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Effect of seaweed concentrate from *Ecklonia maxima* (Osbeck) Papenfuss on *Meloidogyne incognita* infestation on tomato

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Abstract

Seaweed concentrate (SWC), prepared from *Ecklonia maxima,* when applied as a soil drench to tomato seedlings, significantly increased plant growth and reduced infestation by *Meloidogyne incognita.* Foliar applied SWC had little effect on plant growth and increased nematode galling. Ashing SWC reduced the suppressive effect on nematode infestation. In an *in vitro* experiment, SWC lessened infestation of root-knot nematodes on excised roots of a susceptible cultivar of tomato. Application of the same concentrations of SWC to a nematode-resistant cultivar increased the number of egg masses.

Abbreviations: SWC = seaweed concentrate; NAA = naphthaleneacetic acid

Introduction

Root-knot nematodes (*Meloidogyne spp.*) are major crop pests in sub-tropical and tropical countries. Economic losses caused by these nematodes exceed 12% for all commercial crops in these regions (de Leij *et al.*, 1991). As some synthetic nematicides may harm the environment, natural products such as seaweed concentrates (SWCs) are being examined as an alternative means of controlling these pests.

The beneficial effects of seaweed products on the growth and yield of plants are well documented (Metting *et al.*, 1990). Improved insect and pathogen control (Stephenson, 1966; Booth, 1964; Povolny, 1971; Darrah & Hall, 1976) and a reduction in nematode infestation (Darrah & Hall, 1976; Tarjan, 1977; Tarjan & Frederick, 1983) have also been noted as a result of seaweed application. While enhanced plant growth is thought to be attributed to plant growth regulators, in particular cytokinins (Tay *et al.*, 1985; 1987), auxins (Crouch *et al.*, 1992) and ACC (ethylene precursor) (Nelson & Van Staden, 1985) in the extracts, it is not known how these products influence resistance to pest attack.

Featonby-Smith & Van Staden (1983) treated nematode-infested tomato plants with a seaweed concentrate and found a reduction in root-knot nematode galling and increased root growth. Recent work by De Waele *et al.* (1988) demonstrated that a SWC decreased reproduction of *Pratylenchus zeae* on excised maize roots by 47–63% in an *in vitro* experiment. In a pot experiment however, reproduction of *P. zeae* was not affected by the seaweed preparation. In the present study, the

effects of a seaweed concentrate on infestation by *Meloidogyne incognita* on tomato *in vivo* and *in vitro* were investigated.

Materials and methods

Greenhouse experiments

Seeds of *Lycopersicon esculentum* Mill. (*cv* Rana) were germinated in bark in seedling trays. Seedlings of about 10 cm tall were transplanted into 125 mm pots (450 cm^3) containing a sandy soil/bark mix which was either heavily infested with *M. incognita* (Kofoid et White) Chitwood, or treated with methyl bromide to kill resident nematodes. Plants were grown under 60% shade cloth and maintained at temperatures fluctuating between 14 °C and 28 °C. Each treatment comprised 10 plants arranged in a randomized complete block design.

The seaweed concentrate used is marketed as 'Kelpak' and prepared by a cell-burst process by Kelp Products (Pty) Ltd from the brown alga *Ecklonia maxima* (Osbeck) Papenfuss. Treatments in the first experiment were a) no nematodes and no SWC, b) no nematodes and SWC dilutions of 0.2%, 0.4% and 1.0% applied as a soil drench, and 0.4% as a foliar spray, c) nematodes without SWC and d) nematodes plus SWC dilutions of 0.2%, 0.4% and 1.0% as a soil drench and 0.4% as a foliar spray.

Treatments in the second experiment consisted of nematodes plus 1.0% SWC applied (a) as a soil drench, (b) foliar spray, (c) ashed, (d) acid hydrolysed, and (e) filtered through Whatman N° 41 filter paper. A further treatment of resuspended residue substrate (1.0%) was also included.

Ashed SWC was prepared by heating 10 ml of Kelpak in a silica crucible to 450 °C for two hours, dissolving the residues in 10 ml of 1 N HCl, diluting to 1.0 l, and adjusting the pH of the resulting solution to 5.6 with NaOH.

