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Biopotentials of Marine Algae Extracts against Root-Knot Nematode, *Meloidogyne incognita*

Mona A. Abdel Rasoul
Plant Protection Department, Faculty of Agriculture, Damanhour University, Damanhour, Albeheira, 22516
***Corresponding author E-mail: mona.abdelnaby@agr.dmu.edu.eg**

ABSTRACT

Nematicidal activity of four species of marine algae *Ulva fasciata* Delile (UF) (green algae), *Corallina mediteranea*, *Corallina officinalis* (red algae) and *Spirulina platensis* (blue-green algae) at concentrations of (125, 250, 500 and 1000 µg/ml) for aqueous and ethanolic extracts were investigated against the knot-root nematode, *Meloidogyne incognita* in laboratory and greenhouse on tomato plants. In laboratory experiments, all tested algae caused significant mortality of the second larval stage (J2). It was, also, noticed that the tested algae reduced egg hatching. Ethanol extract of all algae possesses highest nematicidal activity than water extract. *U. fasciata*, ethanolic extracts showed the highest nematicidal activity among the tested algae in vitro. The results of greenhouse experiment clarified that the tested ethanolic extracts of all algae, reduced numbers of root galls, egg masses and population of nematodes of tomato plants at concentration 1000 mg/kg soil compared to the inoculated control. *U. fasciata* was the most effective treatment in reducing root galls, egg masses and final population of the nematode except for oxamyl. Results revealed that all tested treatments increased various measures of plant growth characters; fresh shoot and root weights and lengths as well as reduced the root- knot infestation. Among all of the tested algae, *U. fasciata* followed by *C. officinalis* and *C. mediteranea* were the most effective treatments to increase both fresh shoot & root weights and lengths. None of the algae was phytotoxic at the tested treatments. A chemical constituent of *U. fasciata* was recognized by GC-MS. GC-MS analysis, exhibited the presence of organic component such as bis(2-ethylhexyl) phthalate which ranked the first with 63.75%, followed by diethyl phthalate (18.46%). Results stated that marine algae and especially, *U. fasciata* exhibited significant nematicidal activity in-vitro and in-vivo experiments and could be considered as useful natural namaticidal agents.
Keywords: marine algae, nematicidal activity, *Meloidogyne incognita*

INTRODUCTION

The root-knot nematodes are the most profuse multi-cellular and nutrient cycling in soil food chains (Bongers and Bongers, 1998). It is the most important economically damaging genus of phytoparasitic nematodes on horticultural and field crops and it is containing over 90 species (Moens *et al*., 2009). Annually, it was authenticated that root knot nematode caused considerable economic loss ranged from 8 to11% (Lucas *et al.*, 1985). Sikora and Fernandez (2005) considered root-knot nematodes, *Meloidogyne* spp., among the top five major plant pathogens of tomato, which limit the quantity and quality of fruit yield. According to Khan *et al* (2008), crop loss due to nematodes is more than $100 billion worldwide. Yield loss in tomato due to root-knot nematode has been estimated to be up to 61.0% (Nimaladevi and Tikoo, 1992). Meanwhile, it ranged from 32 to 40% as reported by Anwar and Mckenry (2012). *M. incognita* (Kofoid and White) Chitwood (Tylenchida: Heteroderidae) is considered as the most dangerous plant-parasitic nematode species affecting the quantity and quality of the crop yield in most countries (Roberts *et al*., 2005 and Sikora *et al*., 2007). Safe and cost effective criteria might be taken into consideration when control strategy of nematode is establishment (Abid *et al*., 2005). The population of nematodes in the field can be reduced through several approaches such as using natural enemies (Khan and Kim, 2007), magnify cultural practices (Okada and Harada, 2007), cultivating resistant cultivars (Williamson and Kumar, 2006), and enforcement nematicides (Browning *et al*., 2006). The diffused use of nematicides in control of nematodes led to environmental and health problems as well as the development of nematode resistance. Accordingly, it has so urgent to find alternative control strategies for nematodes. One of potential alternatives is the utilization of plant extracts; the marine algae are the renewable living resources which are affluent source of structurally important novel and biologically active metabolites (Bhasker and Miyashita, 2005). Remarkably, marine algae contain a wide range of compounds such as agar, acids, carotenes, alkaloids and phenolic compound. Some of these compounds have a pesticidal activity (Fenical, 1982). Antibiotics in algae, such as bromophenols, tannins, phloroglucinol, and terpenoids, have antinematodal action (Mareggiani *et al.,* 1998).

