for useful discussions and assistance.
This paper was edited by E. C. Bernard.

Early records of medicinal plants used during the Sheng Nung period (ca. 2800 BC) were assembled in 101 BC in a book titled *Sheng Nung Ben Cao Chien,* which contained descriptions of 365 medicines (Huang, 1993). A second important medical text, the *Huang Di Nei Chien,* appeared around 100 BC (Ma and Zhang, 1972). Knowledge of herbs and their medicinal applications advanced during the long period from the Shang-Zhou dynasty through the Sung, Jiang, and Yuan dynasties (2000 BC to 1368). Many important books were written during this period, for example, *Pin Choi Nai Chen, San Hai Chien, Jian Lui Ben Cao, Yung San Chian,* and the *Ben Cao Chien* collection (Huang, 1993). In these texts, thousands of herbal medicines were described for treating different diseases. An important study on Chinese herbs was written during the Ming-Chin dynasty (1368 to 1911) by Li who, in the 16th century, spent more than 30 years studying the medicinal effects of herbs (Huang, 1993). His treatise, the *Pen Ts'ao Kang Mu* (Li, 1975), includes descriptions of more than 1,892 medicines.

Most provinces in China still have colleges and institutes of traditional medicine. Intensive research continues on herbal remedies, and numerous books and papers have been published (e.g., Huang, 1994; Jian Shu New Medical College, 1986; Miao, 1993; Shih, 1972).

English compilations from the Asian literature list almost 300 plants as having antihelminthic or vermifuge (causing the expulsion of worms) properties (Perry, 1980). We have found reports of more than 500 plant species that are components of prescriptions reportedly effective against parasitic helminths (mainly nematodes) and other invertebrate pests of animals and humans (Anonymous, 1977, 1996; Hu and Chen, 1993; Huang, 1994; Jian Shu New Medical College, 1986; Lu, 1972; Shih, 1972; Shu, 1992; Zhang, 1972; Zhang and Xia, 1996; Zhejian Wenli County, 1973; Zhou and Wang, 1987; Zhuang and Li, 1978). Many species are toxic to microorganisms and some have been successfully used against insect and fungal pests (Barbieri et al., 1993; Ferrari et al., 1991; Zhang and Xia, 1996). The plant sources give rise to countless prescriptive combinations for suppressing internal and external parasites of humans.

In this study our objective was to identify plants used in Chinese herbal remedies and to determine their efficacy against plant-parasitic nematodes. Most of the 153 Chinese herbal medicine sources we surveyed are either documented vermicides and vermifuges or are known to be bactericidal or to ''kill pests'' (Frohne and Pfander, 1984; Hu and Chen, 1993; Huang, 1994; Jian Shu New Medical College, 1986; Nanjing College of Traditional Chinese Medicine, 1993; Lu, 1972; Shih, 1972; Shu, 1992; Sukul, 1992; Zhang, 1972; Zhang and Xia, 1996; Zhejian Wenli County, 1973; Zhou and Wang, 1987; Zhuang and Li, 1978).

Materials and Methods

We attempted to interpret and update the classification and current synonymies of plants reported in Chinese literature from different sources and eras. Often there were variations in spelling that were sometimes obvious and sometimes not. In a few cases we were unable to determine the classification of the plant names provided in the source literature. Where possible, we determined the Chinese and English names of the source plants, their growth habit, and purported medicinal effects (Table 1).

Types of observations and studies: Three types of experiments were conducted in a step-wise progression: a Direct Observation (DO) survey in which the response of nematodes to immersion in the plant extract was observed under the microscope; Time Course (TC) studies of the ability of nematodes to move through a screen after varying periods of exposure; and Effective Concentration (EC) studies, which examined the effect of increasing concentrations of plant extracts on nematodes. For many of the extracts, the DO and TC studies were repeated several times with materials from different sources or of different age. Only plant extracts that appeared to affect nematodes in the DO studies were tested in the TC stud-

TABLE 1. Plant sources of Chinese herbal remedies, ordered by family, tested for effects on *Meloidogyne javanica* and *Pratylenchus vulnus*.

Name	Common name	Chinese name	Family	Gr. habit	Duration	Part	pH (extract)	Medicinal uses
Andrographis paniculata Ness	Common andrographis	Chuanxinlian	Acanthaceae	Herb	Annual	Whole plant	7.0	Antidote, reduces fever
Acorus gramineus Soland.	Grassleaf sweetflag	Shichangpu	Acoraceae	Herb	Perennial	Stem	3.3	Expectorant, sedative
Aloe vera L.	Aloe	Kulasuoluhui	Aloeaceae	Herb	Perennial	Leaf resin	3.9	Kills pests, purgative
Angelica dahurica Benth. et Hook. f.	Dahuriae angelica	Xinanbaizhi	Apiaceae	Herb	Perennial	Root	4.0	Kills pests, bactericidal
Angelica pubescens Shan et Yuan	Doubleteeth pubescent angelica	Chongchimao-danggui	Apiaceae	Herb	Perennial	Rhizome/root	4.8	Analgesic, reduces fever
Angelica sinensis Diels	Chinese angelica	Danggui	Apiaceae	Herb	Perennial	Root	5.2	Purgative, analgesic
Bupleurum chinensis DC.	Chinese thorowax	Beichaihu	Apiaceae	Herb	Perennial	Root	4.1	Kills pests, analgesic
Cinnamomum cassia Presl	Cassia	Rougui	Apiaceae	Tree	Perennial	Bark	4.4	Antihelminthic, sedative
Cnidium monnieri Cuss.	Monnier's snowparsley	Shechuang	Apiaceae	Herb	Annual	Fruit	5.8	Tonic, reduces fever
Ferula sinkiangensis M. Shen	Chinese asafetida	Xinjianawei	Apiaceae	Herb	Perennial	Sap	7.1	Antidiarrheal, antihelminthic
Ligusticum sinense Oliv.	Chinese ligusticum	Gaoben	Apiaceae	Herb	Perennial	Rhizome/root	3.4	Analgesic, antidiarrheal
Ligusticum wallichii Sieb. et Zucc.	Szechwan lovage	Chuanxiong	Apiaceae	Herb	Perennial	Rhizome	2.9	Stimulates blood, analgesic
Peucedanum praeruptorum Dunn.	Whiteflower hogfennel	Baihuaqianhu	Apiaceae	Herb	Perennial	Root	3.5	Expectorant
Nerium oleander L.	Oleander	Jiazhutao	Apocynaceae	Shrub	Perennial	Leaf	6.7	Diuretic, analgesic, cardiotonic
Pinellia tuber Breit.	Pinellia	Banxia	Aracaceae	Herb	Perennial	Tuber	3.0	Antihelminthic, expectorant
Arisaema consanguineum Schott.	Jack in the pulpit	Tiannanxing	Araceae	Herb	Perennial	Tuber	6.3	Expectorant, sedative
Colocasia esculenta Schott.	Dasheen	Yu	Araceae	Herb	Perennial	Tuber, leaf	6.4	Antidote

TABLE 1. *Continued*

Name	Common name	Chinese name	Family	Gr. habit	Duration	Part	pH (extract)	Medicinal uses
Homalomena occulta Schokt.	Obscure homalomena	Qiannianjian	Araceae	Herb	Perennial	Rhizome	2.8	Kills pests, antidote
Hedera helix L.	English ivy	Changchunteng	Araliaceae	Herb. vine	Perennial	Leaf	5.2	Antidote, reduces fever
Tetrapanax papyriferus K. Koch	Ricepaper plant	Tongtuomu	Araliaceae	Shrub	Perennial	Pith	5.7	Antihelminthic, diuretic
Areca catechu L.	Betel palm	Binlang	Arecaceae	Palm	Perennial	Fruit	3.2	Reduces fever, antihelminthic
Asarum sieboldii Miq.	Wild ginger	Xixin	Aristolochiaceae	Herb	Perennial	Whole plant	4.7	Anticough, analgesic
Cynanchum stauntoni Schltr.	Willowleaf swallowwort	Baiqian	Asclepiadaceae	Herb	Perennial	Rhizome/root	3.2	Prevents coughs
Cynanchum versicolor Bge.	Blackened swallowwort	Manshenbaiwei	Asclepiadaceae	Herb	Perennial	Root	4.3	Antihelminthic, reduces fever
Artemisia apiacea Hence	Sweet wormwood	Qinghao	Asteraceae	Shrub	Perennial	Whole plant	4.8	Reduces fever, antidote
Artemisia argyi Levl. et. Vant.	Argy wormwood	Aiye	Asteraceae	Herb	Perennial	Leaf	5.0	Bactericidal, purgative
Artemisia capillaris Thunb.	Virgate wormwood	Yinchenhao	Asteraceae	Herb	Perennial	Seedling	6.4	Antihelminthic, reduces fever
Carpesium abrotanoides L.	Common carpesium	Heshi	Asteraceae	Herb	Perennial	Fruit	7.0	Reduces fever, antihelminthic
Carthamus tinctorius L.	Safflower	Honghua	Asteraceae	Herb	Annual	Flower	4.9	Stimulates blood, purgative
Emilia sonchifolia DC.	Emilia	Yidianhong	Asteraceae	Herb	Annual	Whole herb	6.5	Antihelminthic, antidiarrheal
Rhaponticum uniflorum DC.	Uniflower swisscentaury	Loulu	Asteraceae	Herb	Perennial	Root	5.1	Antihelminthic, antidote
Xanthium sibiricum Patr.	Siberian cocklebur	Canger	Asteraceae	Herb	Annual	Fruit	5.0	Kills pests, analgesic
Mahonia bealei Carr.	Chinese mahonia	Shidagonglao	Berberidaceae	Shrub	Perennial	Leaf	4.2	Kills pests, prevents coughs
Sinapis alba L.	White mustard	Baijie	Brassicaceae	Herb	Annual	Seed	4.6	Expectorant, purgative, antihelminthic
Cercis canadensis Hopkins	Western redbud	Jiazhouzijin	Caesalpiniaceae	Tree	Perennial	Fruit	7.2	Unknown
Lonicera japonica Thunb.	Honeysuckle	Rrndong	Caprifoliaceae	Vine	Perennial	Flower	5.2	Bactericidal, antidote

