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Effective Use of Marine Algal Products in the Management of Plant-Parasitic Nematodes[1]

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Abstract: Algal extracts were ineffective against *Meloidogyne* spp., *Panagrellus redivivus,* and *Neoaplectana carpocapsae* at 1.0% aqueous concentrations, with the exception of *Spatoglossum schroederi. S. schroederi* killed *Meloidogyne incognita, M. javanica, M. acrita,* and *Hoplolaimus galeatus* at concentrations of 1.0, 0.75, and 0.50%. Extracts from *S. schroederi* at a concentration of 1.0% were ineffective against *Hirschmanniella caudacrena* and *Belonolaimus longicaudatus. Spatoglossum schroederi, Botryocladia occidentalis,* and *Bryothamnion triquestrum* when used as soil amendments at 0.5–1.0% concentrations (by weight) produced significant reduction of root gall development in tomato plants infected with *M. incognita.* Tomato plant growth was significantly improved by these algae, as well as by *Caulerpa prolifera.* Soil amendments of *S. schroederi* at concentrations of 0.5 and 1.0% significantly reduced root galling of tomato infected with *M. incognita, M. arenaria,* and *M. javanica.* Tomatoes grown in algal–soil mixture produced significantly heavier shoots and roots than plants raised in autoclaved soil. No significant differences in root-knot indices, nor in fresh and dry weights of tomato, were noted between the two concentrations of algal–soil mixture.

Key words: marine algae, *Meloidogyne* spp., *Spatoglossum schroederi,* algal–soil amendments.

Unique biochemical compounds from a wide variety of marine organisms have been studied as potential pharmaceutical or biocidal agents (3,17,19). These organisms contain elaborate secondary metabolites that play a role in the defense of the host against predators and parasites. Marine algae possess a wide range of compounds such as agar, carageenan, alginic acids, carotenes, kainic acid, terpenes, bromine-containing acetogenins, alkaloids, and phenolic compounds (4,7).

Modern agricultural practices depend heavily on chemicals to control pests such as insects, fungi, bacteria, and nematodes. Because such organisms are capable of detoxifying mechanisms, they have rendered ineffective many of the presently used chemicals. Additionally, many chemicals currently used have been found to possess environmentally undesirable side effects to humans and other nontarget organisms. Pesticidal use, therefore, is increasingly becoming more restrictive. Use of selected marine algae as biocidal agents offers a potential novel approach to suppress the nematode pests of agricultural crops.

Chemical analyses of seaweeds indicate that they contain all major and minor plant nutrients, as well as biocidal agents (18). Commercial seaweed fertilizers and extracts have been marketed as soil additives, conditioners, and foliar sprays. Mustard plants receiving commercial seaweed extract outgrew plants receiving commercial liquid synthetic fertilizers (2). Liquified seaweed derived from *Ascophyllum nodosum* increased plant resistance to spider mites, aphids, powdery mildew, and "botrytis damping off" of seedlings (18). Seaweed products have also increased seed germination, plant nutrient *uptake,* frost hardiness, and resistance to pathogenic fungi (1). Kelp derivatives were mildly nematicidal and beneficial to nematode-infested citrus plants (20). Control of ectoparasitic nematodes, primarily *Belonolaimus longicaudatus,* on established centipede grass turf was obtained from application of commercial kelp preparations (15). Growth of the tomato plants was significantly improved with a liquid concentrate of brown alga, *Ecklonia*

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maxima, and root-knot nematode infection was reduced (5).

Antibiotics in algae, such as bromophenols, tannins, phloroglucinol, and terpenoids, have been reported (6,10,12). Marine algae also have been used as anthelminthics against intestinal worms such as *Ascaris, Oxyurus,* and *Trichuris* (4). The red alga *Digenea* is recognized as an effective agent for expulsion of *Ascaris.* Anthelminthic activity has also been reported for the coralline red alga, *Corallina officinalis,* and the brown algae *Durvillaea, Sargassum,* and *Ulva* spp. (10). Kainic acid (KA) from *Digenea simplex* is a neurotoxin that produces selective neuronal lesions and kills nerve dendrites but not axons (14).