Ulva fasciata Delile is a marine macroalga, It is a source of polyphenolic and diterpenoids compounds which have antibacterial properties and potential applications (Beach *et al.*, 1995; Rouxel *et al*., 2001; Silva *et al.,* 2013 and Chakraborty *et al.,* 2010).

Red seaweeds *Corallina* could be review as a prospective source of bioactive molecules that may be salutary for the development of new pharmaceutical agents such as minerals and saturated fatty acids. Both sulfated galactans and carrageenan isolated displayed anticoagulant and antibacterial activity (Borik, 2014).

Spirulina platensis is a micro alga belonging to *Cyanophyceae* class and comprise proteins, carbohydrates, essential fatty acids, vitamins, minerals especially (calcium and potassium) and pigments (Rosario and Josephine, 2015). It displays a great variety of biologically active compounds that possess antifungal effects (Schlegel *et al.,* 1998), antibiotic and toxic effects against plant pathogens (Kiviranta *et al.,* 1993) and antimalarial, antifeedant and herbicides activity (Iwata *et al.,* 1990; Burja *et al.,* 2001).

Based on this background, the present study was initiated to investigate the nematicidal activity of aqueous and ethanolic extracts of four seaweeds *Ulva fasciata*, *Corallina mediteranea*, *Corallina officinalis* and *Spirulina platensis* against tomato knot-root nematode *M. incognita,* in laboratory and greenhouse.

MATERIALS AND METHODS

Chemicals

Oxamyl (Vydate®24% L) (*N, N*-dimethyl-2-methylcarbamoyloxyimino-2-(methylthio) acetamide) was supplied by Dupont company. All other chemicals were purchased from Bio-diagnostics Co., Dokki, and Giza, Egypt.

Collection of algae

Three marine algae samples, *Ulva fasciata* Delile (UF) (green algae; class: Chlorophyceae), *Corallina mediteranea* and *Corallina officinalis* (red algae; class: Florideophyceae) were collected from national institute of oceanography and marine fisheries in Alexandria, while, the four alga, *Spirulina platensis* (blue-green algae; class: Cyanophyceae) was collected from national research institute in Cairo.

Extraction of ethanolic and water bioactive ingredients:

The dried algae were crushed to a fine powder by electric blender and weighed for extraction. Bioactive algae extracts was prepared by immersing the powder in two different flask of each containing 10 g/L for (water and ethanol) and placed at 35°C with regular shaking for one week until the extraction of active ingredients for further use in anti-nematodial testing. The ethanolic extract was concentrated to dryness under reduced pressure in a rotary evaporator (Unipan vacuum rotary evaporator type 350p, Poland) at 35º C and stored in the refrigerator till been used in bioassay tests., while the aqueous extract homogenized and centrifuged at 10,000 g for 15 min. The supernatant was collected and centrifuged again at 8000 rpm for 15min to obtain a clarified mixture. The pooled extract was filtered using filter paper Whitman no.1. Stock solution of ethanolic and water extracts was prepared and further diluted as per dose requirement.

Nematode

M. incognita (Kofoid and White) Chitwood was isolated from inoculated plants of eggplant (*Solanum melongena* L.) in greenhouse. Root-knot nematodes were identified using perineal patterns of adult females as well as the morphology of second stage juveniles (Hartman and Sasser, 1985; Jepson, 1987). Egg masses of root - knot nematode obtained from a pure culture maintained on eggplant roots were placed on sterilized distilled water of sodium hypochloride (NaOCl) solution (Hussey and Barker, 1973) and incubated for 48 h at room temperature at 25 ± 2C° for hatching. The hatched second stage juveniles (J2) were collected daily. Only freshly hatched J2 collected within 48 h were used for experiments. Second stage juveniles (J2) and eggs of *M. incognita* were used for toxicity evaluations.

Nematicidal activity

Nematicidal activity of eight marine algal extracts was evaluated against second-stage juveniles (J2) of *M. incognita* under laboratory conditions. Four concentrations (125, 250, 500 and 1000 µg/ml) of each extracts were prepared in distilled water. Four replicates of each concentration with about 100 specimens of *M. incognita* juveniles in each replicate were used. The control treatment contains distilled water or ethanol. Oxamyl was used as reference nematicide. The treatments were incubated at 25 ± 2C° and the mortality of nematodes was recorded after 48 h. The LC_{50} values were calculated according to Finney (1971).

