The use of seaweed-based products from *Ecklonia maxima* and *Ascophylum nodosum* as control agents for *Meloidogyne chitwoodi and M. hapla* on tomato plants.

Master Dissertation

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Summary- Two commercially available seaweed products, derived from *Ascophyllum nodosum* (An) and *Ecklonia maxima* (Em) were evaluated for their potential as control agent for the root-knot nematodes, *Meloidogyne chitwoodi* and *M. hapla*, cultured on tomato plants *(Solanum lycopersicum* cv. Moneymaker). The numbers of nematodes recovered from plants treated with the seaweed product and distilled water (DW) as control did not differ between treatments within the same species, but differed significantly between the two species after one generation. Percentage hatch from egg masses immersed in the seaweed products for 64 days was significantly reduced with 50% and 100% concentrations of An, for *M. hapla* and *M. chitwoodi* respectively, but not with Em compared with distilled water (DW) control treatment. When pre-exposed to the test solutions for 6h, there was no difference for both products in the number of second-stage juveniles (J2) that infected the plant in test tubes filled with sandy soil. After 24h pre-exposure time, there was a significant reduction in the number of J2 that infected the plant in the test tubes for the treatment with An compared with DW control for both species of nematode. In pluronic gel bioassay on Petri dish, treatment with Em resulted in an increase in the number of *M. chitwoodi* J2 that reached a 0.5cm area around the root when the J2 were pre-exposed for 24h in the solution. There was no difference between treatments after 6h pre-exposure time for this species. When *M. hapla* was the test species, Em resulted in a high number of J2 that reached a 0.5cm area around the root after 6h pre-exposure of J2 to the solution.

Key words- attraction, biological control, hatching, infectivity, *Meloidogyne chitwoodi*, *Meloidogyne hapla*, seaweed products.

The tomato plant (*Solanum lycopersicum*) is one of the world's most grown vegetables. It is a good source of potassium, fibre and vitamins A and C. It has been reported as a rich source of lycopene (Di Mascio *et al.*, 1989), which can be used in the fight against cancer, especially prostate cancer (Mills *et al.*, 1989; Giovannucci *et al*., 1995; Giovannucci, 1999), although the US Food and Drug Administration, after extensive review reported in November 2005, cast significant doubt on the potential for lowering disease risk, showing no link between lycopene and prevention of prostate cancer. However, it is suggested that eating tomatoes does provide benefit, perhaps because as yet undiscovered compounds (other than lycopene) are the beneficial agents.

The tomato plant originated in Central and South America, and it is today widely eaten, especially in Europe and the USA. During the last four decades, the world crop area of tomato has witnessed a 164% increased, while the world consumption has observed a corresponding increase of 314% (Nicola *et al*., 2009). The driving force for the expansion in acreage and export share of tomato (processed and fresh), has been due to its increasing importance in the worldwide market, especially for those countries located close to the major importers. In recent years, the quantity of tomato consumption has increased by 3% on an annual basis (Nicola *et al.*, 2009). The increase in consumption has been geared to the improvement in the quality. In tropical and subtropical climates, the production of tomatoes during the rainy and dry season is limited by unfavourable conditions, some of which include flooding, high temperature, strong winds and high incidence of diseases, especially those caused by nematodes.

Phytoparasitic nematodes are responsible for an estimated $157 billion in agricultural damage globally on an annual basis (ScienceDaily, 2008). They constitute an exceptionally complex crop pest to control. The root-knot nematodes (*Meloidogyne* spp.) are among the three most economically damaging genera of phytoparasitic nematodes on horticultural and field crops. They are distributed worldwide and are obligate parasites of roots of thousands of plant species, including lower and higher herbaceous and woody plants. The genus encompasses more than 90 species with some species having

numerous races. Four most important species, *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, are of utmost economic importance worldwide, responsible for 95% of the infestations of cultivated land (Sasser & Carter, 1982), whereas *M. chitwoodi* and *M. fallax* as well as *M. enterolobii* (= *M. mayaguensis*) and *M. minor* have recently gained importance as quarantine organisms or newly emerging and extremely pathogenic species. Among a total of 43 crops listed as having plant-parasitic nematodes of major importance, *Meloidogyne* occur in 23, ranging from field crops, through pasture and grasses, to horticultural, ornamental and vegetable crops (Stirling *et al*., 1992). Damage caused by root-knot nematodes results in poor growth, loss of quality and yield, as well as reduced resistance to other stresses (*e.g*., biotic, abiotic, and other diseases). Heavy infestations of root-knot nematode can lead to total crop loss. Establishment of root-knot nematodes in deep-rooted perennial crops makes their management and control difficult, especially with the limited options available.

The management and control of damage caused by phytoparasitic nematodes have historically been achieved with the use of crop rotation, plant resistance and other cultural practices or nematicides. However, the invention of a new nematicide is a multifarious task. Due to the soil borne nature of most species of phytoparasitic nematode or the fact that they reside within the roots of the plant during the parasitic phase of their life span, the target of any chemical nematicide usually resides at a reasonable distance away from the site of application of the chemical. In addition, the impermeability of the nematode cuticle and other surface structures to most organic molecules remain a confining factor. As a result, most nematicides tend to be rather toxic or volatile, with poor target specificity, thus having severe adverse effects on human or environmental safety, such as underground water contamination and/or the depletion of the ozone layer (Thomas, 1996; Anonymous, 2000).

During the last two decades, there have been tremendous developments that have had significant effects on the prospects and opportunities for the biological control of plant-parasitic nematodes. Among others, these include the withdrawal of several nematicides from the market because of health and environmental problems associated with their production and use (Thomason,

1987). As a result, increased interests in the development of alternative methods of control, including the use of biological agents have been employed. In addition, nematophagous fungi and bacteria have been demonstrated to show increase in several soils under some perennial crops, and under those grown in monocultures, and thus may control some nematode pests, including cyst and root-knot nematodes (Stirling, 1991). Also interesting to note is the fact that a number of commercial products based on nematophagous fungi and bacteria have been developed, although most of them so far have had only limited success. Their use has been based on empirical research, and it is sensible to consider what might be the key factors for a successful biological control agent for nematodes in order to identify the reasons for the general failure of the products that have been developed.

Biological control is an environmentally sound and effective means of reducing or mitigating pests and pest effects through the use of natural enemies. Despite being environmentally friendly, biological control agents are more inconsistent, less effective and slow in action than control normally achieved with chemicals. This does not obviate the fact that improvements in performance might be expected from more research on individual agents. However, it seems likely that these limitations are inherent in most biological control agents and that the success of their application will depend on integration with other control measures or the combination of two or more biological control agents. With this background, the present study was initiated to investigate the bioactive potential of some major seaweed-based products as control agents for *Meloidogyne* spp. on tomato plants.