Acid hydrolysed SWC was prepared by adding 10 ml of Kelpak to 20 ml 1N HCl, heating in a water bath at 95 °C for two hours, diluting the hydrolysed extract to 1 l, and adjusting the pH to 5.6 using NaOH.

One hundred mls of each treatment was applied to the seedlings at transplanting and thereafter every 14 days. Plants were harvested after ten weeks and shoot and root fresh and dry weights, leaf surface area, flower number, root length and the number of nematode galls per root were measured. The surface area of the leaves was determined using a Li Cor 3100 area meter and root length measured with a Comair root length scanner. Data were statistically analyzed by analysis of variance ($P<0.05$) and a multiple range test.

In vitro *experiment*

Two cultivars of tomato, the nematode resistant (*cv* M1) and the nematode susceptible (*cv* Rana), were used. Treatments for each cultivar of tomato consisted of: no SWC and SWC at 0.2% and 0.4% in Skoog, Tsui & White (STW) culture medium (Orion *et al.*, 1980) before autoclaving. After two weeks, a single sterile egg mass was placed 2 cm away from the growing tip of each root. Each treatment was replicated 20 times and arranged in a randomized complete block design. After four weeks, nematode infestation was calculated as either the mean number of galls or mean number of egg masses per Petri-dish.

Monoclonal root culture: Stock cultures of roots of each tomato cultivar were maintained according to Finnie and Van Staden (1986). The cultures were grown on a rotary shaker (120 rpm) at 25 °C. Roots were subcultured every 10 days until a large stock of material was available for experimentation. The apical 12 mm of each lateral root was removed and transferred to Petri-dishes containing STW medium solidified with 0.8% agar. Cultures were maintained in the dark at 25 °C for 3 weeks or until inoculated with sterile nematode egg masses.

Nematode inoculum: Egg masses removed from heavily infested tomato roots, were surface sterilized with 0.5% Hibitane for 20 min (active ingredient = chlorhexidine gluconate, 50 mg l^{-1})

or 0.1% mercuric chloride for 3 min followed by 3.0% hydrogen peroxide for 20 min and were then washed four times in autoclaved distilled water. The egg masses were transferred to 3-week-old *in vitro* grown roots and the inoculated cultures incubated in the dark at 25 °C to allow galls to develop. Sterile egg masses from these cultures were used to inoculate all experimental material.

Experiment to determine effect of SWC on the hatching of eggs and the survival of larvae prior to entry into the root

Two hundred nematode egg masses were carefully removed from a heavily infested root and 40 egg masses placed in vials containing (a) 10% SWC, (b) 1.0% SWC, (c) 0.4% SWC, (d) 0.2% SWC and (e) distilled water. After 15 and 60 minutes, 20 egg masses were removed from each vial and placed in Petri-dishes containing only aqueous agar (0.49 g l^{-1}). The Petri-dishes were incubated in the dark at 24 °C for 12 hours and the number of hatched larvae counted. Thereafter, the dishes were examined daily to monitor larval activity and survival.

Results

Greenhouse experiments

SWC applied as a soil drench to nematode free plants increased plant fresh weight (Fig. 1A), leaf surface area and the number of flowers (Table 1). Plants treated with 0.4% and 1.0% SWC were significantly larger than control plants ($P<0.05$) (Fig. 1A). Foliar application had no significant effect on growth (Table 1).

Similar results were obtained for plants grown in nematode-infested soil (Fig. 1 B). Foliar applied SWC did not effect plant growth. Applied as a soil drench, SWC increased plant size. SWC (1.0%) significantly ($P<0.05$) increased overall plant growth (Fig. 1B). Application of SWC to the soil significantly ($P>0.05$) reduced nematode infestation irrespective of the treatment used (Fig. 2). SWC applied as a 0.4% foliar spray had little effect on root-knot nematode infestation.