Hatching inhibition

Approximately 100 eggs were transferred to the different concentrations of algal extracts in glass vials. Algae extracts were tested at concentrations of 125, 250, 500 and 1000 µg/ml. Each treatment was replicated four times. The glass vials were incubated at room temperature (25 ± 2C°) and the number of hatched juveniles was counted under a stereo microscope. Hatching inhibition percentages was observed after 7 days and IC_{50} values were calculated by probit analysis (Finney, 1971).

Greenhouse experiment

The ethanolic extracts of all algae were applied to evaluate their efficacies on *M. incognita* at the concentration of 1000 µg/ml and oxamyl at recommended rate (3L per fedden). Uniform tomato (*Lycopersicon esculentum Mill* cv. Elisa) transplants of similar age and size, 30-day-old, were singly transplanted on a plastic pot (20 cm diameter and 15 cm depth filled with 3 Kg mixture of 3 sand: 1 peat moss v: v). Transplants were allowed to recover from transplanting shock for 10 days. Agricultural treatments such as irrigation, fertilization, weed control an integrated crop management were carried out whenever are necessary Each pot was inoculated with an initial inoculum level of (5000 eggs/pot) of root-knot nematode in holes of 5-7 cm depth around the plant within the radius of two centimeters. There were four replicates for each treatment including the untreated un-inoculated and inoculated controls. Greenhouse temperature ranged between 25-30°C. After 60 days, plants root systems were gently cut from the stem. The parameters including number of galls and egg masses per root system and final J2 population were recorded. The reduction in the galls, egg masses and nematode population density expressed as a percentage was calculated at the end of the experiment according to Henderson and Tilton's (1955) equation. Roots were stained for 15 minutes in an aqueous solution of Phloxine B stain (0.15 g/l water) (Holbrook *et al.,* 1983), then gently washed in tap water. Plant growth parameters expressed by shoot and root lengths (in centimeter), and fresh weights (in grams), were recorded and calculated as a percentage of increase.

Gas Chromatography-Mass Spectrometry Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed by using Agilent 6890 equipped with an Agilent mass spectrometric detector. A direct capillary interface and fused silica capillary column PAS-5 ms (30 m x 0.32mm x 0.25µm film thickness) was used. About 1 µl of each sample was injected using helium as carrier gas at approximately 1 ml min^{-1}, pulsed splitless mode and the solvent delay time was 3 min. The mass spectrometric detector was operated in electron impact ionization mode at 70 e.v., scanning from 50-500 m/z. The ion source temperature

was 230˚C. The electron multiplier voltage (EM voltage) was maintained 1650v above auto tune. The GC temperature program was started at 60º C for 2 min. then elevated to 300 Cº at rate 5C° min^{-1}, the injector temperature were at 280 Cº, respectively. The m/z (mass/charge) ratio obtained was calibrated from the mass spectrum graph, which is the fingerprint of a molecule. Peak identification of crude *U. fasciata* extract was performed by comparison with retention times of standards and the mass spectrum obtained was compared with those available in the NIST libraries.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 17.00 software (SPSS Inc., Chicago, IL, USA). Duncan's Multiple Range Test (DMRT) was employed to test for significant differences between the treatments (Duncan, 1955), and the LC_{50} values were calculated using probit analysis (Finney, 1971).

RESULTS

Toxicity of marine algae extracts to J2 of *M. incognita*

In the bioassay test, the effects of ethanolic and aquatic marine algae extracts of *U. fasciata*, *C. mediteranea*, *C. officinalis* and *S. platensis* on J2 of *M. incognita* at concentrations of 125, 250, 500 and 1000 µg/ml were evaluated as shown in Table (1). Results clarified that the inhibitory effect of marine algae extracts on nematode activity or mortality was concentration dependent, *i.e.* toxicity of the nematode increased by increasing of marine algae extracts concentration. It was found that each of the marine algae extracts , *U. fasciata*, *C. officinalis and C. mediteranea* ethanolic extracts exhibited lethal effects and killed 50% of nematodes when their LC_{50} value were from 124.19 to 183.34 µg/ml, while water extracts of them and S. *platensis* ethanolic extracts were moderately toxic with LC_{50} of 289.40 to 561.46 µg/ml, respectively.