The discovery of inhibitory substances biosynthesized by seaweeds dates back to early 1917 (Harder & Oppermann, 1953). Pratt *et al.* (1944) were the first to report antibiotic activities of seaweeds. Evidence of seaweeds containing cytotoxic (Rocha *et al.*, 2007), antibacterial (Tuney *et al.*, 2006), antifungal (Aliya & Shamaeel, 1999; Tang *et al.*, 2002), antiviral (Garg *et al.*, 1992; Serkedjieva, 2004), and larvicidal activity (Manilal *et al.*, 2009) have been reported. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to algicidal, nematicidal, insecticidal and ichthyotoxicity in lower

forms of animals (Smith, 2004). Seaweed products have also been shown to increase seed germination, plants' nutrients uptake, frost resistance, and resistance to pathogenic fungi (Boot, 1964). Stake holders of many countries are encouraging farmers to use commercial seaweed fertilizers as soil additives, conditioners and foliar spray. Tarjan (1977) demonstrated that extracts of *Ascophylum nodosum* can cause a significant reduction in the number of nematodes and an increase in plant weight when applied to citrus seedlings infected with *Radopholus similis,* compared with water-treated control seedlings. Commercial extracts of *A. nodosum* when applied to the soil effectively controlled *Belonolaimus longicaudatus* on centipede grass after one month application (Morgan & Tarjan, 1980). Featonby and van Staden (1983) reported a significant reduction of root-knot nematode infection of tomato plants with the use of commercially-available seaweed concentrate prepared from *Ecklonia maxima*. Suppression of reproduction of *Pratylenchus zeae* on excised root of *Zeae mays* has also been reported by De Waele *et al*. (1988) using the same commercial product. Therefore, the present study focused on experiments to compare the effects of *A. nodosum* and *E. maxima* on hatching, infectivity and attraction of *M. chitwoodi* and *M. hapla*, and on the growth of the tomato plants. Such background information is essential to determine the reality of using these seaweed extracts as biological control agents for *Meloidogyne* spp. on tomato plants.

Materials and methods

NEMATODE CULTURE

To set up cultures of *Meloidogyne hapla* and *M. chitwoodi* for the experiments, seeds of tomato plant, *Solanum lycopersicum* cv. Moneymaker, were sown in small transparent plastic tubes (120 × 20 × 15 mm) filled with moist sterilized (100°C, 18h) sandy soil. The advantage of these tubes is that as they are transparent, egg masses can be seen on the roots growing near the surface through the tubes.

Also, they give room for more nematode cultures within a limited space. However, their limitations stem from the fact that nematode cultures cannot be maintained in them for long as the roots will become 'tube bound'. The seedlings were allowed to geminate to the four-leaf stage and approximately 200 freshly hatched second-stage juveniles (J2) of *M. hapla* and *M. chitwoodi*, obtained from pure cultures maintained under controlled (14h day light, 22±6°C) conditions in the glasshouse of ILVO, Merelbeke, were inoculated into the tube. Freshly hatched J2 used throughout the experiment were extracted from freshly harvested infected roots by the Bearmann funnel technique (Baermann, 1917).

GLASSHOUSE EXPERIMENTS

The effects of the seaweed products from *Ecklonia maxima* (Em) and *Ascophylum nodosum* (An) on the infectivity of the *Meloidogyne* species on tomato plants was examined under controlled glasshouse conditions. Seeds of *Solanum lycopersicum* cv. Moneymaker were germinated in seedling trays. Seedlings were transplanted at the four-leaf-stage into 1.7 l pots containing organic soil (Peat, 20% OM, pH 5.0-6.5, NPK 12-14-24, Saniflor, Belgium). Plants were grown under controlled glasshouse conditions (14 h day light, 22±6°C). The experimental set-up consisted of six treatments (Table 1) each with five replicates arranged in a fully randomized design.

Table 1. *Treatments for the glasshouse experiment. Em* = Ecklonia maxima*; An* = Ascophyllum nodosum.

a) Em +*Meloidogyne chitwoodi*
b) Em + *M. hapla*
c) An + *M. chitwoodi*
d) An + *M. hapla*
e) *M. chitwoodi*
f) *M. hapla*

The two seaweed concentrates used were 'Kelpak' and OSMO®. Kelpak is prepared by a cell-burst process by the South African based company, Kelp Products (Pty) Ltd from the brown alga *E. maxima*. OSMO® liquid fertilizer is an aqueous alkaline extract produced from the brown marine alga, *Ascophylum nodosum*, marketed by OSMO® International NV Belgium. Dilutions for the solutions used were 10ml and 5ml l^{-1} for Em and An, respectively, which are concentrations recommended by the manufacturers for application to plants as a soil drench.

Inoculations were done by boring small holes around the active growing root region into which nematodes were inoculated with the aid of a micro pipette; 4000 J2 per pot were used for each treatment. A hundred milliliter of the solutions were applied to the seedlings at transplanting and thereafter every 5 days for a 20-day period. This was followed by 200ml at the same time interval with in-between application of water following the increased moisture requirement by the plants as they grew.

Plants were harvested after 2 months. Shoot length, fresh weight of the root and the above ground plant parts, as well as the number of fruits were recorded. Roots from pots treated with nematodes were collected, carefully washed, macerated and subjected to extraction of nematodes. Extraction was done with an automated zonal centrifugal machine (Hendrickx, 1995). The principles under which this machine operates are same as those of conventional centrifugation but the process is fully automated. Number of juveniles, eggs, females and males were counted with the use of a binocular microscope. Data were statistically analyzed by analysis of variance with significance level taken as $P < 0.05$.

HATCHING TEST

For the examination of the effect of the seaweed products on hatching of J2, egg masses of *M. chiwoodi* and *M. hapla* were carefully removed from heavily infected tomato roots. Five egg masses

were picked by means of forceps and placed on a 48µm sieve. These sieves are capable of retaining the egg masses as well as loose eggs, while hatched J2 can easily move through the sieves. The sieves containing the egg masses were then placed in small plastic tubes each containing 4ml of the test solutions (Table 2). Care was taken to ensure that all egg masses were completely immersed in the test solutions. The tubes were incubated in the dark at a temperature of 20 ±1°C. The solutions were refreshed at intervals of 4 days and the number of hatched J2 at each interval were counted and recorded. This process continued for 64 days, the time at which the number of hatched J2 was less than five. To check for reversible effects after treatment, the sieves with the egg masses were all transferred into distilled water for 8 days and the number of J2 that hatched were counted. The egg masses were then carefully transferred into counting dishes, covered with 3ml of 10% sodium hypochlorite and homogenized to dissolve the gelatinous layer. With this treatment the eggs containing unhatched J2 were released and counted.