This paper reports the effects of aqueous extracts of seaweeds on nematodes in vitro and the effects of ground seaweed amendments on root-knot nematodes and tomato plants in the greenhouse.

Materials and Methods

Algal extracts: Eighteen species of marine algae from Florida waters were tested for their nematicidal activity (Table 1). Each species was field collected and transported to the laboratory where it was freeze dried. Aqueous extracts of 12 algal species were prepared as follows: Approximately 150–200 g freeze-dried material was ground in chloroform (700–1,000 ml) for 20–30 seconds in a Waring Blender and then filtered using Whatman #1 paper. Ten-minute extractions at 35–40 C, followed by filtering, were repeated two more times for chloroform, then three times each for methanol and water. Aqueous extracts were freeze dried (11).

Algal powders: Freeze-dried algal material was mechanically ground and sifted through a 1-mm-pore screen. Algal powders thus prepared were weighed and mixed with autoclaved field soil to obtain an appropriate algal–soil ratio by weight. Clay pots were filled with these mixtures, and 2-week-old tomato seedlings (*Lycopersicon esculentum* cv. Rutgers) were transplanted. One week after planting, the root system of each tomato seedling was inoculated with a suspension of root-knot nematode eggs.

Table 1. Species of marine algae and their experimental use in assaying for nematicidal activity.

Species	Nematicidal activity: Aqueous extract	Nematicidal activity: Soil additive
Chlorophyta (green)		
Anadyomene menziessi Harvey	+	−
Anadyomene stellata (Wolfen) C. Agardh	+	+
Caulerpa mexicana (Sonder) J. Agardh	+	−
Caulerpa prolifera (Forsskal) Lamouroux	+	+
Caulerpa racemosa (Forsskal) J. Agardh	+	−
Caulerpa sertularioides (Gmelin) Howe	+	−
Caulerpa verticilla J. Agardh	+	−
Penicillus capitatus Lamarck	−	+
Cyanobacteria (bluegreen)		
Microcoleus lyngbyaceus (Kuetzing) Crouan	+	−
Phaeophyta (brown)		
Dictyota dichotoma (Hudson) Lamouroux	−	+
Padina vickersiae Hoyt	+	−
Sargassum cymosum C. Agardh	+	+
Spatoglossum schroederi (Mertens) Kutzing	+	+
Stypopodium zonale (Lamouroux) Papenfuss	+	−
Rhodophyta (red)		
Botryocladia occidentalis (Borgesen) Kylin	−	+
Bryothamnion triquetrum (Gmelin) Howe	−	+
Gracilaria mammillaris (Montagne) Howe	+	+
Laurencia poitei (Lamouroux) Howe	−	+

Nematodes: Hoplolaimus galeatus and *B. longicaudatus* were recovered from cultivated soil, *Hirschmanniella caudacrena* extracted from aquatic plant roots, *Neoaplectana carpocapsae* juveniles obtained from parasitized insects, and *Panagrellus redivivus* cultured in oatmeal culture.

Populations of the root-knot nematodes *M. incognita, M. javanica, M. acrita,* and *M. arenaria* were propagated on tomato plants

in the greenhouse at 22–35 C. To obtain second-stage juveniles (J2), egg masses of root-knot nematodes were incubated in water in a shallow dish for 24 hours at 25 C.

Root-knot egg inoculum for in vivo (greenhouse) experiments was obtained as follows: Infected roots were cleaned of debris and vigorously shaken for 30 seconds in 200 ml of 0.5% sodium hypochlorite solution; eggs were collected on a 25-μm-pore sieve and rinsed with tap water to remove the chemical (13). Eggs were suspended in 1 liter of water, and numbers in 1 ml of the suspension were counted. Between 2,000 and 3,000 eggs in 2 ml water were placed in two small holes on opposite sides of the base of each tomato seedling. The inoculated plants were maintained under greenhouse conditions for 6–8 weeks. Fresh weights of plant shoots and roots were recorded before air drying at 75 C for 36 hours for dry weight determination. Data were analyzed by the Waller-Duncan K ratio *t*-test.