TABLE 1. *Continued*

Name	Common name	Chinese name	Family	Gr. habit	Duration	Part	pH (extract)	Medicinal uses
Dianthus superbus L.	Pink lilac	Qumai	Caryophyllaceae	Herb	Perennial	Whole plant	5.8	Diuretic, purgative
Tripterygium wilfordii Hook. f.	Common threewingnut	Leigongteng	Celastraceae	Shrub	Perennial	Root	5.8	Kills pests
Quisqualis indica L.	Rangoon creeper	Shijunzi	Combretaceae	Shrub	Perennial	Fruit	6.4	Antihelminthic
Ipomoea nil Roth.	White-edge morningglory	Qianniu	Convolvulaceae	Herb. vine	Annual	Seed	5.6	Purgative, antihelminthic
Cornus officinalis Sieb. et Zucc.	Asiatic cornelian cherry	Shanzhuru	Cornaceae	Tree	Perennial	Fruit	3.4	Antihelminthic, bactericidal
Cucurbita moschata Duch.	Pumpkin	Nangua	Cucurbitaceae	Herb. vine	Annual	Fruit	5.1	Antihelminthic
Cucurbita pepo L.	Pumpkin	Nangua	Cucurbitaceae	Herb. vine	Annual	Seed	5.9	Antihelminthic
Cucurbita pepo L.	Winter squash		Cucurbitaceae	Herb. vine	Annual	Fruit	8.5	Unknown
Luffa cylindrica L.	Luffa	Sigua	Cucurbitaceae	Herb	Annual	Sponge	6.5	Antihelminthic, antidote
Trichosanthes kirilowii Maxim.	Snakegourd root	Gualou	Cucurbitaceae	Vine	Perennial	Root	7.8	Anti-inflammatory
Platycladus orientalis Franco	Chinese arborvitae	Cebai	Cupressaceae	Tree	Perennial	Leafy twigs	4.3	Antihelminthic, reduces fever
Cyperus rotundus L.	Nutgrass galingale	Shacao	Cyperaceae	Herb	Perennial	Rhizome	4.8	Kills pests, bactericidal
Ephedra sinica Stapf.	Chinese ephedra	Caomahuang	Ephedraceae	Shrub	Perennial	Rhizome/root	5.5	Stimulates circulation, cures asthma
Rhododendron molle G. Don	Chinese azalea	Naoyanghua	Ericaceae	Shrub	Perennial	Flower	5.4	Anti-inflammatory, analgesic, antihelminthic
Croton tiglium L.	Purging croton	Baidou	Euphorbiaceae	Tree	Perennial	Fruit	6.1	Purgative, antihelminthic
Euphorbia fischeriana Steud.	Fischer euphorbia	Lueixianlangdu	Euphorbiaceae	Herb	Perennial	Root	4.4	Kills pests, expectorant
Euphorbia hirta L.	Garden euphorbia	Feiyangcao	Euphorbiaceae	Herb	Annual	Whole herb	5.3	Bactericidal, antidote
Euphorbia pekinensis Rupr.	Peking euphorbia	Daji	Euphorbiaceae	Herb	Perennial	Root	3.5	Anti-inflammatory, purgative
Manihot esculenta Crantz	Common cassava	Mushu	Euphorbiaceae	Shrub		Tuber	4.4	Eliminates swelling, antidote
Ricinus communis L.	Castorbean	Bima	Euphorbiaceae	Shrub	Perennial	Leaf	4.7	Antidote, purgative
Sapium sebiferum Roxb.	Tallowtree	Wujiu	Euphorbiaceae	Tree	Perennial	Leaf	4.5	Diuretic, antidote, antihelminthic
Speranskia tuberculata Bail.	Tuberculate speranskia	Digouye	Euphorbiaceae	Herb	Perennial	Whole herb	7.7	Stimulates blood, analgesic

TABLE 1. *Continued*

Name	Common name	Chinese name	Family	Gr. habit	Duration	Part	pH (extract)	Medicinal uses
Erythrina variegata Merr.	Oriental variegated coralbeum	Citong	Fabaceae	Tree	Perennial	Bark	4.4	Reduces fever, analgesic, antihelminthic
Gleditsia sinensis Lam.	Chinese honeylocust	Zaojia	Fabaceae	Tree	Perennial	Fruit	4.4	Antihelminthic, expectorant
Senna alexandrina P. Mill.	Indian senna	Fanxie	Fabaceae	Shrub	Perennial	Leaf	6.0	Purgative
Sophora flavescens Ait.	Light-yellow sophora	Kushen	Fabaceae	Shrub	Perennial	Root	4.1	Antihelminthic, antidote
Hydnocarpus anthelmintica Pier.	Chaulmoogra seed	Dafengzhi	Flacourtiaceae	Tree	Perennial	Seed	5.3	Kills pests, antidote
Gentiana scabra Bge.	Chinese gentian	Longdan	Gentianaceae	Herb	Perennial	Root	3.8	Reduces fever
Ginkgo biloba L.	Maidenhair tree	Yinxing	Ginkgoaceae	Tree	Perennial	Fruit	4.4	Anticough, antihelminthic
Illicium verrum Hook. f.	Star anise	Bajiaohuixiang	Illiaceae	Tree	Perennial	Fruit	3.8	Cures colds, analgesic
Juglans regia L.	English walnut	Hutao	Juglandaceae	Tree	Perennial	Leaf	7.8	Kills pests, antidote
Schizonepeta tenuifolia Brig.	Fineleaf schizonepeta	Jingjie	Labiatae	Herb	Annual	Whole plant	6.7	Bactericidal, reduces fever
Ocimum basilicum L.	Basil	Iuone	Lamiaceae	Herb	Annual	Seed	8.4	Stimulates circulation, antidote
Prunella vulgaris L.	Common selfheal	Xiakucao	Lamiaceae	Herb	Perennial	Fruit-spike	6.9	Bactericidal, reduces fever
Scutellaria barbata D. Don	Barbed skullcap	Banzhilian	Lamiaceae	Herb	Perennial	Whole plant	4.9	Antidote, anti-inflammatory
Akebia quinata Deene	Akebia	Mutong	Lardizabalaceae	Vine	Perennial	Fruit	3.9	Kills pests, analgesic
Lindera strychnifolia Willar.	Combined spicebush	Wuyao	Lauraceae	Shrub	Perennial	Root	3.6	Antihelminthic, eliminates bloat
Allium cepa L.	Onion	Yangcong	Liliaceae	Herb	Perennial	Bulb	4.3	Bactericidal
Allium sativum L.	Garlic	Dasuan	Liliaceae	Herb	Perennial	Bulb	5.0	Bactericidal, antihelminthic
Allium sp.	Onion	Yangcong	Liliaceae	Herb	Perennial	Bulb/leaf	4.8	Kills pests
Allium tuberosum Rottler	Tuber onion	Jiu	Liliaceae	Herb	Perennial	Stem	7.8	Antidote