Effect of tested marine algae extracts on hatching inhibition

The results shown in Table (2) indicated that the tested algae extracts either watery or ethanolic, significantly and drastically, reduced hatching of eggs at the various concentrations from 125 to 1000 µg/ml. It was noticed that the inhibition of hatching was more obvious with all ethanolic extracts of algae compared to the watery ones. At the highest concentration (1000 µg/ml), these compounds caused 90% reduction in hatching. The results, also, revealed that *U. fasciata* was highly toxic with IC_{50} value 195.82 µg/ml. Meanwhile, *C. officinalis* and *C. mediteranea* were moderately toxic with IC_{50} values of 239.08 and 284.94µg/ml, respectively.

Table 1. In vitro toxicity of marine algae extracts against J2 of *M. incognita.*

Treatment	extract	LC_{50}[a] (µg/ml)	95% Confidence Limits (µg/ml)		Slope ± S.E.[b]	χ^{2} [c]	Toxicity Index
			Lower	Upper			
Oxamyl	-	27.29	15.60	44.01	1.39 ± 0.122	12.35	100
U. fasciata	EE	124.19	98.33	147.69	2.56 ± 0.26	0.55	21.97
	WE	289.40	236.66	346.96	1.65 ± 0.20	1.06	9.43
C. officinalis	EE	151.97	122.44	179.14	2.36 ± 0.26	4.65	17.96
	WE	309.86	248.66	379.64	1.46 ± 0.20	0.58	8.81
C. mediteranea	EE	183.34	145.23	219.42	1.90 ± 0.22	2.91	14.88
	WE	449.11	377.35	546.06	1.73 ± 0.20	0.63	6.08
S. platensis	EE	561.46	432.69	809.37	1.14 ± 0.19	0.23	4.86
	WE	992.84	718.42	1737.09	1.19 ± 0.21	0.24	2.75

[a] The concentration causing 50% larval mortality, [b] Slope ± standard error of the concentration–mortality regression line, c Chi square, EE, ethanolic extract: WE, water extract.

Table 2. In vitro effect of marine algae extracts on egg hatching inhibition of *M. incognita*

Treatment	extract	IC_{50}[a] (µg/ml)	95% Confidence Limits (µg/ml)		Slope ± S.E [b]	χ^{2} [c]	Toxicity Index
			Lower	Upper			
Oxamyl	-	37.64	20.37	70.99	1.43 ± 0.13	16.69	100
U. fasciata	EE	195.82	148.21	241.05	1.55 ± 0.20	0.44	19.22
	WE	326.27	261.03	403.29	1.41 ± 0.19	0.09	11.54
C. officinalis	EE	239.08	176.53	301.92	1.27 ± 0.20	0.26	15.74
	WE	552.56	449.12	726.60	1.47 ± 0.20	0.96	6.84
C. mediteranea	EE	284.94	215.85	361.13	1.23 ± 0.19	0.19	13.21
	WE	608.63	492.92	805.78	1.50 ± 0.20	1.22	6.18
S. platensis	EE	599.53	479.02	812.66	1.38 ± 0.20	0.99	6.28
	WE	939.91	730.61	1379.03	1.56 ± 0.22	0.32	4.00

[a] The concentration causing 50% larval mortality, [b] Slope ± standard error of the concentration–mortality regression line, c Chi square, EE, ethanolic extract: WE, water extract.

In vivo nematicidal activity of marine algae ethanolic extracts to *M. incognita* on tomato plants:

In pot experiments under greenhouse, the number of galls and egg masses per root system, and final population as affected by the tested extracts algae are presented in Table (3). Untreated inoculated control (UI control) recorded the maximum number of galls and egg masses per root system, and final population. The nematicidal activity of the four selected extracts, significantly, affected root galls, egg masses and final population of *M. incognita* infecting tomato plants at the application rate of 1000 mg/kg. After two months of a

single application, *M. incognita* produced variable number of galls on roots as affected by the tested extracts. It was noticed that U. *fasciata* extract was the highest effective treatment on root galls reduction as it recorded the lowest number of galls (106) with 58.76% reduction On the other side, the number of galls produced by plants treated with either C. *officinalis* (142) or *C. mediteranea* (160) was not so high enough to be significant . On the other hand,it worthy to mention that all the tested algae extracts were less active than oxamyl (79.67%) in suppressing the number of galls per plant.

Table 3. The efficacy of marine algae ethanolic extract against *M. incognita* galls, egg masses on roots and second stage J2 population in soil of tomato plants.