Experimental procedures: To determine activity of algal extracts on immobility of J2, *M. incognita, P. redivivus,* and *N. carpocapsae* were placed in a 1.0% concentration of each of the 12 marine algae in aqueous solution. Ten hand-picked nematodes were transferred to a Bureau of Plant Industry (BPI) watchglass containing 1 ml of the aqueous extract solution. Each treatment was replicated three times. Nematode activity was observed at the end of a 24-hour period.

In the second test, extracts of a brown alga, *Spatoglossum schroederi,* at concentrations of 1.0, 0.75, 0.5, and 0.1% in aqueous solution, were tested against *M. incognita, M. javanica, M. acrita,* and adults of *H. galeatus.* Adults of *H. caudacrena* and *B. longicaudatus* were tested in 1.0% concentration of *S. schroederi.*

In the third experiment, the effect of 12 mixtures of algal powder and soil mixtures on subsequent tomato growth and nematode infection was determined. Algae were mixed with soil at 1.0% by weight and placed in 13-cm-d pots each containing a single 3-week-old tomato seedling. There were six replicates per treatment. After 1 week, plants were inoculated with nematode eggs. The algae treatments were as follows: 1) *Laurencia poitei,* 2) *Penicillus capitatus,* 3) *Bryothamnion triquestrum,* 4) *Gracilaria mammillaris,* 5) *Anadyomene stellata,* 6) *Dictyota dichotoma,* 7) *Caulerpa prolifera,* 8) *Botryocladia occidentalis,* 9) *Sargassum cymosum,* 10) *Spatoglossum schroederi,* 11) a mixture of 0.5% *S. schroederi* and 0.5% *S. cymosum,* 12) a split treatment of *S. schroederi* in which 0.5% concentration was initially applied with 0.25% being applied 2 and 4 weeks later, and 13) controls consisting of soil with no added algae.

A fourth experiment was designed principally to confirm the results of the preceding test. The following freeze-dried, ground algae were added to soil in 13-cm-d pots at a concentration of 1.0% (w/w) *A. stellata, B. triquestrum, C. prolifera,* and *S. schroederi.* A fifth alga, *B. occidentalis,* was used at a concentration of 0.37% (w/w) due to limited availability. Each pot contained a 10-week-old tomato seedling inoculated a month previously with eggs of *M. incognita.* The algae were sprinkled evenly over the surface of the soil, mixed to a depth of 6 mm, and then watered. Controls without algae were similarly treated. There were eight replicates per treatment. The test was terminated 8 weeks after treatments were applied.

In the fifth experiment, dried, ground *S. schroederi* was mixed with autoclaved soil at concentrations of 0, 0.5, and 1.0% by weight. The soil was placed in 13-cm-d pots, and a single 3-week-old tomato seedling was planted in each pot. Each treatment was replicated five times. After 3 weeks, pots were inoculated with 3,000 eggs of *M. arenaria, M. incognita,* or *M. javanica.* The test was terminated 6 weeks after inoculation.

Results

All 12 algal extracts, with the exception of *Spatoglossum schroederi,* proved ineffective against the three test nematode species at the applied concentrations. The *S. schroederi* extract immobilized juveniles of

TABLE 2. Effect of aqueous extracts of *Spatoglossum schroederi* on plant parasitic nematodes in vitro.

Concentration	Juveniles active (%)					
	Mi	Mj	Ma	Hg	Hc	Bl
1.0	0	0	0	0	70	0
0.75	0	0	0	30		
0.50	0	0	50	50		
0.10	100					

Mi = *M. incognita*, Mj = *M. javanica*, Ma = *M. arenaria*, Hg = *H. galeatus*, Hc = *H. caudacrena*, Bl = *B. longicaudatus*.