The means ± SD of the hatching data obtained for both *M. chitwoodi* and *M. hapla* were fitted to the logistic model $y = c/(1 + \exp(-b \times (\text{time} - m)))$, where y is the cumulative percentage hatch. The model is described by three parameters: the time at which 50% hatch is reached (m), the hatching rate (b) and the final hatching percentage (c) (Oude Voshaar, 1994). These parameters were calculated for all the replicates of the treatments separately and subjected to analysis of variance (one-way ANOVA). Observations were reported as significant or non-significant using the LSD test with significance at $P < 0.05$.

Table 2.*Treatments for the hatching experiment*

***Meloidogyne chitwoodi* (Mc)**	***M. hapla* (Mh)**
Distilled water (DW) control	Distilled water (DW)control
10% Em+Mc	10% Em+Mh
25%Em+Mc	25%Em+Mh
50%Em+Mc	50%Em+Mh
100%Em+Mc	100%Em+Mh
10%An+Mc	10%An+Mh
25%An+Mc	25%An+Mh
50%An+Mc	50%An+Mh
100%An+Mc	100%An+Mh

INFECTIVITY OF HATCHED JUVENILES AFTER EXPOSURE TO THE SEAWEED PRODUCTS

To investigate the effect of the seaweed products on infectivity of *M. chitwoodi* and *M. hapla*, tomato plants were inoculated with J2 of both species after they had been exposed to the seaweed products. Therefore, small transparent plastic tubes of 120 x 20 x 15 were filed with moist sterilized (100°C for 18h) sandy soil and seeded (one seed per tube) with tomato (*Solanum lycopersicum* cv. Moneymaker). The seeds were allowed to germinate to the four-leaf-stage under controlled glasshouse conditions maintained at 20°C and 14h lighting with daily application of water with a jet spray to maintain the moisture content of the soil. The tomatoes were inoculated with approximately 200 freshly hatched J2. The experiment consisted of twelve treatments (Table 3) each replicated five times and fully randomized. Plants were harvested after ten days and the tubes immerged in water while the soil was gently washed away and the nematodes stained *in vitro* with acid fuchsin (Byrd *et al*., 1983). The number of nematodes that penetrated the plant roots were counted and recorded. Data were analyzed by one-way ANOVA and results reported as significant or not using the LSD test, with significance at $P < 0.05$.

Table 3. *Treatments for the infectivity test and pluronic gel bioassay with 6 h and 24 h pre-exposure time to the test solutions.*

Meloidogyne chitwoodi		***M. hapla***	
6h	24h	6h	24h
DW	DW	DW	DW
Em	Em	Em	Em
An	An	An	An

IN VITRO BIOASSAY WITH PLURONIC GEL

The effect of the seaweed products on the sense organs of *M. chitwoodi* and *M. hapla* after prior exposure of the J2 to the products was examined by assessing their attraction to tomato roots on Pluronic gel. Therefore, pluronic F-127 gel (NF Prill Poloxamer 407, BASF) was prepared according

to Wang *et al.* (2009). The advantage of the gel compared with agar is that nematodes move in the gel in three-dimensions, thus allowing for maximum sensation of root diffusate within the gel unlike on agar where there is only the possibility for surface movement. In addition, the transparency of the gel allows for easy monitoring of the nematode movement within the gel.

Seedlings of tomato plant were germinated by placing them on moist filter paper on Petri dishes in the dark for 1 week. Thirty milliliters of gel was poured into each Petri dish and seedlings of tomato plant were added one seedling per plate. The gel was allowed to solidify at room temperature. The roots were in the gel for 6h to release attractants after which approximately 200 freshly hatched J2 of each *Meloidogyne* species, which had been previously exposed to the test products (DW, An and Em) for 6h and 24h (Table 3), were inoculated onto the gel. Each treatment was replicated five times. The J2 were inoculated as far away from the root as possible. A line was marked 0.5cm around the root. After 20h, the number of nematodes within the 0.5cm area of the root was scored. Data were analyzed statistically by one-way ANOVA and results reported as significant or not using the LSD test with significance at $P < 0.05$.

Results

GLASSHOUSE POT TEST

Plant growth features

Both seaweed products applied as a soil drench significantly ($P<0.05$) increased the fresh weight of roots of the tomato plants compared to the control with *M. chitwoodi* (Fig.1 A). However, the reduction was not significantly different to the control with *M. hapla*. There were no significant differences in shoot length or the fresh weight of the shoot between all treatments (Fig.1 B&C). These

symptoms were observed on the leaves as they started dying back from the edges. Likewise, the tomato fruits started developing blossom end rot. As a result of the blossom end rots, the data for fruit number was excluded from the experiment.