Treatment	No. Galls	Reduction %	No. Egg Masses	Reduction %	Population J2 in soil	Reduction%
UU control	0.0^{f}	-	0.0^{e}	-	0.0^{e}	-
UI control	258.25^{a}	-	218.75^{a}	-	675.0^{a}	-
U. fasciata	106.5^{d}	58.76	70.75^{d}	67.66	481.5^{cd}	28.67
C. officinalis	142.5^{c}	44.72	106.75^{c}	51.20	517.5^{bc}	23.33
C. mediteranea	160.5^{c}	37.85	124.75^{c}	42.97	535.5^{bc}	20.66
S. platensis	196.5^{b}	23.91	160.75^{b}	26.51	571.5^{b}	15.33
Oxamyl	52.5^{e}	79.67	20.75^{e}	90.51	427.5^{d}	36.67

*** Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test.**
***UU control: untreated un-inoculated control, UI control: untreated inoculated controls**

Results reflected that all the tested algae and oxamyl, significantly, reudced the number of egg masses . U. *fasciata* was the most effective treatment to decrease the number of egg masses with 67.66% reduction followed by *C. officinalis* and *C. mediteranea* achieving 51.20 and 42.97% reduction, respectively.

Application of the tested algae, significantly, reduced the population of J2 in soil. U. *fasciata* was the superior treatment which suppressed the final population of *M. incognita* with a value of 28.67 reduction percent without any significance different from oxamyl However , *S. platensis* exhibited the less performance with a value of 15.3% reduction .

In pot experiments under greenhouse as illustrated in Fig.1 (A,B), *M. incognita* reduced all studied plant growth parameters in the untreated inoculated treatments as compared with the treated or uninoculated plants. Tomato plant biomass was markedly increased due to most of the used treatments. Oxamyl gave the highest increases in the total fresh shoot and root weights and lengths achieving values of 78.77% and 58.94% ,respectively. Beyond oxamyl, U. *fasciata* was the most effective treatment to increase both fresh shoot & root weights and lengths with 63.10% and 47.64% without any significant differences from each other, consecutively. Meanwhile, *C. officinalis* and *C. mediteranea* recorded the intermediate values of fresh shoot and root weights (45.1 and 33.6 % increase) and (30.9 and 23.4 % increase) for lengths. On the other hand, *S. platensis* showed the least activity towards both fresh shoot & root weights and lengths with values of 11.1 and 11 % increase, respectively. herefore, these results indicated that none of the compounds was phytotoxic even at the tested concentration**.**

GC-MS of *U. fasciata* extract:

GC-MS analysis of ethanolic extract of *U. fasciata* exhibited mixture of organic compounds. A total of 15 peaks were illustrated with retention times as shown in Figure 2 and Table 4. Chemical constituents were identified using spectrum data base NIST 11 software installed in GC-MS. The GC-MS analysis of the ethanolic extract showed that the main chemical-constituent was bis(2-ethylhexyl) phthalate (Rt = 40.98 min) (63.75 %) followed by diethyl phthalate (Rt = 21.55 min) (18.46%), which, may be involved in biological activity with other compounds.

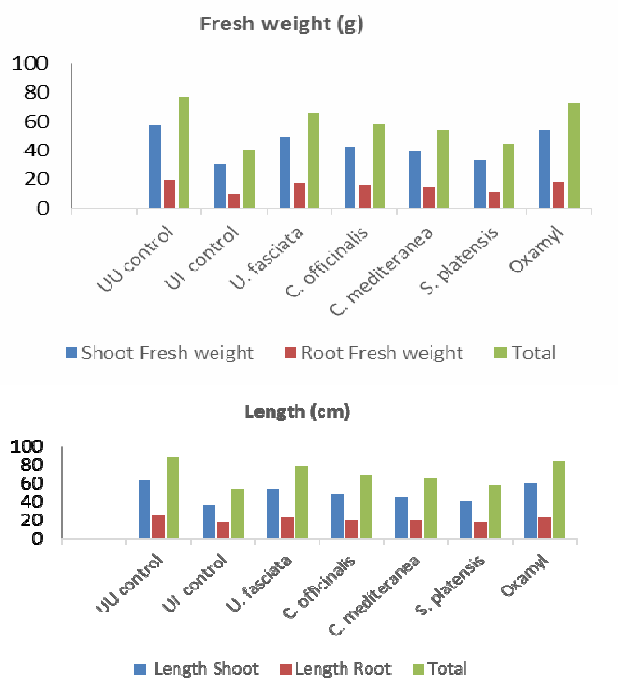

Fig. 1. Effect of marine algae ethanolic extract against *M. incognita* on vegetative growth parameters (A, fresh weight and B, length) of tomato plants grown in the greenhouse.