M. incognita and *P. redivivus* within 24 hours, but not *N. carpocapsae* juveniles.

Juveniles of *M. incognita* and *M. javanica* were immobilized at 1.0, 0.75, and 0.5% *S. schroederi* concentrations. Half of the juveniles of *M. acrita* remained viable at a concentration of 0.5% (Table 2). Juveniles of *M. incognita* exposed to a 0.1% aqueous concentration of *S. schroederi* were not affected by the solution during the 24-hour test period. Mobility of *H. galeatus* was also affected by the *S. schroederi* extract with increased survival at the lower concentrations. *Hirschmanniella caudacrena* was not affected by the *S. schroederi* extract.

Nematodes affected by the extract were vacuolated within 24 hours. Nematicidal activity may be due to acidity. When buffered with morpholino-ethano-sulfonic acid to pH of 6.2, extracts of *S. schroederi* were ineffective against *M. incognita*. The acidic nature of the extract (pH 2.3) is not known.

Plants growing in soil treated with *S. schroederi*, *B. occidentalis*, and *C. prolifera* at a concentration of 1.0% by weight showed the most significant reductions in nematode root-knot gall development over the 7-week period (Table 3). Split applications of *S. schroederi*, alone or in combination with *S. cymosum*, did not produce results that were significantly different from the treatment where the alga was mixed with the soil at the beginning of the test. Compared with controls, *Sargassum cymosum* also significantly reduced the amount of galling from *M. incognita* infection.

Growth of the nematode-infected plants was significantly increased by soil treatments with *S. schroederi*, *B. occidentalis*, and *C. prolifera* (Table 4). The algal-treated plants grew taller, formed more foliage, and had a significantly higher dry shoot weight than the control.

All treatments except *A. stellata* signifi-

TABLE 3. Effect of 12 algal–soil mixtures on subsequent growth of tomato seedlings, with and without root-knot nematode infection.

Algae	Without nematodes		Root-knot nematode infected		
	Dried aerial parts (g)	Dried roots (g)	Dried aerial parts (g)	Dried roots (g)	Root-knot indices†
Laurencia	1.21 de	0.23 de	1.23 e	0.35 bc	3.95 bc
Penicillus	1.54 cd	0.25 cde	1.69 bcde	0.38 bc	4.38 ab
Bryothamnion	1.92 abc	0.39 abc	1.71 bcde	0.44 abc	3.65 bcd
Gracilaria	1.87 bc	0.30 bcde	1.15 e	0.31 c	4.83 a
Anadyomene	2.26 ab	0.38 abc	1.77 bcde	0.39 bc	4.23 ab
Dictyota	0.94 e	0.22 e	1.63 bcde	0.45 abc	4.18 ab
Caulerpa	2.38 ab	0.35 abcde	2.69 a	0.53 abc	2.31 f
Botryocladia	2.48 a	0.46 a	2.39 ab	0.59 ab	2.51 ef
Sargassum	1.12 de	0.22 e	1.12 e	0.44 abc	3.40 cd
Spatoglossum	2.37 ab	0.43 ab	2.29 abc	0.67 a	3.16 de
Spat. + *Sarg.*‡	2.14 ab	0.38 abc	1.56 cde	0.41 abc	3.28 cd
Spat. split§	1.93 abc	0.36 abcd	2.07 abcd	0.43 abc	2.57 ef
Control	0.97 e	0.27 cde	1.32 de	0.34 bc	4.21 ab

Means followed by the same letter within the same vertical column are not significantly different from one another ($P = 0.05$).

† Root-knot nematode infection was rated using a root gall index of 1–5 as follows: 1 = no galling; 2 = 1–25%; 3 = 26–50%; 4 = 51–75%; and 5 = over 75% of the root system with galls.

‡ *Spat.* + *Sarg.* = *Spatoglossum* combined with *Sargassum*.

§ *Spat.* split = three applications of *Spatoglossum*, 2 weeks apart.