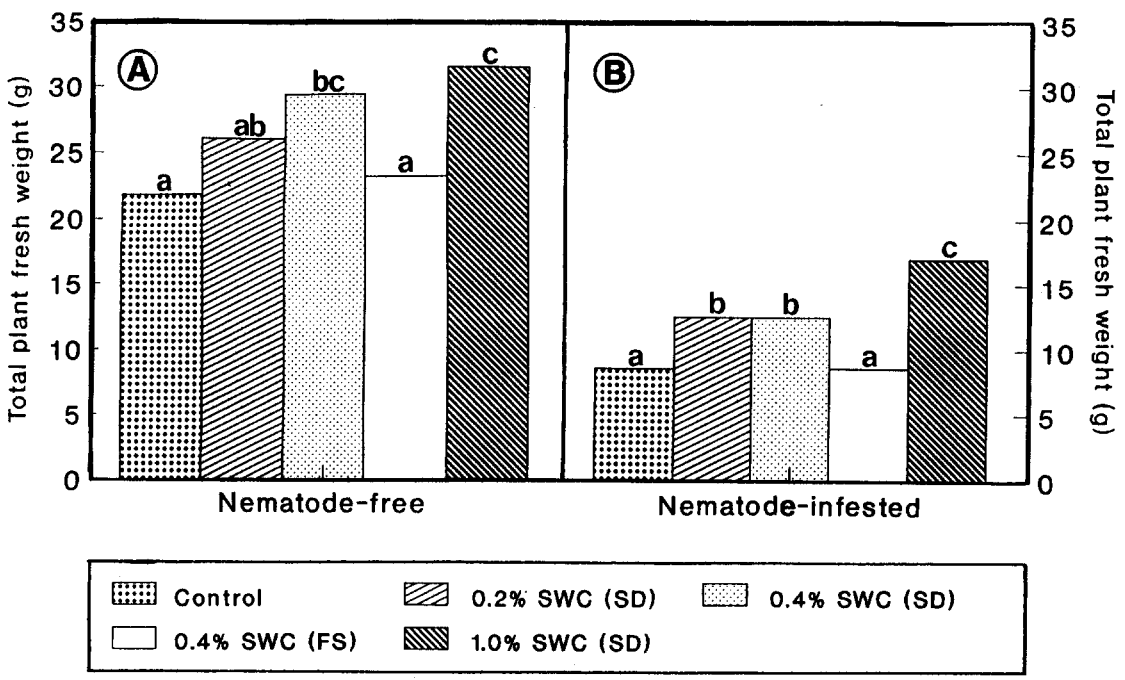

Fig. 1. The effect of SWC on the total fresh weight of 10-week-old tomato plants grown in nematode free (A) and nematode-infested (B) soil (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different ($P>0.05$).

Table 1. The effect of SWC on shoot and root fresh weight, root length, leaf surface area and flower number when applied to nematode free tomato plants (SD = soil drench; FS = foliar spray). Values within a row followed by the same letter are not significantly different ($P > 0.05$).

	Control	0.2% SWC (SD)	0.4% SWC (SD)	1.0% SWC (SD)	0.4% SWC (SD)
Shoot fresh weight (g)	15.5 a	18.5 ab	20.4 bc	22.5 c	16.4 a
Root fresh weight (g)	5 a	6.5 abc	7.1 bc	7.5 c	6.1 ab
Total fresh weight (g)	20.5 a	25 ab	27.5 bc	30 c	22.5 a
Root length (m)	20.1 ab	27.2 bc	25.2 ab	33.0 c	19.3 a
Leaf surface area (cm^2)	346 a	384 ab	441 b	459 b	348 a
Number of flowers	7 a	9 a	9.2 b	9.4 b	7.3 a

Nematode-infected plants were smaller than those grown in nematode free soil.

In the second greenhouse experiment only nematode infestation was recorded. Application of 1.0% SWC to the soil reduced the number of galls found on the roots by about 65% (Fig. 3). This nematotoxic effect was decreased if the SWC was ashed. Applying SWC as a foliar spray increased nematode galling. Filtered SWC, applied as a soil drench, almost eliminated nematode infestation. Plants treated with the acid hydrolyzed extract did not have significantly fewer galls than either the control or the 1.0% SWC soil treatment.