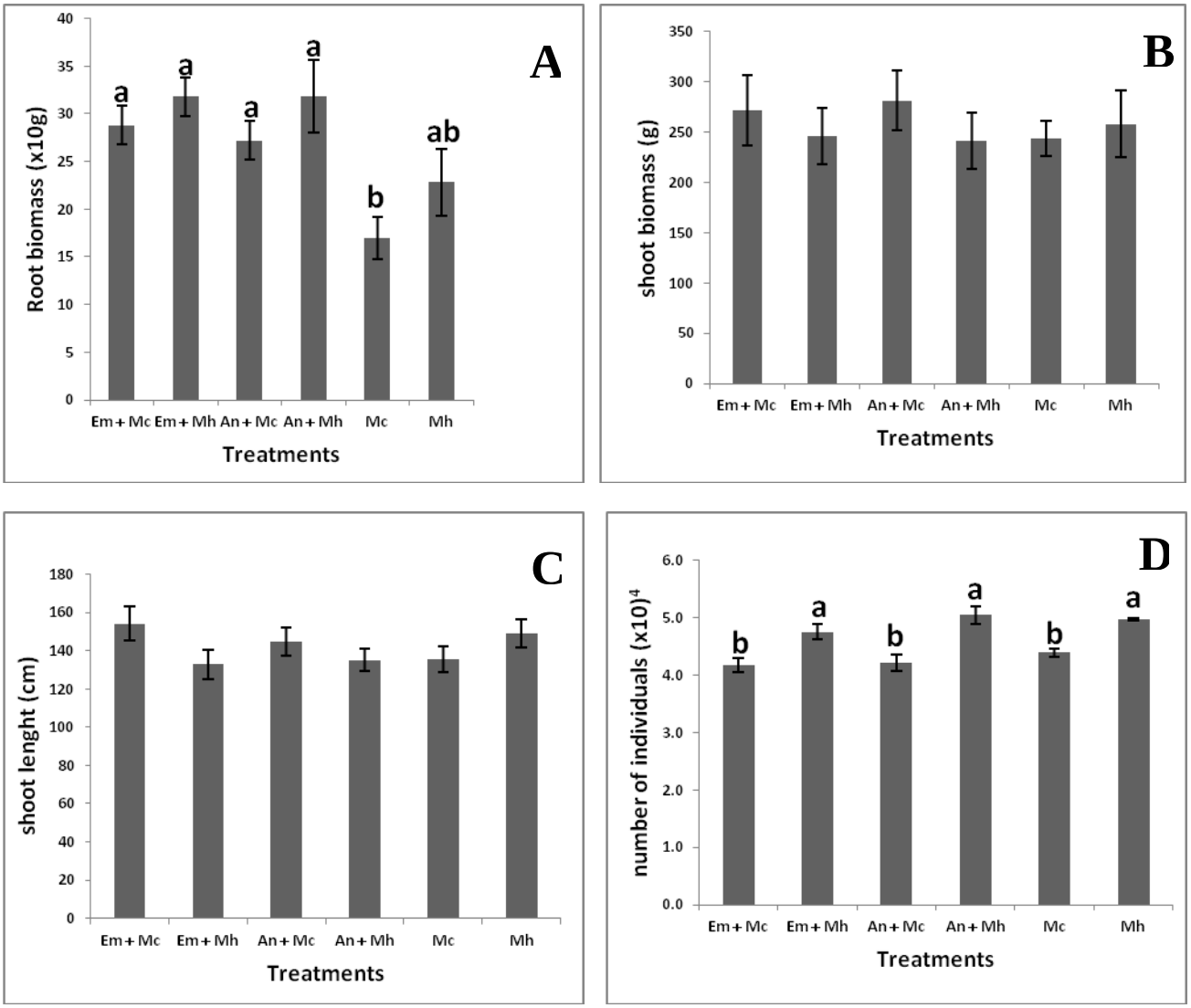

Fig. 1. *Means ± SE of root biomass (A), shoot biomass (B), shoot length (C) and number of nematodes (C). Significant differences (P < 0.05) are marked with different letters, LSD test at P < 0.05.*

Nematode infection

The number of nematodes that infected the plants did not differ significantly between treatments within the same species. However, there were significant differences between the two *Meloidogyne* species in their level of attack on the plant root system. The population of *M. hapla* that

infected the plants was significantly (P<0.05) greater than that of *M. chitwoodi (*Fig.1D). The galls produced by *M. chitwoodi* differed from those caused by *M. hapla*. Galls formed by *M. hapla* were larger and distinct, while those formed by *M. chitwoodi* were not easily visible with the naked eye.

Table 4: *F and P-values for the different parameters estimated***.**

Parameter	F	P
Root biomass (g)	3.263082	0.021793
Shoot biomass (g)	0.321553	0.895050
Shoot lenght (cm)	1.392927	0.262243
Number of nematodes	10.62430	0.000018

HATCHING TEST

Upon exposure of the egg masses of *M. hapla* and *M. chitwoodi* to the seaweed products there was significant reduction (P<0.05) in the percentage hatch. This reduction was achieved with An100% concentration for *M. chitwoodi* (Fig. 2A) and An50% concentration for *M. hapla* (Fig. 2B). There was no significant difference between treatments in the rate of hatching as well as the time at which 50% hatch was attained. For the first two successive counts, it was observed that freshly hatched J2 of *M. chitwoodi* in An100% were paralysed. Also, the percentage hatch of J2 of *M. chitwoodi* in An100% was less compared with the hatch in the distilled water control and the rest of the treatments. Subsequently, hatched J2 of *M. chitwoodi* in this treatment showed normal activity with respect to mobility. There was no significant effect on hatching from all treatments with Em as well as the 10% and 25% concentrations of An. When transferred to DW, there were no changes in hatching for all the treatments.