TABLE 4. Effect of five marine algae on growth of tomato seedlings infected with *Meloidogyne incognita.*

Treatment	Dried aerial parts (g)	Dried roots (g)	Root-knot indices
Bryothamnion	5.87 a	0.78 a	3.44 c
Anadyomene	3.60 cd	0.40 c	5.00 a
Caulerpa	5.57 ab	0.62 ab	4.16 b
Botryocladia	4.57 bc	0.61 ab	4.56 ab
Spatoglossum	5.44 ab	0.56 bc	2.44 d
Control	3.11 d	0.48 bc	4.75 a

Means followed by the same letter within the same vertical column are not significantly different from one another ($P = 0.01$).

cantly improved ($P = 0.01$) the top growth of the tomato plants (Table 4), but only the *B. triquestrum* treatment resulted in a significantly heavier root system than controls. *Spatoglossum schroederi* was effective in reducing visible root-knot nematode symptoms; root-knot indices for plants growing in soil treated with *B. triquestrum* and *C. prolifera* were also significantly lower than those for controls.

Plants grown in all the algal soil mixtures were taller than the control and had profuse green foliage. Aerial and root weights of plants grown in treated soil were significantly heavier ($P = 0.05$) than those plants grown in soil without algal amendment (Table 5). Aerial growth comparison of tomato plants infected with *M. arenaria* at both concentrations of *S. schroederi* are shown in Figure 1. There was a significant reduction in gall development on roots of plants infected with all three species of *Meloidogyne.* No consistent significant differences in root-knot indices or plant weights were noted between the two concentrations of *S. schroederi.*

DISCUSSION

These experiments show that *S. schroederi* inhibits development of *Meloidogyne* spp. and enhances growth of tomato plants with or without nematodes. *B. occidentalis, B. triquestrum,* and *C. prolifera* also are promising algae that warrant further studies. *Spatoglossum* spp. have already drawn the attention of scientists because of their unique biochemical properties (16). The cytotoxic diterpenoid spatol, extracted from *Spatoglossum schmittii,* killed the fertilized eggs of Pacific sea urchins, *Lytenchinus pictus* (8). Spatol also inhibited division in neoplastic cell lines (9). The presence of spatol or related compounds in *S. schroederi* is unknown. An alternative mechanism of biocidal activity may be caused by the acidity of *S. schroederi.* Recent research on kainic acid, isolated from the red alga *Digenea simplex,* suggests the neurotoxic mechanism of cytotoxicity.

The research reported here establishes *S. schroederi* as a promising candidate for the management of plant-parasitic nematodes of cultivated crops in Florida. It also has been shown that not all algae or algal

TABLE 5. Effect of two concentrations of *Spatoglossum schroederi* as soil amendments on tomato plants infected with three species of root-knot nematodes.

Root-knot nematode species	*Spatoglossum* concentration (%)	Weight (g)				Root-knot indices
		Fresh aerial parts	Dried aerial parts	Fresh roots	Dried roots	
M. arenaria	1.0	34.46 a	4.13 a	7.86 a	1.16 a	1.60 a
	0.5	28.14 a	3.07 a	4.52 a	0.63 b	2.00 a
	0.0	11.64 b	1.17 b	2.58 c	0.37 b	2.80 b
M. incognita	1.0	25.96 a	2.69 a	4.92 a	0.71 a	3.00 a
	0.5	19.22 ab	1.91 ab	2.96 b	0.41 b	2.80 a
	0.0	12.78 b	1.36 b	3.24 ab	0.40 b	3.80 b
M. javanica	1.0	28.24 a	3.08 a	7.40 a	0.90 a	2.00 a
	0.5	20.74 a	2.05 ab	4.56 b	0.50 b	2.20 a
	0.0	10.20 b	0.97 b	1.88 c	0.30 c	3.00 b

Means followed by the same letter for each species and within each vertical column are not significantly different from one another ($P = 0.05$).

FIG. 1. Effect of *Spatoglossum schroederi* on *M. arenaria*-infected tomato plants. *S. schroederi* was applied as a soil amendment at 1.0% (A), 0.5% (B), or 0% (C) 3 weeks before inoculations with nematodes. Treatment D received neither alga nor nematodes.

products possess nematicidal properties. Accordingly, distinction should be made in separating the marine algae yielding only nutritional effects from those achieving pest management. Many marine organisms possess unique and diverse kinds of secondary metabolites whose agricultural, as well as pharmaceutical, application remain largely an untapped resource (3).

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