TABLE 1. *Continued*

Name	Common name	Chinese name	Family	Gr. habit	Duration	Part	pH (extract)	Medicinal uses
Asparagus cochinchinensis Merr.	Cochinchinese asparagus	Tingmendong	Liliaceae	Herb	Perennial	Root	4.0	Antihelminthic, reduces fever
Narcissus tazetta L.	Narcissus	Suixian	Liliaceae	Herb	Perennial	Bulb	6.0	Stimulates blood, purgative
Ophiopogon japonicus Ker.-Gawl.	Dwarf lilyturf	Maimendong	Liliaceae	Herb	Perennial	Root	4.1	Purgative
Paris polyphylla Smith		Qiyeyizihua	Liliaceae	Herb		Rhizome	3.9	Reduces fever, antidote
Rhodea japonica Roth.	Omoto nipponlily	Wanninqing	Liliaceae	Herb	Perennial	Leaf	6.7	Antihelminthic, antidote
Veratrum nigrum L.	Falsehellebore	Heililu	Liliaceae	Herb	Perennial	Rhizome/root	4.8	Kills pests, antidote
Lobelia chinensis Lour.	Chinese lobelia	Banbianlian	Lobeliaceae	Herb	Perennial	Whole herb	4.5	Antihelminthic, anti-inflammatory
Magnolia officinalis Rehd. et Wils.	Magnolia	Houbo	Magnoliaceae	Tree	Perennial	Bark	5.1	Astringent
Hibiscus syriacus L.	Rose of sharon	Mujin	Malvaceae	Shrub	Perennial	Bark	3.9	Reduces fever, antihelminthic
Azadirachta indica Adr. Juss.	Chinaberry	Kujian	Meliaceae	Tree	Perennial	Bark	3.4	Antihelminthic
Azadirachta indica Adr. Juss.	Chinaberry	Kujian	Meliaceae	Tree	Perennial	Fruit	3.4	Antihelminthic
Toona sinensis Roem.	Chinese toona	Xianxhun	Meliaceae	Tree	Perennial	Bark	5.5	Antihelminthic, reduces fever
Sinomenium acutum Rehd.	Orientvine	Qingfengteng	Menispermaceae	Herb	Perennial	Stem	5.0	Kills pests, antidote
Stephania tetrandra Moore	Fourstamen stephania	Fangji	Menispermaceae	Vine	Perennial	Root	3.5	Kills pests, reduces fever
Albizzia julibrissin Durazz.	Silktree albizzia	Hehua	Mimosaceae	Tree	Perennial	Bark	4.2	Kills pests, anti-inflammatory
Morus alba L.	Mulberry	Sang	Moraceae	Tree	Perennial	Leaf	7.4	Antihelminthic, reduces fever
Myristica fragrans Houtt.	Nutmeg	Roudoukou	Myristicaceae	Tree	Perennial	Fruit	4.5	Antidiarrheal
Eucalyptus globulus Labill.	Tasmanian bluegum	Lanan	Myrtaceae	Tree	Perennial	Leaf	7.8	Bactericidal, antihelminthic

TABLE 1. *Continued*

Name	Common name	Chinese name	Family	Gr. habit	Duration	Part	pH (extract)	Medicinal uses
Eugenia caryophyllata Thunb.	Dovetree	Dingxiang	Myrtaceae	Tree	Perennial	Clove	3.9	Antihelminthic, purgative
Bletilla striata Reichb. f.	Common bletilla	Baiji	Orchidaceae	Herb	Perennial	Tuber	3.8	Kills pests
Oxalis corniculata L.	Creeping woodsorrel	Cujiangcao	Oxalidaceae	Herb	Perennial	Whole herb	3.1	Antihelminthic, antidote
Phytolacca octandra L.	Redink plant	Shanglu	Phytolaccaceae	Herb	Perennial	Root	4.7	Diuretic, anti-inflammatory
Pinus sp.	Pine	Songshu	Pinaceae	Tree	Perennial	Leaf	4.4	Antihelminthic, antirheumatic
Plantago asiatica L.	Plantain	Cheqian	Plantaginaceae	Herb	Perennial	Whole herb	5.1	Bactericidal, expectorant
Coix lacryma-jobi L.	Job's tears	Yiyi	Poaceae	Grass	Annual	Seed	6.4	Tonic, diuretic
Hordeum vulgare L.	Barley	Damai	Poaceae	Grass	Annual	Seed	4.4	Antidiarrheal
Triticum aestivum L.	Wheat	Xiaomai	Poaceae	Herb	Annual	Whole plant	4.5	Cardiotonic
Polygonum aviculare L.	Common knotgrass	Bianxu	Polygonaceae	Herb	Annual	Whole herb	5.8	Antihelminthic, anticancer
Polygonum flaccidum Meisn.		Laliao	Polygonaceae	Herb	Annual	Whole herb	6.2	Antihelminthic, anti-inflammatory
Rheum officinale Baill.	Chinese rhubarb	Daihuang	Polygonaceae	Herb	Perennial	Stem	4.0	Reduces fever, purgative
Rumex crispus L.	Curly dock	Zhouyesuanmuo	Polygonaceae	Herb	Perennial	Root	5.9	Kills pests, purgative
Rumex japonicus Houtt.	Japanese dock	Yangti	Polygonaceae	Herb	Perennial	Leaf	6.5	Kills pests, purgative
Rumex obtusifolius L.	Bitter dock	Dunyesuanmuo	Polygonaceae	Herb	Perennial	Leaf	7.4	Kills pests, antidote
Dryopteris crassirhizoma Nakai	Fern	Guanzhong	Polypodiaceae	Fern	Perennial	Root	4.3	Antihelminthic, bactericidal, antiviral
Polyporus mylittae Cook et Mass.	Fungus	Leiwan	Polyporaceae	Fungus		Whole fungus	3.3	Antihelminthic
Portulaca oleracea L.	Purslane	Machixian	Portulacaceae	Herb	Annual	Whole herb	5.1	Antihelminthic, reduces fever
Punica granatum L.	Pomegranate	Shiliu	Punicaceae	Tree	Perennial	Bark	3.9	Antidiarrheal, antihelminthic

TABLE 1. *Continued*

Name	Common name	Chinese name	Family	Gr. habit	Duration	Part	pH (extract)	Medicinal uses
Anemone altaica Fisch.	Lrkutus anemone	Aetaiyinlienhua	Ranuncluaceae	Herb	Perennial	Rhizome	4.2	Antihelminthic, antidote
Coptis chinensis Franch.	Coptis	Huanglian	Ranunculaceae	Herb	Perennial	Root	3.7	Bacteriostatic, reduces fever
Pulsatilla chinensis Regel	Chinese pulsatilla	Baitouweng	Ranunculaceae	Herb	Perennial	Root	4.9	Reduces fever, antidote, antidiarrheal
Ranunculus sceleratus L.	Poisonous buttercup	Shilongrui	Ranunculaceae	Herb	Perennial	Whole herb	4.7	Kills pests, anti-inflammatory
Agrimonia pilosa Ledeb.	Hairyvein agrimonia	Xianhecao	Rosaceae	Herb	Perennial	Whole plant	4.8	Cardiotonic
Crataegus pinnatifida Bge.	Hawthorn	Shanzha	Rosaceae	Shrub	Perennial	Fruit	2.6	Antihelminthic, purgative
Eriobotrya japonica Lindl.	Loquat	Pipa	Rosaceae	Tree	Perennial	Leaf	5.8	Prevents coughs, expectorant
Photinia serratifolia Kalkm.	Chinese photinia	Shinan	Rosaceae	Shrub	Perennial	Leaf	4.4	Antihelminthic, analgesic
Prunus armeniaca L.	Bitter almond	Xinzi	Rosaceae	Tree	Perennial	Seed	5.8	Antihelminthic, expectorant
Prunus mume Sieb. et Zucc.	Japanese apricot	Wumei	Rosaceae	Tree	Perennial	Fruit	2.8	Antihelminthic
Prunus persica Batsch	Peach	Tao	Rosaceae	Tree	Perennial	Seed	5.9	Antihelminthic, stimulates blood
Rosa laevigata Michx.	Cherokee rose	Jinyinzi	Rosaceae	Shrub	Perennial	Fruit	4.1	Antihelminthic, antidiarrheal
Rubus chingii Hu	Palmleaf raspberry	Hupenzi	Rosaceae	Shrub	Perennial	Fruit	4.8	Liver tonic
Gardenia angusta Merr.	Cape jasmine	Shanzhi	Rubiaceae	Shrub	Perennial	Fruit	4.0	Reduces fever, sedative
Dictamnus dasycarpus Turcz.	Densefruit pittany	Baixian	Rutaceae	Tree	Perennial	Bark	3.9	Diuretic, antihelminthic
Evodia rutaecarpa Benth.	Medicinal evodia	Wuzhuyu	Rutaceae	Shrub	Perennial	Fruit	5.6	Antihelminthic, analgesic
Phellodendron chinense Schneid.	Chinese corktree	Huanpishu	Rutaceae	Tree	Perennial	Bark	4.5	Kills pests, antidote
Zanthoxylum bungeanum Maxim.	Chinese pricklyash	Huajiao	Rutaceae	Shrub	Perennial	Fruit	6.6	Antihelminthic, analgesic