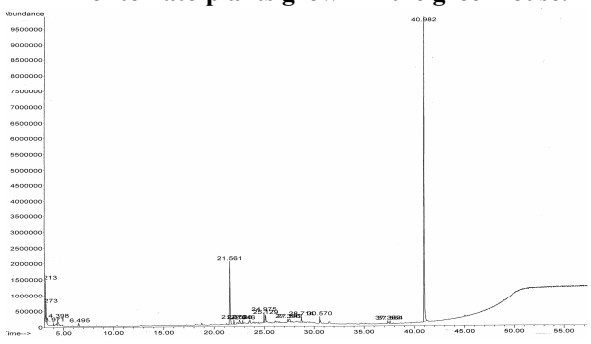

Figure 2. GC/MS chromatogram of *U. fasciata* ethanolic extract

Table 4. Chemical composition of the ethanolic extract of *U. fasciata* analyzed by GC-MS.

Peak No.	RT (min)	Component name	Peak area (%)	Molecular formula	Molecular weight (g/mol)
1	3.215	Dimethoxydimethyl silane	2.36	$C_4H_{12}O_2Si$	120.22
2	3.274	3-Methyl-1-butanol	1.45	$C_5H_{12}O$	88.15
3	3.970	Formamide, N-methoxy	0.39	$C_2H_5NO_2$	75.06
4	4.395	Dimethyl Sulfoxide	2.36	C_2H_6OS	78.12
5	6.491	Butane,1,1- diethoxy-3-methyl	1.05	$C_9H_{20}O_2$	160.25
6	21.559	Diethyl phthalate	18.46	$C_{12}H_{14}O_4$	222.24
7	21.975	Hexadecane	0.97	$C_{16}H_{34}$	226.44
8	24.972	Heptadecane	1.96	$C_{17}H_{36}$	240.47
9	25.133	Pentadecane,2,6,10,14-tetramethyl	2.00	$C_{19}H_{40}$	268.52
10	27.366	Octadecane	0.61	$C_{18}H_{38}$	254.49
11	27.544	Hexadecane, 2,6,10,14-tetramethyl	1.53	$C_{20}H_{42}$	282.54
12	28.715	Phthalic acid isobutyl undecyl ester	1.54	$C_{23}H_{36}O_4$	376.53
13	30.566	Dibutyl phthalate	0.91	$C_{16}H_{22}O_4$	278.34
14	37.365	Cinnamic acid, methyl ester	0.65	$C_{10}H_{10}O_2$	162.18
15	40.982	Bis (2-ethylhexyl) phthalate	63.75	$C_{24}H_{38}O_4$	390.56

DISCUSSION

Tomato (*Solanum lycopersicum* L.) plays a key role in Egypt's horticultural industry and it is among the most important vegetables grown by smallholder farmer in Egypt. According to agricultural statistics bulletin 2013, tomatoes rank the first in relation to total area, productivity per area and total yield comparing with the other vegetable crops (Statistics yearbook, 2013). Root-knot nematodes are important pests of tomato worldwide (Barker and Koenning, 1998; Asif *et al*., 2016). A positive effect of bio potentials of marine algae extracts against root-knot nematode, *M. incognita* was demonstrated as the obtained results indicated that all extracts showed more than 50% nematicidal activity in 1000 µg/ml. The results of this study indicated that ethanolic and aquatic extract of *U. fasciata*, *C. officinalis* and *C. mediteranea* showed nematicidal activity against the root knot nematode *M. incognita*. However, ethanolic extract of *U. fasciata* exhibited the maximum nematicidal activity as compared to other extracts.