In vitro *experiment*

Fewer galls developed on the roots of the nematode resistant cultivar. Seaweed treatment had

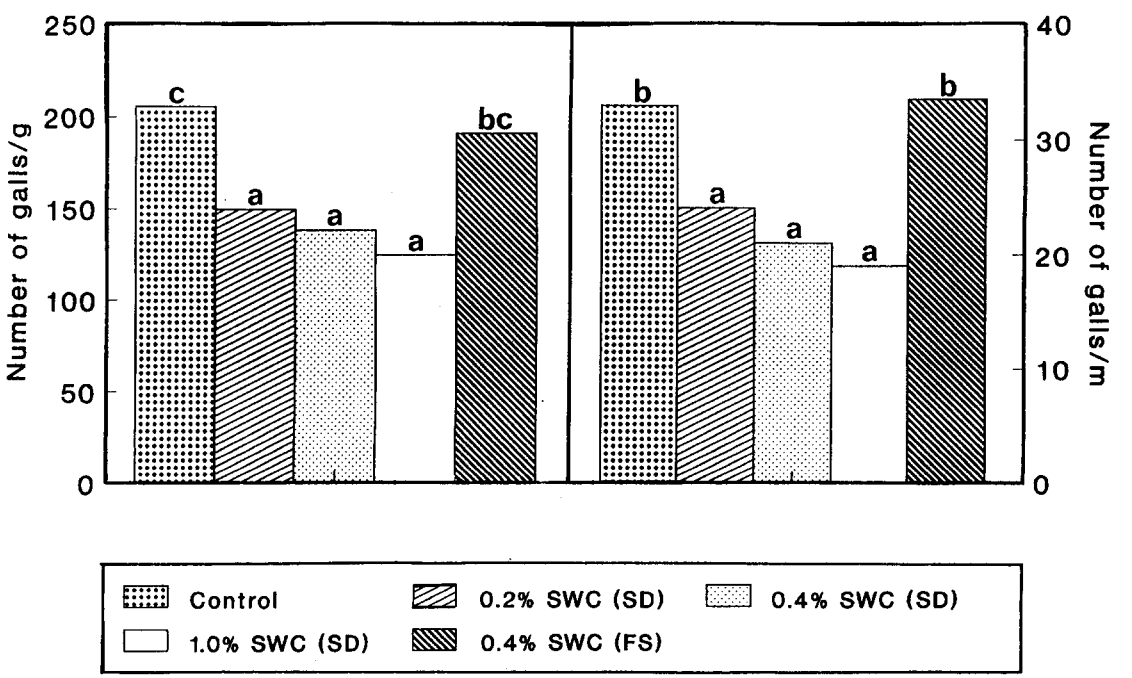

Fig. 2. The effect of SWC on the degree of nematode infestation on the roots of 10-week-old tomato plants (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different ($P > 0.05$).