TABLE 1. *Continued*

Name	Common name	Chinese name	Family	Gr. habit	Duration	Part	pH (extract)	Medicinal uses
Zanthoxylum nitidum DC.	Shingleaf pricklyash	Rudijinniu	Rutaceae	Vine	Perennial	Root	3.8	Anti-inflammatory, analgesic
Zanthoxylum piperitum DC.		Shujiao	Rutaceae	Herb	Annual	Fruit	4.9	Diuretic, antihelminthic
Sapindus mukorossi Gaertn.	Chinese soapberry	Wuhuanshu	Sapindaceae	Tree	Perennial	Seed	4.2	Kills pests, expectorant
Houttuynia cordata Thunb.	Heartleaf houttuynia	Jicai	Saururaceae	Herb	Perennial	Whole herb	4.2	Bactericidal, reduces fever
Dichroa febrifuga Lour.	Antifebrile dichroa	Huanchagshann	Saxifragaceae	Shrub	Perennial	Root	5.4	Kills pests, expectorant
Brucea javanica Merr.		Yadanzi	Simaroubaceae	Shrub	Perennial	Fruit	4.8	Antihelminthic
Smilax glabra Roxb.	Glabrous greenbrier	Tufuling	Smilacaceae	Shrub	Perennial	Rhizome	2.8	Kills pests, antidote
Capsicum frutescens L.	Hot pepper	Lajiao	Solanaceae	Herb	Annual	Fruit	5.4	Kills pests, bactericidal
Capsicum sp.	Hot pepper	Lajiao	Solanaceae	Herb	Annual	Fruit	4.6	Kills pests, bactericidal
Hyoscyamus niger L.	Henbane	Tianxianzi	Solanaceae	Herb	Ann./per.	Seed	4.0	Antihelminthic, analgesic
Lycium chinense Mill.	Barbary wolfberry	Gouqi	Solanaceae	Shrub	Perennial	Fruit	4.4	Antihelminthic, liver tonic
Lycopersicon esculentum Mill.	Tomato	Fanqie	Solanaceae	Herb	Annual	Fruit, leaf	4.4	Bactericidal, purgative
Nicotiana tabacum L.	Tobacco	Yancao	Solanaceae	Herb	Annual	Leaf	6.5	Anti-inlfammatory antidote, antihelminthic
Stemona sessilifolia Miq.	Stemona	Baibu	Stemonaceae	Herb	Perennial	Root	4.0	Antihelminthic, bactericidal, anticough
Torreya grandis Fort.	Grand torreya	Fei	Taxaceae	Tree	Perennial	Fruit	4.6	Antihelminthic
Ulmus macrocarpa Hance	Elm	Daguoyu	Ulmaceae	Tree	Perennial	Fruit	7.3	Antihelminthic
Verbena officinalis L.	Herb of the cross	Mabiancao	Verbenaceae	Herb	Annual	Whole plant	6.4	Purgative
Vitex rotundifolia L.	Shrub chastetree	Danyemang	Verbenaceae	Shrub	Perennial	Fruit	5.4	Antihelminthic, reduces fever

TABLE 1. *Continued*

Name	Common name	Chinese name	Family	Gr. habit	Duration	Part	pH (extract)	Medicinal uses
Curcuma zedoaria Rosc.	Zedoary	Ezhu	Zingiberaceae	Herb		Stem	3.7	Analgesic, purgative
Zingiber officinale Rosc.	Ginger	Jiang	Zingiberaceae	Herb	Perennial	Stem	6.7	Antidote
Tadehagi triquetrum Ohashi	Triquetrous tadehagi	Hulucha		Shrub	Perennial	Whole herb	4.5	Kills pests, reduces fever

ies, and only those with an apparently strong effect on nematode mortality were tested in the EC studies.

Preparation of plant extracts: Many of the herbal medicine sources used in this study were purchased from specialty Chinese herb stores in San Francisco and Sacramento, California. For others, which were gathered fresh, the plant material was air-dried for about 2 days prior to use. Throughout the drying period, the plant material was turned gently to allow it to dry completely without overheating. Large and hard seeds were crushed, and dried stems and leaves were chopped into small pieces. Separation of extracts from the parent plant material was extremely time-consuming when the highest levels of clarity were required. We used more rapid extraction procedures when the experiment would not be compromised by reduced extract clarity. Consequently, three preparation methods were used. In all cases our stock concentration was 1 g of plant material/10 g of distilled water.

For the DO survey, dried plant material was soaked in distilled water for 1 day. The solution was then filtered through a Whatman No. 2 filter paper to obtain a particle-free liquid. For TC studies, the plant material was soaked in distilled water for 1 day and then the solution and plant material were processed in a blender for 2 minutes. The mixture was then squeezed through a cotton cloth to separate the liquid, which was centrifuged at 850*g* for 20 minutes. The supernatant was filtered through a Whatman No. 2 filter paper. For EC studies, the herbal extracts were prepared as for the TC studies, but centrifuged at 14,500*g* for 30 minutes. The supernatant fluid was filtered through Whatman No. 2 filter paper to obtain a clear liquid.

Direct observation survey: Approximately 50 individuals of *Pratylenchus vulnus* (mixed stages) or 200 *Meloidogyne javanica* second-stage juveniles (J2) were placed in distilled water in a BPI dish, with three replicate dishes for each trial. The excess water was removed with a pipet, leaving an amount just adequate for nematode movement. One milliliter of plant extract stock solution was placed into each dish. After the nematodes settled to the bottom, the solution in the dish was removed with a pipet and replaced with 1 ml of fresh plant extract. For both *P. vulnus* and *M. javanica,* nematode activity was recorded at 1, 3, 5, 8, and 24 hours after addition of the extract. After 24 hours, *M. javanica* J2 were transferred to distilled water for an additional 24 hours to check for recovery. Where there were no apparent effects on the nematodes during the exposure and recovery periods of the DO survey, the extracts were usually omitted from further studies.

For human medicinal use, herbs typically are heated in water for 30 to 60 minutes to release the active ingredients from the plant material. We compared the effects of extracts prepared by heating the plant material in water to about 90 °C for 1 hour with the same materials prepared by our standard extraction method for DO studies.

Time course studies: Depth-compensating mobility screens, similar to floating hatch chambers (Zheng and Ferris, 1991), were prepared. The screens consisted of a 0.5-cm length of 1-cm-diam. tube covered at one end with a fine nylon sieve (17-μm aperture). The end of the tube covered by the nylon sieve was inserted into a hole in the center of a 3-cm-diam. styrofoam disk so that the sieve was flush with the bottom of the disk. When placed in a plant extract solution, or in water, the disk floated and nematodes placed inside the tube were always immersed to the same depth. Ten milliliters of plant extract were placed in a 6-cm-diam. petri dish. A depth-compensating mobility screen was floated in each dish, and 1 ml of nematode suspension containing approximately 100 individuals of *P. vulnus* or 250 individuals of *M. javanica* was pipetted into each screen. The mobility screen was carefully transferred into another petri dish containing 10 ml of fresh plant extract after 1 hour, and the number of individuals that had moved through the screen into the first dish was counted. For *P. vulnus,* this procedure was repeated at 1-hour intervals for up to 4 hours, after which the screen was transferred to a petri dish containing distilled water. The number of active *P. vulnus* in the final dish was counted after 20 hours in the water. One concentration level (stock solution), replicated five times, was used for each extract. For *M. javanica,* the length of exposure to the extract was increased in three separate experiments (5, 8, and 24 hours' exposure) to ensure that the full range of effects on nematode activity was included. The three experiments were similar to those conducted for *P. vulnus,* except that counts and transfers were made at 1, 3, and 5 hours for experiment one; 1, 3, 5, and 8 hours for experiment two; and 1, 3, 5, 8, and 24 hours for experiment three. In all experiments, the depth-compensating screens were transferred to distilled water after the last count, and the number of active *M. javanica* J2 was determined after a further 24 hours in water. Each treatment had three replicates.

Effective concentration studies: One milliliter of a nematode suspension containing approximately 200 to 250 individuals of *P. vulnus* or *M. javanica* was placed into centrifuge tubes. Different amounts of plant extract were placed into each of the centrifuge tubes so that they contained final extract concentrations of 0, 5, 15, 30, 60, 75, and 90% of the stock solution. The resulting suspension was mixed gently. Three replicates were prepared for each concentration level. After exposure of the nematodes to the plant extract for 24 hours, the tubes containing the extracts and nematodes were centrifuged at 750*g* for 3 minutes. The supernatant fluid from each tube was removed with a pipet until about 1 ml remained. Distilled water was added to make a total volume of 12 ml. The tubes were again centrifuged at 750*g* for 3 minutes. The supernatant fluid was removed and the nematode-containing solution remaining at the bottom of each tube (approximately 1 ml) was pipetted into a mobility screen floating on distilled water in a petri dish. After 20 hours, the floating mobility screens were removed from the petri dishes and the nematodes in the dishes were counted. The relationship between plant extract concentration and nematode mortality was subjected to probit analysis so that standardized EC_{50} and EC_{90} levels (concentrations necessary to cause 50% and 90% nematode mortality, respectively) could be determined for comparison across plant sources. In some cases, the EC_{90} levels were estimated by extrapolation since they were not achieved within the concentration range tested.

Effect of extract pH on nematodes: To determine the possibility that any observed effects on nematodes were due to plant extract pH, we measured the pH of each extract in its stock solution (Table 1). To determine if pH was a confounding factor in extract effectiveness, 50 individuals of *P. vulnus* or 100 *M. javanica* J2 were placed in BPI dishes containing aqueous solutions of varying pH (from 2.0–8.5, with pH increasing in steps of 0.5). Nematodes were observed at hourly intervals for 24 hours (*M. javanica*) or 72 hours (*P. vulnus*). Following immersion in the pH solutions, the nematodes were transferred to a mobility screen in distilled water for 24 hours. Their activity and condition were determined by their ability to move through the screen.

Results

The plant sources of extracts effective against one or both species of plant-parasitic nematodes included annuals and perennials and ranged in growth habit from grasses and herbs to woody plants. They represent 46 plant families (Tables 1, 2). The plant components used for extraction of the effective herbal remedy include roots, rhizomes and bulbs, stems, leaves, seeds, bark, and whole plants (Table 1).