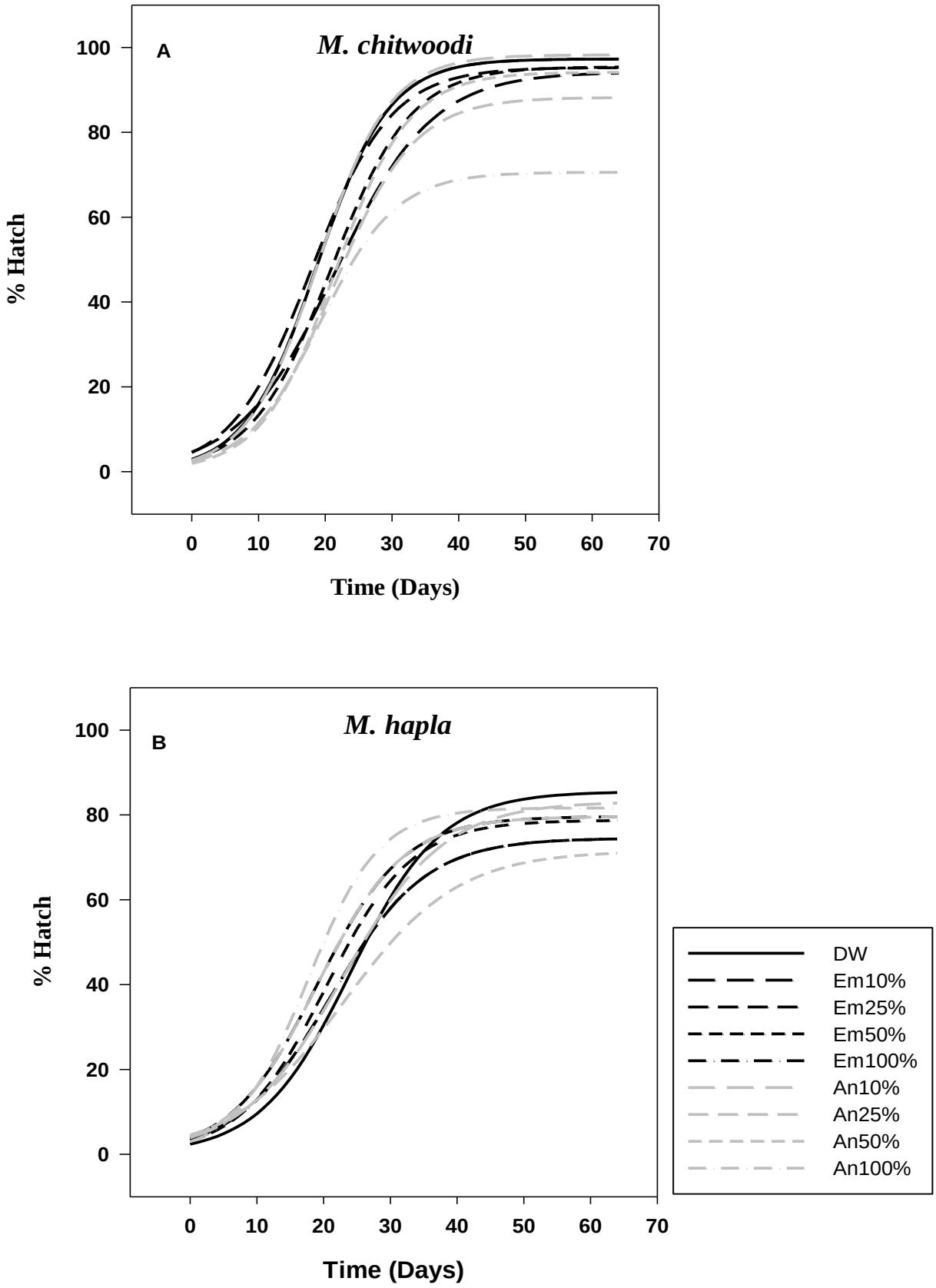

Fig. 2. *Cumulative hatching curves for* M. chitwoodi *(A) and* M. hapla *(B) based on the means±SE of the test parameters; the time (t) at which 50% hatch is reached (m), the hatching rate at the time (t) at which 50 % hatch is reached (b) and the final hatching percentage (c).*

Table 6. *F and P-values for the parameters for cumulative hatching curve*

Parameters	F-value	P-value
c	1,924062	0,035524
b	0,740983	0,747711
m	0,235039	0,999062

Table 5. *Coefficients of the logistic model describing the cumulative hatching curve of J2 of* M. hapla *and* M. chitwoodi *in distilled water (DW),* Ecklonia maxima *(Em) and* Ascophylum nodosum *(An) at 10%, 25%, 50% & 100% concentrations respectively. Function: y = c/(1+exp(-b*(t-m))). Mean ± the standard deviation of: the time (t) at which 50% hatch is reached (m), the hatching rate at the time (t) at which 50 % hatch is reached (b) and the final hatching percentage (c). Significant differences between treatments are indicated with different letter (LSD test:* P<*0.05).*

	M. chitwoodi				*M. hapla*			
Treatments	c	b	m	R^2	c	b	m	R^2
DW	$97.29\pm1.11_{a}$	$0.19\pm0.01_{a}$	18.79±0.38	0.98	$85.51\pm3.23_{a}$	$0.15\pm0.02_{a}$	24.01±1.27	0.92
Em 10%	$94.23\pm2.92_{ab}$	$0.14\pm0.01_{b}$	21.50±1.10	0.93	$79.63\pm4.32_{b}$	$0.15\pm0.03_{a}$	19.01±2.05	0.82
Em 25%	$95.28\pm1.72_{ab}$	$0.17\pm0.01_{ab}$	17.94±0.64	0.96	$74.51\pm2.27_{ab}$	$0.14\pm0.02_{a}$	21.12±1.01	0.94
Em 50%	$95.45\pm1.24_{ab}$	$0.17\pm0.01_{ab}$	20.88±0.44	0.98	$78.75\pm4.32_{b}$	$0.16\pm0.03_{a}$	20.36±2.06	0.82
Em 100%	$97.29\pm1.11_{a}$	$0.19\pm0.01_{a}$	18.79±0.41	0.98	$74.51\pm2.99_{a}$	$0.14\pm0.02_{a}$	21.12±1.34	0.89
An 10%	$98.26\pm1.21_{a}$	$0.19\pm0.02_{ab}$	18.94±1.03	0.93	$83.14\pm9.20_{a}$	$0.13\pm0.03_{b}$	22.85±4.59	0.64
An 25%	$88.24\pm2.23_{b}$	$0.17\pm0.02_{ab}$	21.40±0.85	0.95	$79.61\pm4.67_{b}$	$0.15\pm0.02_{ab}$	19.01±2.36	0.83
An 50%	$94.17\pm1.66_{ab}$	$0.18\pm0.01_{a}$	21.48±0.58	0.97	$71.56\pm2.98_{a}$	$0.12\pm0.03_{a}$	22.97±1.34	0.89
An 100%	$70.60\pm4.58_{c}$	$0.18\pm0.06_{a}$	19.30±2.21	0.72	$81.67\pm3.35_{a}$	$0.09\pm0.02_{a}$	17.58±1.41	0.90

INFECTIVITY OF HATCHED JUVENILES AFTER EXPOSURE TO THE SEAWEED PRODUCTS

When pre-exposed to the two seaweed products and DW for 6h, there were no significant differences in the number of J2 of *M. chitwoodi* that infected the plants (Fig. 3A). After 24h pre-exposure to the two seaweed solutions, there was a significant reduction (P<0.05) in the number of J2 of *M. chitwoodi* that infected the plant compared with infectivity in the distilled water control treatment (Fig. 3A). However, the reduction achieved with pretreatment with An was much greater than with Em. There was also a significant reduction (P<0.05) for *M. hapla* in the number of J2 that infected the plant after 24h pre-exposure time for the treatment with An (Fig. 3B).

Six hours pre-exposure of the J2 of *M. hapla* did not show any significant difference between the treatment with An and DW, but the treatment with Em demonstrated an unexpected increased in the number of J2 that infected the plant (Fig. 3B).