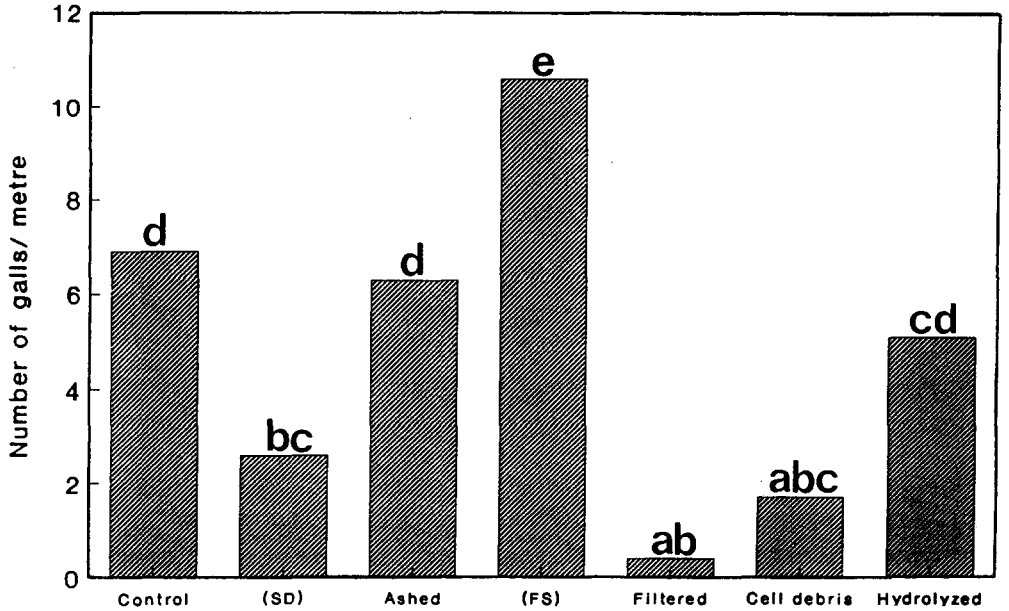

Fig. 3. The effect of filtered, ashed and acid hydrolyzed SWC on the degree of nematode infestation (number of galls per unit weight) of 10-week-old tomato plants. (SD = soil drench; FS = foliar spray). Bars with the same letter are not significantly different ($P > 0.05$).

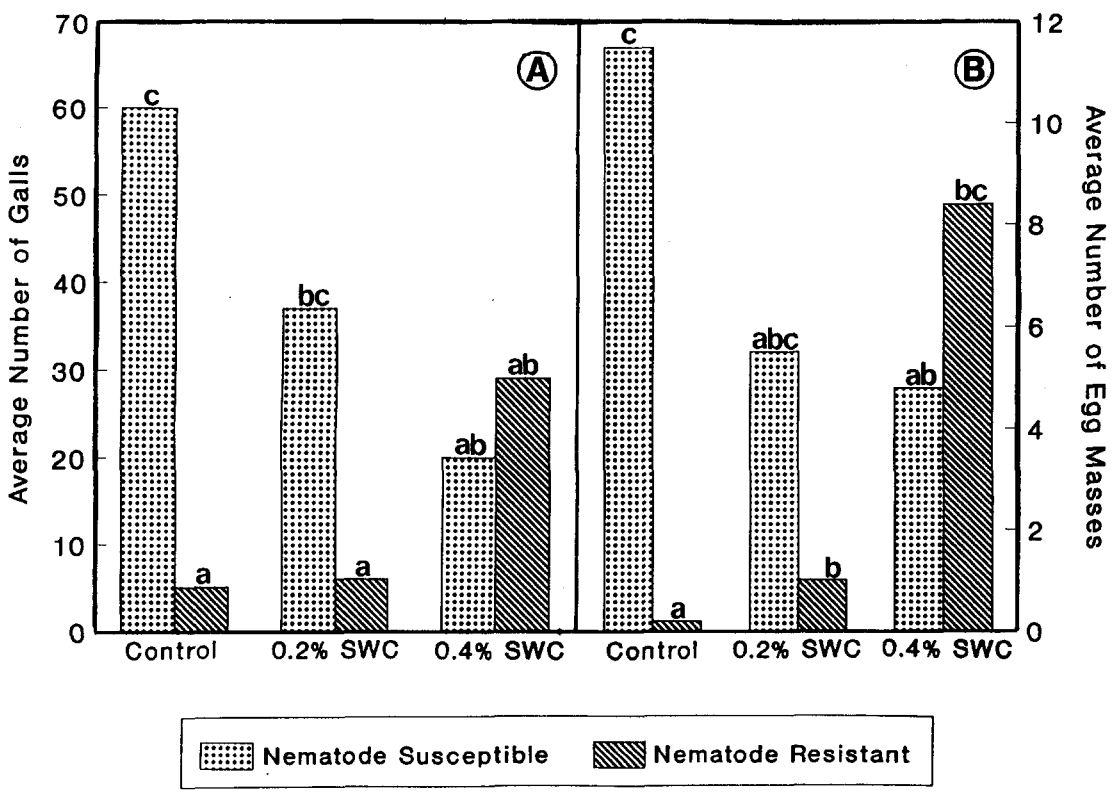

Fig. 4. The effect of SWC on the number of galls (A) and egg sacs (B) on *in vitro* cultured tomato roots. Bars with the same letter are not significantly different ($P > 0.05$).

little effect on gall number (Fig. 4A) but at 0.4% it significantly increased the number of egg masses (Fig. 4B). In the susceptible cultivar, SWC applied at a concentration of 0.4% significantly decreased the number of galls and egg masses (Figs 4A, B). Two-way analysis of variance indicated a significant interaction between SWC and the reduced incidence of nematode infestation in the roots (results not shown).