Direct observation and time course studies: Effects of plant extracts on nematode mobility varied. In some cases the nematodes twisted and moved spasmodically as the extracts were added to the BPI dishes. In other cases the nematodes ceased moving and straightened. The duration and intensity of the effect varied for each extract and in some cases differed between the two nematode species (Table 2).

The DO studies and TC studies suggested five patterns (A–E) of nematode response to extracts. In three of the patterns (A,B,C) the nematodes were unaffected or recovered from the treatment (Fig. 1A). In two patterns the nematodes were affected by the treatment and did not recover (Fig. 1B, Table 2). In pattern A, almost all of the nematodes were moving at each observation and activity levels did not diminish over the duration of the test. In pattern B, the nematodes were unaffected initially but became inactive after 24 hours of exposure and then recovered (>20% of nematodes active) after a further 24 hours in water. In pattern C responses, the nematodes were inactivated after 1 to 4 hours of exposure, but then recovered during the remainder of the exposure period and became fully active after a further 24 hours in water. In patterns D and E, the nematodes did not recover from exposure to extracts and fewer than 20% were active after immersion in water for 24 hours. The pattern D response frequently included some effect on mobility during the first hour, but the permanent effect required longer exposure, up to 24 hours. In pattern E, nematodes succumbed rapidly to the plant extract and did not recover.

Pratylenchus vulnus exhibited two types of response when exposed to extracts in the TC study. In the first response type, only a few nematodes moved through the mobility screens during the first hour of exposure and fewer than 15% had moved through the screen after 4 hours. In several cases, 60% or more of the inactivated nematodes recovered after transfer to distilled water (Fig. 2A). Despite this recovery, these plants remain assigned to pattern E due to the inactivity of nematodes after a longer exposure period (24 hours) in the DO studies. In the second response type, many nematodes moved through the mobility screens during the first hour, but progressively fewer did during 2 to 4 hours of exposure. Also, at least 20% of the relatively few inactivated nematodes moved through the mobility screens after transfer to distilled water (Fig. 2B).

For *M. javanica* in the TC study, the greatest movement through the mobility screens of J2 exposed to the herbal extracts oc-

TABLE 2. Response patterns of *Meloidogyne javanica* (*Mj*) and *Pratylenchus vulnus* (*Pv*) to extracts of plant sources of Chinese herbal remedies.[a]

Plant	Pattern		Plant	Pattern	
	MJ	*Pv*		*Mj*	*Pv*
Allium sativum	E	E	*Illicium verrum*	C	C
Andrographis paniculata	E	E	*Morus alba*	C	C
Azadirachta indica (seed)	E	E	*Phytolacca octandra*	C	C
Brucea javanica	E	E	*Sapindus mukorossi*	C	C
Hedera helix	E	E	*Sapium sebiferum*	C	C
Stemona sessilifolia	E	E	*Allium tuberosum*	C	B
Ulmus macrocarpa	E	E	*Cercis canadensis*	C	B
Allium sp.	E	E	*Cinnamomum cassia*	C	B
Angelica pubescens	E	E	*Colocasia esculenta*	C	B
Artemisia capillaris	E	E	*Gardenia angusta*	C	B
Asparagus cochinchinensis	E	E	*Gleditsia sinensis*	C	B
Bletilla striata	E	E	*Juglans regia*	C	B
Carthamus tinctorius	E	E	*Lindera strychnifolia*	C	B
Cucurbita moschata	E	E	*Luffa cylindrica*	C	B
Cucurbita pepo (pumpkin)	E	E	*Ocimum basilicum*	C	B
Cynanchum stauntoni	E	E	*Pinus sp.*	C	B
Ephiopogon japonicus	E	E	*Prunella vulgaris*	C	B
Euphorbia fischeriana	E	E	*Prunus armeniaca*	C	B
Euphorbia hirta	E	E	*Pulsatilla chinensis*	C	B
Ginkgo biloba	E	E	*Rosa laevigata*	C	B
Lycium chinense	E	E	*Speranskia tuberculata*	C	B
Manihot esculenta	E	E	*Xanthium sibiricum*	C	B
Nerium oleander	E	E	*Zanthoxylum bungeanum*	C	B
Nicotiana tabacum	E	E	*Zingiber officinale*	C	B
Prunus persica	E	E	*Trichosanthes kirilowii*	C	A
Ranunculus sceleratus	E	E	*Agrimonia pilosa*	B	E
Rheum officinale	E	E	*Eucalyptus globulus*	B	E
Rhododendron molle	E	E	*Angelica sinensis*	B	E
Rumex crispus	E	E	*Plantago asiatica*	B	E
Sinapis alba	E	E	*Platycladus orientalis*	B	E
Triticum aestivum	E	E	*Rhaponticum uniflorum*	B	E
Azadirachta indica (bark)	E	D	*Rumex obtusifolius*	B	E
Ipomoea nil	E	D	*Artemisia apiacea*	B	D
Scutellaria barbata	E	D	*Ephedra sinica*	B	D
Capsicum sp.	E	D	*Zanthoxylum nitidum*	B	D
Angelica dahurica	E	C	*Cornus officinalis*	B	C
Asarum sieboldii	E	C	*Hordeum vulgare*	B	C
Capsicum frutescens	E	C	*Lycopersicon esculentum*	B	C
Coptis chinensis	E	C	*Verbena officinalis*	B	C
Dianthus superbus	E	C	*Zanthoxylum piperitum*	B	C
Homalomena occulta	E	C	*Akebia quinata*	B	B
Paris polyphyllata	E	C	*Cyperus rotundus*	B	B
Ricinus communis	E	C	*Dichroa febrifuga*	B	B
Artemisia argyi	E	B	*Lonicera japonica*	B	B
Eugenia caryophyllata	E	B	*Magnolia officinalis*	B	B
Evodia rutaecarpa	E	B	*Mahonia bealei*	B	B
Oxalis corniculata	E	B	*Myristica fragrans*	B	B
Smilax glabra	E	B	*Narcissus tazetta*	B	B
Albizzia julibrissin	E	A	*Phellodendron chinense*	B	B
Allium cepa	D	E	*Photinia serratifolia*	B	B
Crataegus pinnatifida	D	E	*Polygonum flaccidum*	B	B
Emilia sonchifolia	D	E	*Rubus chingii*	B	B
Houttuynia cordata	D	E	*Rumex japonicus*	B	B
Lobelia chinensis	D	E	*Sinomenium acutum*	B	B
Portulaca oleracea	D	E	*Tadehagi triquetrum*	B	B
Veratrum nigrum	D	E	*Tripterygium wilfordii*	B	B
Cucurbita pepo (squash)	D	D	*Vitex rotundifolia*	B	B

TABLE 2. *Continued*

Plant	Pattern MJ	Pattern Pv	Plant	Pattern Mj	Pattern Pv
Gentiana scabra	D	D	*Polyporus mylittae*	B	A
Ligusticum sinense	D	D	*Sophora flavescens*	B	A
Ligusticum wallichii	D	C	*Cnidium monnieri*	A	E
Arisaema consanguineum	D	B	*Torreya grandis*	A	E
Bupleurum chinensis	D	B	*Cynanchum versicolor*	A	E
Areca catechu	C	E	*Peucedanum praeruptorum*	A	D
Coix lacryma-jobi	C	E	*Punica granatum*	A	C
Dryopteris crassirhizoma	C	E	*Tetrapanax papyriferus*	A	C
Hibiscus syriacus	C	E	*Aloe vera*	A	B
Prunus mume	C	E	*Anemone altaica*	A	B
Quisqualis indica	C	E	*Pinellia tuber*	A	B
Schizonepeta tenuifolia	C	E	*Stephania tetrandra*	A	B
Senna alexandrina	C	E	*Acorus gramineus*	A	A
Euphorbia pekinensis	C	E	*Dictamnus dasycarpus*	A	A
Polygonum aviculare	C	E	*Erythrina variegata*	A	A
Carpesium abrotanoides	C	D	*Ferula sinkiangensis*	A	A
Croton tiglium	C	C	*Hyoscyamus niger*	A	A
Curcuma zedoaria	C	C	*Rhodea japonica*	A	A
Eriobotrya japonica	C	C	*Toona sinensis*	A	A
Hydnocarpus anthelmintica	C	C			

[a] Response patterns A–E are illustrated in Figure 1.

curred during the first hour, fewer moved during the next 3 to 8 hours, and almost none between 8 and 24 hours. Most extracts affected at least 50% of the nematodes during the exposure period, but it was not immediately clear whether the nematodes were killed or whether they were only temporarily narcotized. The pattern of recovery of nematodes immobilized during exposure to the herbal extracts provided insight into the nature and longevity of suppressive effects (Fig. 3A–D). For extracts of most plant species, recovery rate in water declined with length of exposure to the extract, and in some recovery was very low after 5 hours of exposure (Fig. 3C,D). For several extracts in which *M. javanica* exhibited pattern E when exposed for 24 hours, there was partial to nearly full recovery after 24 hours in distilled water for shorter exposure periods (5 to 8 hours). For example, over 98% of the J2 were immobilized after 8 hours in whole-plant extracts of *Scutellaria barbata,* but 92% of those nematodes recovered after 24 hours in distilled water (Fig. 3A).