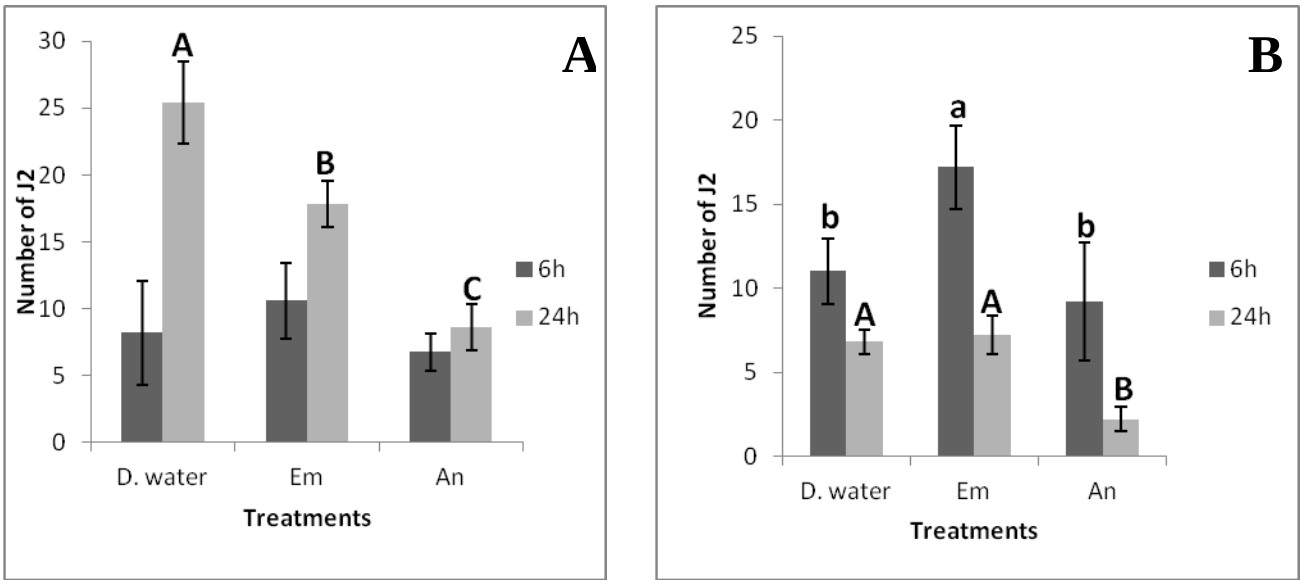

Fig. 3. *Infectivity of hatched J2 of* M. chitwoodi *(A) and* M. hapla *(B) after 6h & 24h pre-exposure time to the test solutions. Bars show means±SE of number of J2 that infected the roots. Significant differences are marked with different letters, LSD test (P<0.05).*

Table 7. *F and P-values for infectivity and gel bioassay.*

Experiment	F-value	P-value
Gel bioassay	12,82940	0,000000
Infectivity	7,387634	0,000000

IN VITRO BIOASSAY WITH PLURONIC GEL

The number of J2 found within the 0.5cm area of the root after 6h pre-exposure of the J2 of *M. chitwoodi* did not differ significantly between the treatments (Fig. 4A). When exposed to the test solutions for 24h prior to inoculation, there was a significant increase (P<0,05) in the number of J2 of *M. chitwoodi* that could be seen within 0.5cm area of the root in the treatments with Em (Fig. 4A). Treatments with DW control and An both resulted in low numbers of J2 that could reach the 0.5 cm

area around the root for *M. hapla*. The number was much lower for J2 pre-exposed for 24h in An than for DW although the difference was not significant (LSD test; *P<0.05*) (Fig 4B). A similar situation was observed when *M. hapla* was the test species, but unlike *M. chtwoodi*, *M. hapla* recorded an increase in the number of J2 seen within the 0.5cm area for the treatment with Em after 6h pre-exposure time (Fig. 4B).

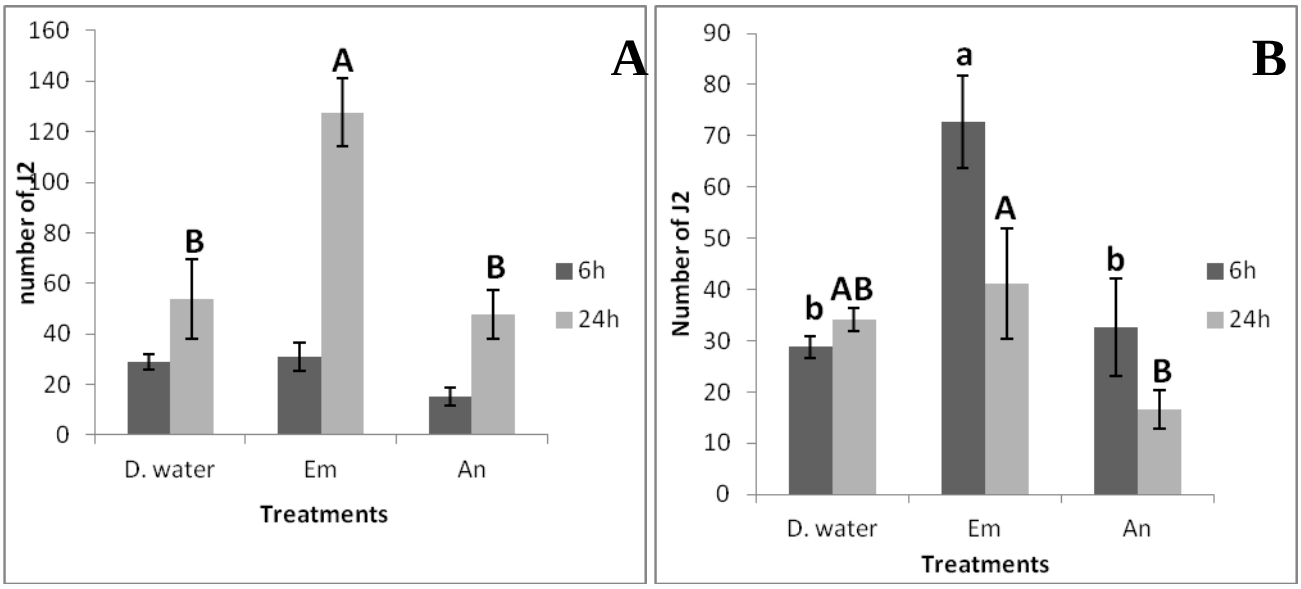

Fig. 4: *Attraction of hatched J2 of* M. chiwoodi *(A) and* M .hapla *(B) after 6h & 24h pre-exposure time to the test solutions. Bars show means±SE of the number of J2 that could be seen within 0.5cm area around the root. Significant differences are marked with different letters, LSD test (P<0.05).*

Discussion

An ample range of the beneficial effects of seaweed extract applications on plants, such as improved crop performance and yield, early seed germination and establishment, enhanced postharvest shelf-life of perishable products and elevated resistance to biotic and abiotic stress have been well documented (Beckett & van Staden, 1989; Hankins & Hockey, 1990; Blunden, 1991; Norrie & Keathley, 2006). Observations from the glasshouse pot test in the present study are a confirmation of previous findings by Featonby-Smith and Van Staden (1983) that seaweed concentrates improve the growth of nematode infected tomato plants. Although there have been a number of reports demonstrating significant reductions of the level of root-knot nematodes attack on plants treated with

seaweed extract from An and Em (Wu *et al*., 1997, Whapham *et al*., 1994; Crouch & van Staden, 1993; Featonby-Smith & van Staden, 1983), the experiments have mostly been done on tropical root-knot nematode species. In the present study, when tomato roots were challenged with the Columbia root-knot nematode, *M. chitwoodi*, and the Northern root knot nematode, *M. hapla*, there was no significant difference between treatments in the level of attack on the root of the tomato plants upon application as a soil drench of the two seaweed products used. However, there was a significant difference between the two nematode species in their level of attack. The number of *M. hapla* that infected the tomato plants was consistently higher than *M. chitwoodi* for all treatments with the two seaweed products and the DW control. The reason for this is unclear, although differences in biology, for example temperature preferences for activity, may have been influential.