Effect of SWC on the hatching of eggs and survival of larvae

After 12 hours egg masses from each treatment had released large numbers of larvae. The egg masses continued to release larvae over three or four days. Monitoring the larvae over a period of three weeks showed no differences between treatments.

Discussion

The results of the greenhouse experiments confirmed previous reports (Featonby-Smith & Van Staden, 1983) that SWC improves the growth of nematode infested tomato plants. In the present study, foliar applied SWC did not enhance plant growth and encouraged nematode galling. A significant reduction in nematode infestation was noted when SWC was applied as a soil drench. It is not understood why these two different modes of application resulted in opposite effects. A reduction of nematode galling following application to the roots suggests that the product is affecting the early stages of gall development. A decrease in activity when the SWC was ashed indicated that an organic component may be responsible for this effect. Furthermore a reduction in nematode infestation after filtering suggests that a filterable component of the SWC may be involved. To get a better understanding of the mode of seaweed action on nematode infestation, *in vitro* experiments were conducted to determine the stage at which SWC influences the nematodes in the roots.

Two cultivars of tomato, one nematode-resistant and the other nematode-susceptible, were examined. Nematode-resistant plants are those that limit nematode reproduction, whereas susceptible plants facilitate nematode reproduction. In the *in vitro* experiments the resistant cultivar bore fewer galls than the susceptible cultivar. However, incorporation of 0.4% SWC into the medium increased the number of egg masses in the resistant cultivar. A direct nematotoxic effect of SWC was excluded as a possible mode of action since SWC was shown to have no detrimental effect on the hatching of eggs and survival of juveniles prior to entry into the root.

Sawhney & Webster (1979) had earlier suggested that plant growth regulators may affect host susceptibility or resistance to nematodes. It is thought that high levels of plant growth regulators, e.g. auxins and cytokinins (Sawhney & Webster, 1975; Cutler & Krusberg, 1968; Kochba & Samish, 1972), ethylene precursors (Orion & Minz, 1969), or auxin precursors such as tryptophan (Lewis & McClure, 1975) favour susceptibility (Veech, 1981). Resistance, on the other hand, has been induced by the application of auxin and cytokinin, but only when applied separately. The application of either NAA or kinetin to a tomato cultivar susceptible to *M. incognita* inhibited larval penetration and root-knot nematode development (Dropkin *et al.*, 1969; Sawhney & Webster, 1979).

In a tomato cultivar resistant to *Meloidogyne*, the exogenous application of a kinetin mixture (Dropkin *et al.*, 1969), or an auxin and kinetin (Kochba & Samish, 1971; Sawhney & Webster, 1975), encouraged giant cell and gall formation in the infected roots. Sawhney & Webster (1975) noted that although galls were produced, only a few larvae developed to maturity indicating that resistance was not completely broken. In the present study SWC applied to root cultures resistant to *Meloidogyne* encouraged galling and the production of egg masses. It is feasible that high levels of cytokinins and auxins in SWC may be affecting the plant-nematode interaction.

The occurrence of plant growth regulators in commercial seaweed concentrates is well docu-

mented. These include cytokinins and their glucosides (Tay *et al.*, 1985, 1987) and several indoles including indole-3-acetic acid, indole-3-carboxylic acid, indole-3-aldehyde and N,N-dimethyltryptamine (Crouch *et al.*, 1992). Although the results presented above are difficult to explain simply in terms of hormone levels, it is highly probable that these plant growth regulators are involved. An interaction between endogenous auxins and cytokinins in SWCs in host plants may well be instrumental in affecting resistance of tomato to *M. incognita*.

Acknowledgements

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