Occasionally, results of the DO and TC studies were contradictory. For example, most J2 were inactivated in the TC study after 24 hours' exposure to extracts of *Zingiber officinale* and there was minimal recovery in distilled water. However, in the DO study, using a different source of the herb, a considerable number of individuals remained active in the BPI dishes after 24 hours of exposure. Consequently, we assigned this extract to pattern C for *M. javanica* (Table 2).

Exposure to the same extracts prepared by soaking alone, or by soaking and heating for 1 hour, produced varied results. For 14 of the 25 herbs tested on *P. vulnus,* extracts prepared with heat were less effective than those prepared by soaking. In particular, *Schizonepeta tenuifolia, Senna alexandrina, Eucalyptus globulus,* and *Brucea javanica* were much more effective when prepared without heat. In these cases the active principal may be heat-sensitive. By contrast, extracts of *Hibiscus syriacus, Sophora flavescens* were more effective when prepared with heat. For nine of the herbs, the method of extract preparation made almost no difference to the nematicidal effect. For example, in extracts of *Polyporus mylittae* prepared by the two methods, almost all nematodes continued to move; in extracts of *Coix lacryma-jobi,* no

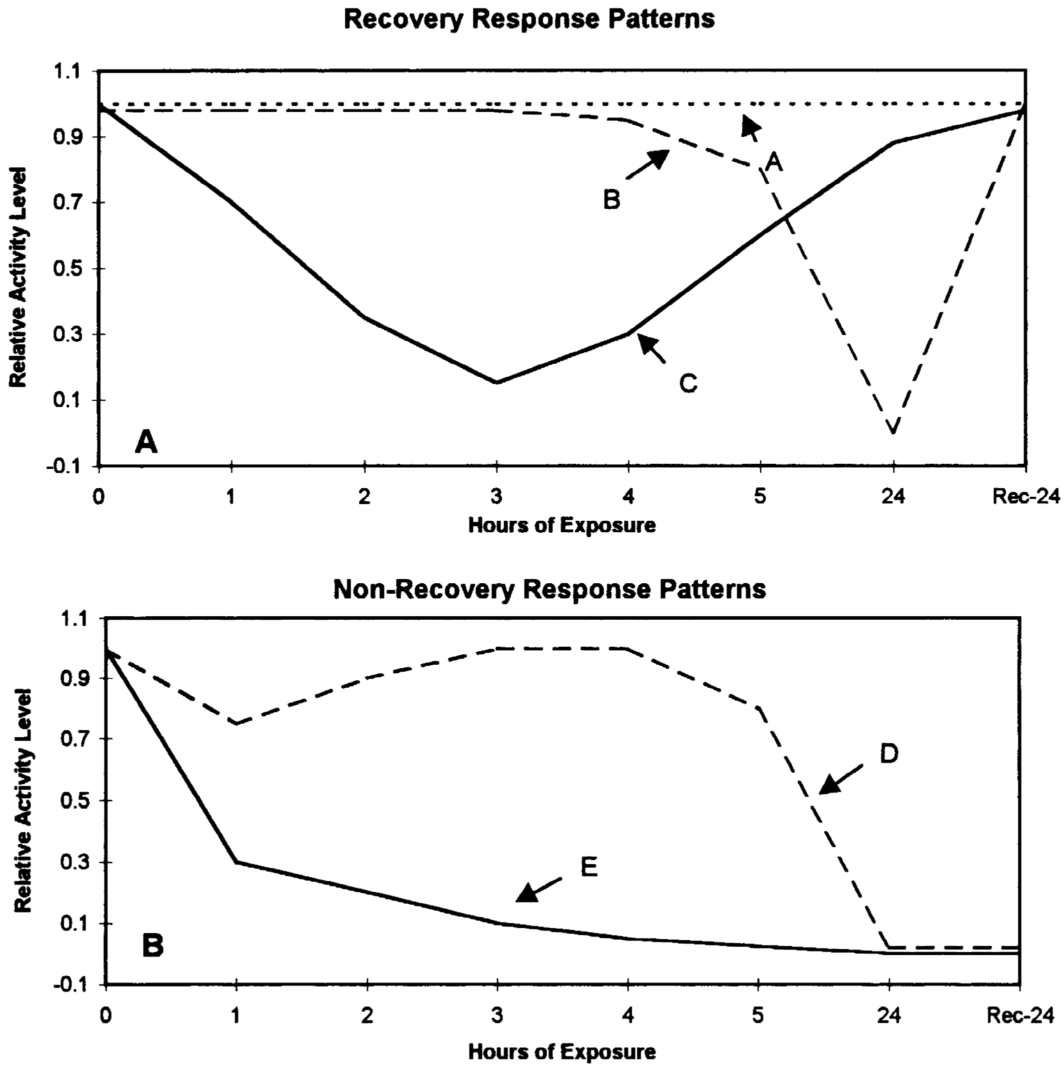

FIG. 1. Relative activity levels of juveniles of *Meloidogyne javanica* or mixed life stages of *Pratylenchus vulnus* after varying periods of exposure to plant extracts (activity level in extract relative to activity level in water) and after a subsequent 24-hour recovery period in water (Rec-24). A) Response patterns in which nematodes were unaffected by the exposure (Pattern A) or in which >20% of the individuals recovered motility during the exposure period (Pattern B) or during a subsequent 24 hours in water (Pattern C). B) Response patterns in which nematodes either became inactive after several hours in the plant extract (Pattern D) or became inactive rapidly (Pattern E) and <20% of the individuals recovered during a subsequent 24 hours in water.

nematodes moved in either the soaked or heated extract.

Effective concentration studies: Of the 45 plants with extracts having effects from which fewer than 20% of both *M. javanica* and *P. vulnus* recovered, and the 42 plants with extracts from which fewer than 20% of one of the nematode species recovered (Fig. 1B, Table 2), we conducted concentration-response analyses for 24 representative extracts on *M. javanica* and seven on *P. vulnus.* The nature of the relationship between nematode survival after 24 hours of exposure and concentration of plant extract (based on a stock solution of 1 g plant material/10 ml water) varied (Fig. 4).

Concentration-response analyses allowed comparison across plant sources of the extract concentration necessary to achieve specified nematode mortality. In some cases,

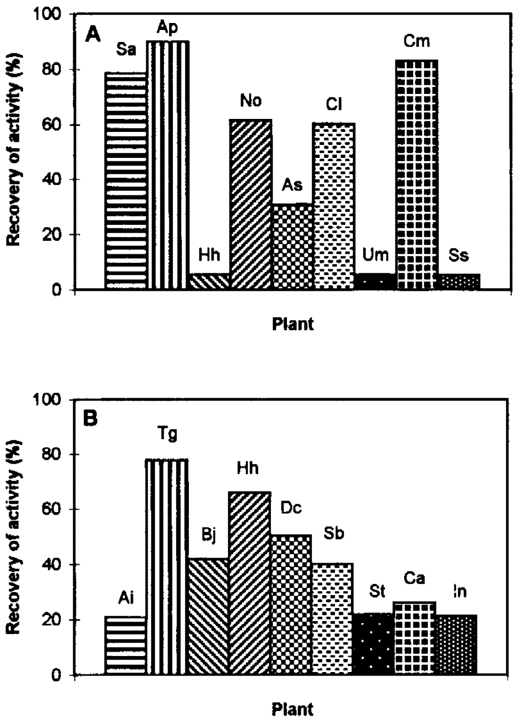

FIG. 2. Recovery of mixed life stages of *Pratylenchus vulnus* when transferred to water for 24 hours after being inactivated by 4 hours' exposure to plant extracts. A) Nematode movement inhibited rapidly in the extract, and ability to move through a screen diminished after 1 hour; Sa = *Senna alexandrina,* Ap = *Andrographis paniculata,* Hh = *Hedera helix* (leaf), No = *Nerium oleander,* As = *Allium sativum,* Cl = *Coix lacryma-jobi,* Um = *Ulmus macrocarpa,* Cm = *Curcubita moschata,* Ss = *Stemona sessifolia.* B) Nematode movement inhibited less rapidly, but ability to move through a screen diminished after 4 hours; Ai = *Azadirachta indica,* Tg = *Torreya grandis,* Bj = *Brucea javanica,* Hh = *Hedera helix* (stem), Dc = *Dryopteris crassirhizoma,* Sb = *Scutellaria barbata,* St = *Schizonepeta tenuifolia,* Ca = *Carpesium abrotanoides.*

a high level of nematode mortality was achieved at relatively low concentrations of the extract (Fig. 4A); in others, much higher concentrations of the extract were necessary to induce significant mortality (Fig. 4B–F). Since we were unable to prepare extracts at concentrations greater than 200% of the stock solution for many of the plants tested, indications of higher concentrations necessary to achieve specified mortality are extrapolations based on probit analysis. We standardized comparisons across extracts by calculation of EC_{50} and EC_{90} levels (Table 3). In some cases, 50 and 90% mortality levels could be achieved at relatively low concentrations of the plant extract stock solution, whereas in others, while 50% mortality was achieved at low concentrations, much higher concentrations were needed to achieve 90% mortality (Table 3).