There were no significant differences in length and fresh weight of the above ground plant part. However, there were visible differences between treatments in the aerial plant parts.

In many species of parasitic nematodes, the hatching behaviour is considered an essential component of the life cycle for optimizing the chances of successful infection by synchronization with host availability (Perry, 2002). *Meloidogyne chitwoodi* and *M. hapla* have a remarkable difference in this aspect of their biology. It has been shown (Santo & O'Bannon, 1981) that *M. chitwoodi* is able to reproduce at lower soil temperatures than *M. hapla*. However, Inserra *et al.* (1983) had demonstrated that temperatures of approximately 15, 20 and 25°C gives similar effects on the hatch of both species. The maximum percentage hatch obtain during the present study was higher for *M. chitwoodi* (98%) than for *M. hapla* (85%). The high percentage hatch obtained in DW confirms the statement by Perry (1987) that *Meloidogyne* is a genus with normal rates of hatch under favourable environmental conditions. This is also consistent with earlier report by Inserra *et al.* (1983) that root diffusates of tomato, potato and wheat does not increase hatch of *M. chitwoodi* and *M. hapla.* However, recent findings by Perry and Wesemael (2008) show exceptions, suggesting that responses to root exudates may be more important than previously realized. This is supported by a more recent study by Oka and Mizukubo (2009) who demonstrated an increased numbers of J2 of *M. incognita* that hatched in

hydroponic culture media in which tomato and okra had been grown as compared with those in water or fresh culture medium.

Reduction in hatching has been clearly demonstrated by the 100% and 50% concentrations of An compared with the DW control treatment. This, together with the amount of reduction in the number of J2 that infected the tomato plant root after 24h pre-exposure to An confirms previous reports (Whapham *et al*., 1994; Wu *et al*., 1997, 1998) that extracts from this seaweed product reduces infection of tomato plants by J2 of *Meloidogyne* spp. Preliminary experiment had shown that the seaweed extract has no toxic effect on the J2 upon exposure to the product for longer period of time and this confirms an earlier report by Wu *et al*. (1996). In addition, the fact that hatched J2 after being in the product for 4 days were still actively moving is an additional support. Whapham *et al*. (1994) showed that J2 of *M. javanica* hatched directly into seaweed extract from An had a greatly reduced level of attack on tomato plant roots. The role of extract of the seaweed products on hatching and infectivity therefore can be considered as an indirect effect.

Ascophyllum nodosum extracts are said to contain various betaines and betaine-like compounds (Blunden *et al.*, 1986). Upon application to plants, betaines act as a compatible solute that alleviates drought stress as well as osmotic stress induced by salinity. Suggestions have also been made concerning the possible roles of betaines in enhancing leaf chlorophyll content of plants following their treatment with seaweed extracts from An (Blunden and Gordon 1986; Blunden *et al*., 1997). The enhancement in chlorophyll content may be due to a decrease in chlorophyll degradation (Whapham *et al*., 1993). Genard *et al*. (1991) attributed yield enhancement effects due to improved chlorophyll content in leaves of various crop plants treated with seaweed to the betaines present in the seaweed extract. A variety of enzymes are involved in the hatching process of nematodes (Perry 1997) and their role in the hatching of *Meloidogyne* has been discussed by Bird (1968). The extract of An consist of a variety of compounds and some of these compounds could possibly have interacted with and interrupted activities of the enzymes (lipase, proteinase, chitinase or collagenase) that are correlated

with the hatching percentage; these enzymes are capable of increasing the flexibility of the egg shell (Perry *et al*., 1992), which is a characteristic for hatching of these *Meloidogyne* spp.

Migration by root knot nematodes through the soil can be limited or stopped by adverse conditions of moisture, porosity, oxygen availability, toxins and temperature (Curtis *et al*., 2009). Sandy soils are well known as good medium for root-knot nematodes, and low compaction of the soil appears to favour the movement of these nematodes (Eo *et al*., 2007). In the bioassay with pluronic gel on Petri dish, the behaviour of both *Meloidogyne* spp are somewhat consistent with their infectivity after prior exposure to the seaweeds products. *Meloidogyne chitwoodi* appeared to be more attracted to the roots on both the gel and in sandy soil after 24h pre-exposure to Em, even two folds more than the DW control in the attraction test. The same holds for *M. hapla,* but unlike *M. chitwoodi, M. hapla* was more attracted to the root after 6h pre-exposure to Em. This could either be due to the fact that the J2 became adapted to the conditions in the solution after the said time period, or that this seaweed product has some chemical properties that enhance sensory perceptions of the roots by the nematodes after these time periods.

Perry (2005) provided a useful generalized framework to visualize attractants by classifying them as long-distance, short-distance and local attractants. Root knot nematodes are said to be attracted to the root area by long-distance attractants. Short-distance attractants attracts the nematodes to roots themselves while local attractants are responsible for orientation to the preferred inversion site by endo-parasitic nematodes. Early *in vitro* experiments demonstrated the attraction of nematodes to roots as well as within sand to zones where roots had been growing (Prot, 1980). Wang *et al*. (2009) using *in vitro* assays with pluronic gel, demonstrated that *M. javanica* and *M. incognita* moved to roots much more rapidly than *M. hapla*. However, it could be that the two tropical root-knot nematodes were more favoured by the temperature of the gel than the northern root-knot nematode. Pluronic gel becomes semi-solid at room temperature while at temperatures below 20^{o}C, it is in the liquid state when concentrated at 20-30%.They also found aggregations of J2 when the nematodes were in contact with root tips, indicating that a signal from the root is involved in the attraction. In the present study, a

similar situation was observed in treatment with Em. Wang *et al*. (2009) suggested that lower oxygen or a volatile attractant is involved in this aggregation behaviour.