Mode of action of these findings could be attributed to that marine algae synthesized a wide range of compounds such as: agar, carrageenan, alginic acids, carotenes, bromine containing acetogenins, alkaloids, and phenolic compound (Fenical, 1982). In this respect, Featonby-Smith and Van Staden (1983) showed that presence of cytokinins in marine algae extracts may be responsible for nematicidal activity. It was found by Mareggiani *et al.,* (1998) that the specific chemical basis for the anti-nematodal activity remains obscure although fractions containing steroids and terpenoid glycosides appear to be toxic *in vitro* to *M. incognita*. Recent results showed that algae possess unique and diversified types of elaborate secondary metabolites containing biocidal agents such as: steroids, triterpenoids, saponins, tannins, alkaloids, and phenols. According to Wu *et al.* (1997), betaines of marine algae extracts can suppress the growth of nematodes. A terpenoid compound in marine algae is known to have nematicidal effects (Abid *et al.,* 1997). In addition, antibiotics in algae, such as bromophenols, tannins, phloroglucinol, and terpenoids, have antinematodal activity (Mareggiani *et al.,* 1998). A correlation of elevated levels of phenolic with resistance or response of plants to nematode infection was previously stated (Ara *et al*., 2002). Furthermore, it was reported that phenolic and lignification were associated with plant resistance to a variety of pests and pathogens (Chitwood, 2002).The early reports confirmed our results which clarified that application of marine algae extracts reduce the nematode infestation in tomato plants. The present results evidenced that ethanolic extract of *U. fasciata*, *C. officinalis* and *C. mediteranea*, significantly, reduced egg masses and galls produced by *M. incognita* nematodes at 1000 mg/kg in greenhouse experiments. Consequently, a clear enhancement of tomato vegetative growth parameters was observed. The inhibitory effects of marine algae extracts on the population of root knot nematode attacks on plants treated were previously reported by Khan *et al. (* 2005) and Rizvi, and Shameel (2006). The present results demonstrated that the marine macro algae; *U. fasciata* was the most efficient biopotentials of marine algae extracts against root-knot nematode, *M. incognita*. GC-MS analysis of ethanolic extract of *U. fasciata* detected many compounds, the major chemical constituents were bis (2-ethylhexyl) phthalate and diethyl phthalate, which, concordant with (Sivakumar *et al*. 2014).

Based on the present study, it can be concluded that the marine algae can be used for the bio-control of root-knot nematodes and this method of control is considered as a cheap environment friendly and it is free from hazards. It is potentially one alternative source to chemical nematicides that are being currently used in nematode control programs. This study possesses the following suggestions: *U. fasciata* which is promising marine algae, locally available can be cultivated in coastal areas; the algae bioactive can be extracted by cost effective method with high yield. Therefore, the marine algae *U. fasciata* may be of great help to prevent the root diseases in tomato plants.

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REFERENCES

Abid, M., Sultana, V., Zaik, M. J., and Maqbool, M. A., (1997). "Nematicidal properties of *Stoechospermum marginatum*, a seaweed," Pakistan Journal of Phytopathology. 9: 143-147.

Abid, M.; Zaki, M.J. Khan, M.Q. and Sattar, A. (2005). Use of marine algae for the management of root-knot nematode (*Meloidogyne javanica*) in okra and tomato plants. Int. J. Phycol. Phycochem., 1: 187-192.

Anwar, S. A. and Mckenry, M.V., (2012). Incidence and population density of plant-parasitic nematodes infecting vegetable crops and associated yield losses. Pakistan J. Zool., 44: 327-333.

Ara, J., Sultana, V., Ehtechamul_Haque, S., Athar, M., and Qasim, R., (2002). Antibacterial activity of marine macro_algae from Karachi coast, *Bull. Polish Acad. Sci.*,50:199-206.

Asif, M.; Khan, A.; Tariq, M. and Siddiqui, M. A.2016. Sustainable management of root knot nematode meloidogyne incognita through organic amendment on *Solanum lycopersicum L*. Asian Journal of Biology. 1(1): 1-8.

Barker, K. R. and Koenning, S. R. (1998). Developing sustainable systems for nematode management. Annual Review Phythopathology 36:165 –205.

Beach, K. S., Smith, C. M., Michael, T. M. and Shin, H. W. (1995). Photosynthesis in reproductive unicells of *Ulva fasciata* and *Enteromorpha flexuosa*: Implications for ecological success. Marine Ecology Progress Series, 125:129-137.

Bhasker, N.and Miyashita, K. (2005). "Lipid composition of *padina tetratomatica* (*Dictyotales,Pheophyta*) a brown sea weed of the west coast of India", Indian Journal of fisheries. 52:263-268.

Bongers, T. and Bongers M. (1998). Functional diversity of nematodes. Applied Soil Ecology. 10:239-251.

Borik. R. M. (2014). Volatile compounds extraction, fractionation and identification from the red alga *Corallina officinalis*. World Applied Sciences Journal 30 (6): 741-746.