Effective concentrations of various plant sources at the 50% level were similar for both *M. javanica* and *P. vulnus* (Table 3). For both nematode species, differences were greater among plant sources in the concentration of material necessary to achieve 90% mortality than those necessary for 50% mortality (Table 3) due to the exponential nature of the upper extreme of the concentration-response curves (Fig. 4). However, since determined by extrapolation, those data are less reliable than the EC_{50} levels. In some cases the nematode-suppressive factor in the extract is apparently present at very low concentrations, for example in the seeds and bark of *Azadirachta indica* (Fig. 4F, Table 3).

Effect of extract pH on nematodes: Nematode activity was not affected by immersion in solutions of pH 4.0 to 8.5 for *M. javanica* or pH 3.0 to 8.5 for *P. vulnus.* Nematodes were inactivated at pH 2.0; they did not recover when transferred to distilled water after immersion for 24 hours in solutions of pH 2.5 or less. The effects of pH, and the proportional recovery, were intermediate in solutions between pH 2.5 and 4.0. In a solution of pH 3.5, most nematodes moved freely at 3 hours but activity decreased with additional time of exposure.

The pH values for most herbal extracts were between 3.5 to 7.5, with the majority below 7.0 (Table 1). Several extracts with pH values less than 3.5 had little apparent effect on nematodes, while others with very low pH were effective against one or both nematode species (Tables 1,2). Many effective extracts had near-neutral pH levels (6.5–7.5).

DISCUSSION

The plant sources of Chinese herbal remedies used in this study were selected mainly for their purported efficacy against helminth and other pest infestations of hu-

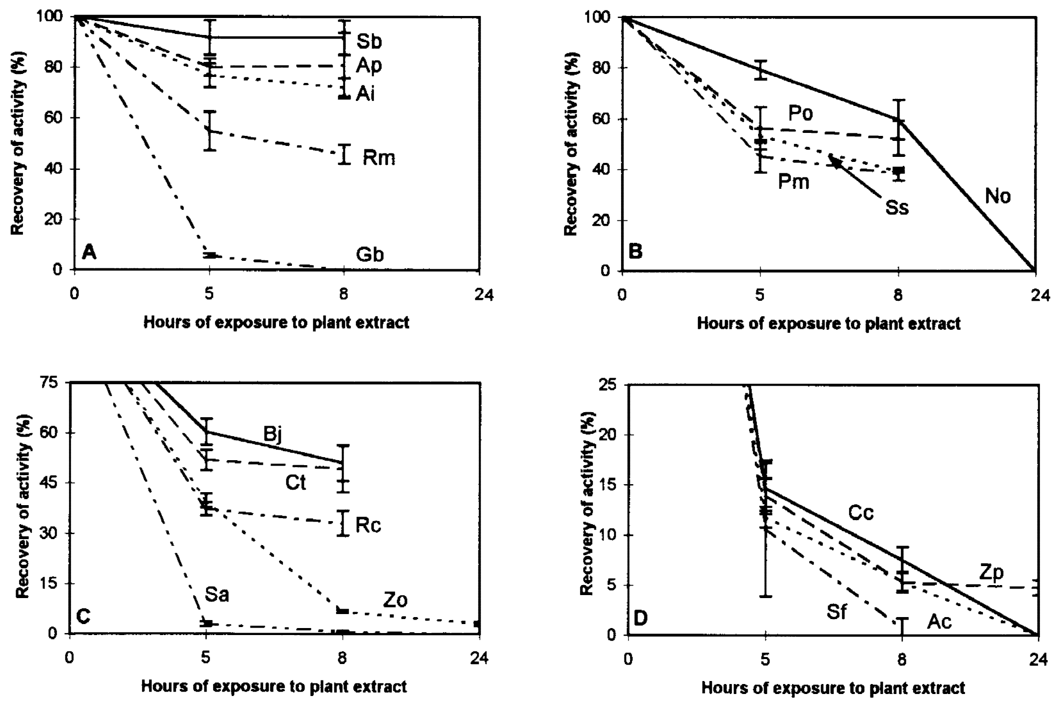

FIG. 3. Recovery of juveniles of *Meloidogyne javanica* when transferred to water after being immobilized during increasing periods of exposure to plant extracts. Graphs A–D arranged approximately in order of decreasing levels of recovery in water after exposure to extracts of different plant sources. In some cases maximum exposure time was 8 hours; in others it was 24 hours. Data are means of three replications; bars indicate standard errors.

mans. In some cases they are described as antihelminthic; in other cases they are vermifuges and promote expulsion of the parasites from the host. Different modes of action are probable; either the effects are directly on the parasite or the effects are on the digestive system of the host. Of the 153 plant sources investigated in this study (Table 1), 64 of the remedies have been reported as antihelminthics, 21 are purgatives, and another 29 are designated to ''kill pests'' (Chinese = *shä chóng*.) However, the designations are not exclusive and one remedy may have several effects (Huang, 1993). Extracts of 36 of the 64 purported sources of antihelminthic remedies killed one or both of the nematode species in this study; 22 narcotized the nematodes but did not kill them; and 6 had no apparent effect on either species. Extracts of 13 of the ''purgative'' sources killed nematodes, while 6 others narcotized them only. Of those materials indicated as ''killing pests,'' 13 killed nematodes and 15 had narcotic effects from which the nematodes recovered. Some other sources effective against nematodes are described in the literature as being analgesic, bactericidal, or fever-reducing (Tables 1, 2).

Plant extracts vary in their chemical and physical characteristics, and in the microflora and microfauna they support. In some cases the observed effects on nematodes may have been due to specific microbes or microbial metabolites associated with the plant extracts. Our protocols, including repetition and step-wise increase in rigor, reduced the probability of materials providing false positive indications. In a few cases the pH of the extract may have affected nematodes, particularly those at pH 2.8, but generally both nematode species were very tolerant of low pH. Any pH effects would probably be less pronounced for materials incorporated into soil due to buffering effects of the soil chemistry.

We infer that the effective molecules resulting in nematode death or inactivation