Seaweed extracts from *A. nodosum* used as a soil drench in the glasshouse pot test to improve the growth of *Meloidogyne* infected tomato as well as reduction in hatch and inhibition of infectivity of J2 *in vitro* has been clearly demonstrated. However, the level of control achieved with these applications alone may be insufficient under normal agricultural conditions and thus such treatments would have to be incorporated into an integrated control programme. This would include other nematode control measures, for example, the addition of organic supplements to the soil (Godoy *et al*., 1983) and the feasible use of nematicides.

Further research and development of biological control methods must be given high priority and people in general and farmers in particular must be educated about the dangers posed by handling and use of chemical nematicides. The general public is advised to demand farm products where chemical nematicides are not used. All these will lead to a general enlightenment about the benefits of biological control agents and will compel the governments to make policy decisions reducing the use of chemical nematicides and increasing the use of a green substitute.

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Appendices

Appendix 1: Commercial seaweed products used in the agriculture and horticulture industries (Khan *et al.*, 2009)

Product name	Seaweed name	Company	Application
Acadian	*Ascophyllum nodosum*	Acadian Agritech Chance & Hunt	Plant growth stimulant
Acid Buf	*Lithothamnium*	Limited Agri Gro Marketing Inc	Animal feed
Agri-Gro Ultra.	*calcareum*	Algas y Bioderivados	Plant growth stimulant
AgroKelp	*Ascophyllum nodosum*	Marinos, S.A. de C.V.	Plant growth stimulant
Alg-A-Mic	*Macrocystis pyrifera*	BioBizz Worldwide N.V.	Plant growth stimulant
Bio-Genesis™ High Tide™.	*Ascophyllum nodosum*	Green Air Products, Inc	Plant growth stimulant
Biovita	*Ascophyllum nodosum*	PI Industries Ltd	Plant growth stimulant
Emerald RMA	*Ascophyllum nodosum*	Dolphin Sea Vegetable Company	Health product
Espoma	Red marine algae	The Espoma Company	Plant growth stimulant
Fartum	Unspecified	Inversiones Patagonia S.A.	Biofertilizer
Guarantee	*Ascophyllum nodosum*	MaineStream Organics	Plant growth stimulant
Kelp Meal	*Ascophyllum nodosum*	Acadian Seaplants Ltd	Plant growth stimulant
Kelpak	*Ascophyllum nodosum*	BASF	Plant growth stimulant
Kelpro	*Ecklonia maxima*	Tecniprocesos Biologicos, S.A. de C.V	Plant growth stimulant
Kelprosoil.	*Ascophyllum nodosum*	Productos del Pacifico, S.A. de C.V.	Plant growth stimulant
Maxicrop	*Ascophyllum nodosum*	Maxicrop USA, Inc.	Plant growth stimulant
Nitrozime.	*Ascophyllum nodosum*	Hydrodynamics International Inc	Plant growth stimulant
Profert	*Ascophyllum nodosum*	FBAS	Plant biostimulant
Sea Winner	*Durvillea antarctica* Unspecified	China Ocean University Product Development Co., Ltd	Plant biostimulant
Seanure	Unspecified	Farmura Ltd	Plant growth stimulant
Seasol	*Durvillea potatorum*	Seasol International Pty Ltd	Plant growth stimulant
Soluble Seaweed Extract	*Ascophyllum nodosum*	Technaflora Plant Products, LTD	Plant growth stimulant
Stimplex	*Ascophyllum nodosum*	Acadian Agritech	Plant growth stimulant
Synergy	*Ascophyllum nodosum*	Green Air Products, Inc.	Plant growth stimulant
Tasco_	*Ascophyllum nodosum*	Acadian Agritech	Animal feed

Appendix 2: Composition and physical properties of OSMO® (*Ascophyllum nodosum)*

Ascophylum nodosum Seaweed (Dry Matter) 300g/ L

Composition		Growth Stimulants	
Carbohydrates	10-15%	Adenine	0.006%
Alginic acid	2-5%	IAA	0.009%
Mannitol	1-2%	ABA	0.003%
		Betaines	0.013%

Micro Nutrients		Macro Nutrients	
Boron	5- 20ppm	Nitrogen	0.25-0.4 %
Copper	1- 2ppm	Phosphorus (P_2O_5)	0.01- .03%
Iron	10- 50ppm	Potassium (K_2O)	3- 4 %
Manganese	1- 3ppm	Calcium	0.1- 0.3 %
Molybnenum	1- 2ppm	Sulphur	1- 2.5 %
Zinc	2- 25ppm	Magnesium	0.1- 0.3%

Trace Ellements	Physical Properties	
Aluminium	Color	Dark brown to black
Antimony	Physical appearance	Rich, fluid water sol.
Barium	Odour	Typical for Seaweed
Bromine	Specific gravity	1.10-1.15Kg/L
Cadmium	pH	9- 10
Chlorine	Solubility	100 % in water
Chromium	Stability	Stable at normal T and Light Conditions
Cobalt	Oxidizing or reducing	None
Fluorine	Flammability- flash point	None
Iodine	Explodability	None
Lead	Storage ability	Preservatives added to protect against bacteria and mould.
Mercury		
Nickel	Corrosion characteristics	Will corrode unprotected metal surfaces
Selenium	Toxicity	Non-toxic/biodegradable
Silicon	Compatibility	With most commonly used sprays
Sodium		
Strontium		
Tin		
Tungsten		
Vanadium		

Appendix 3. Composition and physical properties of 'Kelpak' (*Ecklonia maxima)*

Ecklonia maxima Seaweed (Fresh Material) 34.26%

Composition		Additives	
Carbohydrates	1.2%	*Ecklonia maxima* seaweed	34.26 %
Protein	0.2%	(fresh material)	
Amino acids	0.1%	Acetic Acid (98%)	0.09 %
Ashes	2.6%	Hydrogen Peroxide (50%)	0.07 %
Moisture	96.0%	Water	62.86 %
		Mono ammonium phosphate	2.70 %
		Green dye	<0.01 %

Micro Nutrients		Macro Nutrients	
Boron	3.2 mg	Nitrogen	0.41 %
Copper	1.8 mg	Phosphorus (P_2O_5)	1.89 %
Iron	2.2 mg	Potassium (K_2O)	0.61 %
Magnesium	56.4 mg	Sodium	0.11 %
Manganese	0.8 mg	Calcium	0.01 %
Zinc	0.9 mg		

Plant Growth Regulators		Physical Properties	
AUXINS (7 natural auxins identified)	10.7 mg	State	Liquid
CYTOKININS (12 natural cytokinins identified)	0.03 mg	Viscosity	24 cps
		Specific gravity	1.02
		pH	4.6
		Solubility	99 %
		Boiling point	101°C
		Receding surface tension (dynes/cm)	59,82