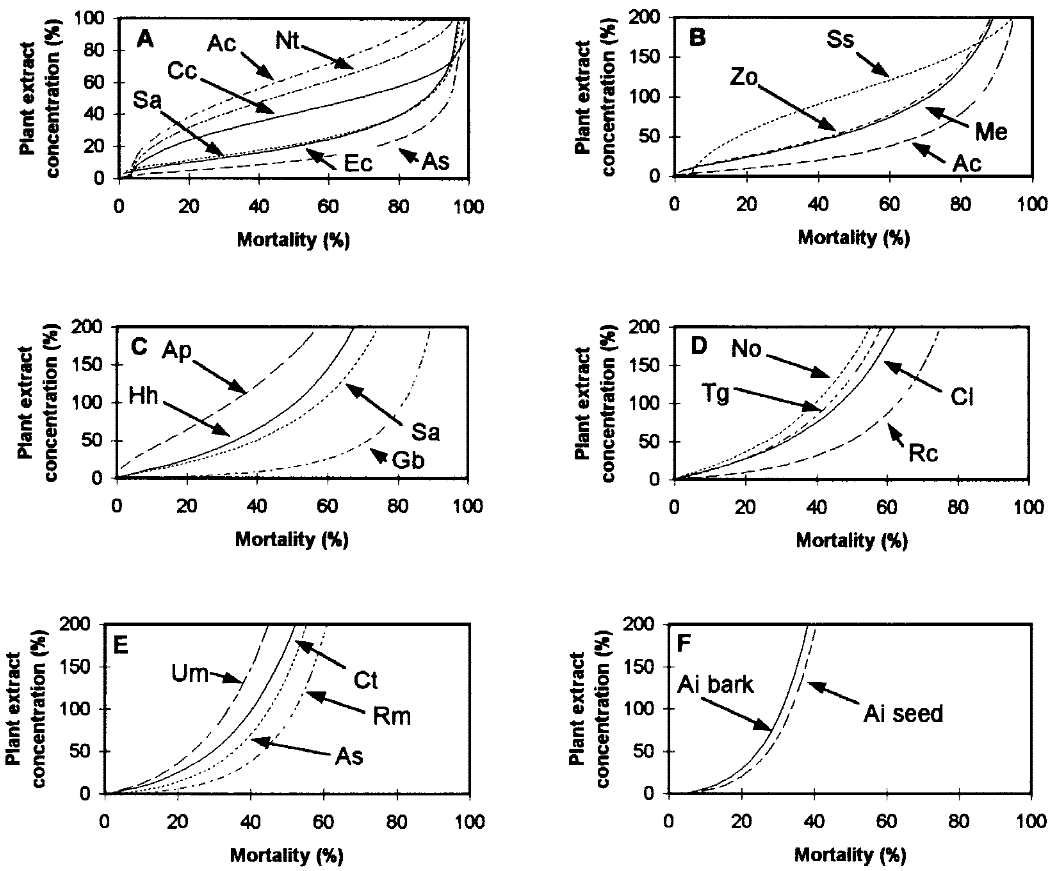

Fig. 4. Relationship between concentration of aqueous plant extract and percent mortality of juveniles of *Meloidogyne javanica* after 24 hours' exposure, where extracts are based on 1 g dried plant tissue in 10 ml water, representing a 100% concentration (stock solution). Extrapolations to high levels of mortality are based on probit analysis. Graphs A–F are arranged approximately in order of increasing concentration of extract necessary to achieve similar levels of mortality. A) Ac = *Allium cepa,* As = *Allium sativum,* Cc = *Coptis chinensis,* Ec = *Eugenia caryophyllata,* Nt = *Nicotiana tabacum,* Sa = *Sinapis alba.* B) Ac = *Asparagus* cochinchinensis, Me = *Manihot esculenta,* Ss = *Stemona sessifolia,* Zo = *Zingiber officinale.* C) Ap = *Andrographis paniculata,* Gb = *Ginkgo biloba,* Hh = *Hedera helix,* Sa = *Senna alexandrina.* D) Cl = *Coix lacryma-jobi,* No = *Nerium oleander,* Rc = *Ricinus communis,* Tg = *Torreya grandis.* E) As = *Asarum sieboldii,* Ct = *Croton tiglium,* Rm = *Rhododendron molle,* Um = *Ulmus macrocarpa.* F) Ai bark = bark of *Azadirachta indica,* Ai seed = seed of *Azadirachta indica.*

are generally plant metabolites. Medicinal or otherwise active components of many of these plant extracts have been identified. They include alkaloids, glucosides, glycosides, organic acids, and essential oils (Hsu et al., 1982, 1985; Huang, 1993). Most of the plant products associated with resistant responses to nematodes are included in these classes of compounds (Kaplan and Keen, 1980). There may be several candidate molecules in a single extract, and aggregate effects are probable. Some of the metabolites that occur in several of the plant species have already been tested for nematicidal activity. For example, coumarins and coumarin derivatives that occur in extracts of *Angelica* spp., *Dichroa febrifuga,* and *Hyoscyamus niger* (Huang, 1993) have been associated with incompatible responses of plants to nematode infection (Kaplan and Keen, 1980). Of these three plants, however, only extracts of *Angelica* spp. were effective in our studies (Table 2). Limonene, which occurs in extracts of *Pinus* sp., *Schizonepeta tenuifolia,* and *Dictamnus dasycarpus* (Huang, 1993), is effective against arthropods but tests of effects on *Heterodera schachtii* were inconclusive due to associated phytotoxicity (Vi-

TABLE 3. EC_{50} and EC_{90} values (percentage concentrations, 1g/10 ml basis) for juveniles of *Meloidogyne javanica* and juveniles and adults of *Pratylenchus vulnus* in extracts of selected plant species.[a]

		Meloidogyne javanica		*Pratylenchus vulnus*	
Plant	Part	EC_{50}	EC_{90}	EC_{50}	EC_{90}
Allium cepa	Bulb	64.0	102.8		
Allium sativum	Bulb	11.1	37.8	15.4	63.2
Andrographis paniculata	Whole plant	164.1[d]	##[c]		
Asarum sieboldii	Whole plant	140.6	##		
Asparagus cochinchinensis	Root	28.3	135.8	16.3	73.6
Azadirachta indica	Seed	#[b]	##		
Azadirachta indica	Bark	#	##		
Coix lacryma-jobi	Seed	117.0	##		
Coptis chinensis	Root	40.8	66.5		
Croton tiglium	Fruit	177.2	##		
Cucurbita pepo	Seed			84.2	##
Eugenia caryophyllata	Clove	20.0	56.3	16.3	61.0
Ginkgo biloba	Fruit	15.3	##		
Hedera helix	Leaf	97.3	##		
Manihot esculenta	Tuber	57.5	##	98.1	##
Nerium oleander	Leaf	160.4	##	81.0	##
Nicotiana tabacum	Leaf	54.4	88.3		
Rhododendron molle	Flower	85.4	##		
Ricinus communis	Leaf	52.0	##		
Senna alexandrina	Leaf	75.0	##		
Sinapis alba	Seed	21.4	54.9		
Stemona sessilifolia	Root	106.2	182.7	95.2	##
Torreya grandis	Fruit	135.9	##		
Ulmus macrocarpa	Fruit	#	##		
Zingiber officinale	Stem	60.3	##		

[a] Numbers are means of three replicates. Lack of a number indicates a combination that was not tested.
[b] # indicates that estimated EC_{50} level is greater than 2 g/10 ml water and may be impossible to prepare in aqueous solution.
[c] ## indicates that estimated EC_{90} level is geater than 2 g/10 ml water and may be impossible to prepare in aqueous solution.
[d] EC levels greater than 100% of stock solution concentration are estimated by probit analysis and extrapolation.

glierchio and Wu, 1989). Extracts of *S. tenuifolia* were effective against *P. vulnus* in the present study but the others were ineffective. Chlorogenic acid, which adversely affects nematode coordination (Kaplan and Keen, 1980), occurs in *Artemisia capillaris, Crataegus pinnatifida,* and *Lonicera japonica* (Huang, 1993). Extracts of the first two of these plants were effective against *M. javanica* and *P. vulnus* in our study. We do not know the concentrations of coumarins, limonene, chlorogenic acid, or any other potentially active chemicals in our aqueous extracts of the plant sources.

In comparisons of plant-extract preparation by soaking and by soaking and heating, we found different effects on nematodes in laboratory tests depending on the plant source. In some cases the active principal may be heat-sensitive; in other cases the amount of material extracted may increase with heat. Throughout these studies we used aqueous extracts of the plant sources. In some cases the Chinese literature recommends alcohol or other solvents as extractants, with or without heat. We used cold water as the extractant mainly due to our interest in practical implementation of these materials in agriculture without the need for industrial processing. However, we recognize that there is commercial potential in some of the active molecules in these plant sources. Plant extracts effective in the stock solution concentration may warrant further investigation with more effective extraction techniques.

In treatment of human afflictions, Chinese herbal medicines do not act as quickly as many conventional medicines. Generally, patients must continue taking the herbal remedies over a longer period of time to obtain health effects. Similarly, in control of plant-parasitic nematodes, a larger amount of herbal extract (compared to conventional nematicides), as well as longer exposure times, may be necessary to obtain a suppressive effect. In the early stages of these studies, we used an exposure time of 5 hours to study nematode suppression. However, a longer exposure time to some of the extracts was required to affect nematode movement. In addition, some extracts had different effects on nematodes as exposure time increased, indicating the possibility of minimum effective exposure times. Time-course studies revealed that at shorter intervals the nematodes were merely inactivated or narcotized in many plant extracts. However, there was little recovery after 24 hours' exposure to extracts of the most effective materials.

The herbal extracts varied in their efficacy at different concentrations. The arbitrary selection of a stock solution concentration of 1 g of plant material/10 ml water provides no measure of active ingredients and therefore is not useful as a standard for comparing the efficacy of materials. However, it provided an important basis for the EC studies and will allow calibration of future soil and field trials through the application of the amount of extract or plant material that produced a standard effect on the nematodes. Thus, for comparative trials of materials in soil, we could use the EC_{90} for a 24-hour exposure. As a caveat to the EC data, we emphasize that in several cases the EC_{90} level was not achieved in the concentration ranges tested (<100% stock solution concentration). Where the estimated EC_{90} levels were >200% of stock solution concentration, the data are not presented because, with most of these plant materials, it was not possible to prepare stock solution concentrations containing more than 2 g of plant material/10 ml water.

Although the plants reported on herein are sources of traditional Chinese medicines, many are cosmopolitan and grow throughout the world, either in cultivation, as ornamentals, or as weeds. Many are also sources of traditional remedies of other cultures (Watt and Breyer-Brandwijk, 1962). The source materials are generally readily available, either as the studied species or a related species. The challenge of integrating these materials as nematode suppressants into agricultural production systems varies with the nature of the plant and the localization of the effective compound. Integration of a suppressive herbaceous cover crop into a rotation may be less challenging than developing methods for extracts from woody plants or portions of plants. Some of the effective materials are derived from relatively small seeds (e.g., *Sinapis alba*) and a few from tree bark (e.g., some extracts from *Azadirachta indica*) (Tables 1, 2). In those cases it is difficult to conceive of incorporation of vast amounts of plant material or aqueous extracts into agricultural soils. Rather, the materials may be the source of effective pesticide molecules, or the plants may be potential sources of genes to be used in transgenic constructs.

Thus far we have focused on extracts from single plant species. Our TC studies provide evidence that modes of action differ in that sometimes effects are clearly lethal while in other cases they are only narcotic and allow nematode recovery. We hypothesize that combinations of materials with different modes of action will lead to effective synergistic interactions. The net practical result may be the opportunity for application of smaller amounts of material to achieve nematode suppression. This would be particularly important when there are phytotoxic or allelopathic effects at high concentrations of the extracts. We envision the development of prescriptive combinations of materials to target different nematode species and for use in different cropping systems (Ferris and Zheng, 1998).

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