Browning, M.; Wallace, D.B.; Dawson, C.; Alm, S.R. and Amador, J.A. (2006). Potential of butyric acid for control of soil-borne fungal pathogens and nematodes affecting strawberries. Soil Biol. Biochem., 38(2): 401- 404.

Burja, A.M., Banaigs, B., Abou-Mansour, E., Burgess, J.G. and Wright, P.C. (2001). Marine cyanobacteria - a prolific source of natural products. Tetrahedron. 57:9347-9377.

Chakraborty, K., Lipton, A.P., Paulraj, R., Vijayan, K.K. (2010). Antibacterial labdane diterpenoids of *Ulva fasciata* Delile from southwestern coast of the Indian Peninsula. Food Chem. 119(4):1399-1408.

Chitwood, D. J. (2002). Phytochemical based strategies for nematode control. Annu. Rev. Phytopathol., 34:201-225.

Duncan, D. (1955). Multiple ranges and multiple F test. Biometrics. 11:1-42.

Featonby-Smith, B. C. and Van Staden, J. (1983). The effect of seaweed concentrate on the growth of tomato plant in nematode infested soil. Scientia Horticulturae. 20: 137-146.

Fenical, W. (1982). Natural products chemistry in the marine environment, Science. 215: 923–928.

Finney, J. C. (1971). Probit analysis, Cambridge University Press, London: 33 P.

Hartman, K.M. and Sasser, J.N., (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: An advanced treatise on *Meloidogyne* (eds. K. R. Barker, C. C. Carter and J. N. Sasser). Vol. 2. Methodology. North Carolina State University Graphics, Raleigh, pp. 69-77.

Henderson, C. F. and Tilton, E. W. (1955). Tests with acaricides against the brown wheat mite. Journal of Economic Entomology, 48: 157-160.

Holbrook, C. C., Knauft, D. A. and Dikson, D. W. (1983). A technique for screening peanut for resistance to *Meloidogyne arenaria*. Plant Disease. 57: 957-958.

Hussey, R. S. and Barker, K. R. (1973). A comparison of methods of collecting inocula of *Meloidogyne spp*. including a new technique. Plant Disease Report. 57:1925-1928.*ISSN 1818-4952*.

Iwata, K, Inayama, T. and Katoh, T. (1990). Effect of *Spirulina platensis* on plasma lipoprotein lipase activity in fructose induced hyperlipidemia in rats. J. Nutr. Sci.Vitaminol. 36:165-171.

Jepson, S.B. (1987)**.** Identification of root-knot nematodes (*Meloidogyne species)*. CAB International, Wallingford, UK.

Khan, Z., Park, S.D., Shin, S.Y., Bae, S.G., Yeon, I.K., and Seo, Y.J., (2005). Management of *Meloidogyne incognita* on tomato by root dip treatment in culture filtrate of the blue_green alga, *Microcoleus vaginatus*, Bioresour.Technol., 96:1338–1341.

Khan, Z. and Kim, Y. H. (2007). A review on the role of predatory soil nematodes in the biological control of plant parasitic nematodes. Appl. Soil Ecol., 35(2): 370-379.

Khan, I.A., Sayed, M., Shaukat, S.S. and Handoo, Z.A. (2008). Efficacy of four plant extracts on nematodes associated with papaya in Sindh, Pakistan. Nematologia Mediterranea.36: 93-98.

Kiviranta, A. Abdel-Hameed, K. Sivonen, S. I. Niemela, and G. Carlberg, (1993). Toxicity of cyanobacteria to mosquito larvaescreening of active compounds. Environmental Toxicology and Water Quality, vol. 8, no. 1, pp. 63–71,.

Lucas, G. B., Lucas. L. T., and Campbell, L. C. (1985). Introduction to plant Diseases: Identification and Management, 2nd Ed., AVI ublishing Company, Inc., pp. 313.

Mareggiani, G., Leicach, S., and Laner, P.(1998). Toxicidad de extractos que contienen metabolitos secundarios de distintos organos de *Melia azedarach* L. alnematodo del nudo de la raiz, *Fitopatologia*. vol. 33, pp. 122–129.

Moens M., Perry R.N., and Starr J.L. (2009). *Meloidogyne* species - a diverse group of novel and important plant parasites. In: Root-knot Nematodes. Perry R.N., Moens M., Starr J.L. (eds.). CABI International, Cambrige, MA, USA: 1-17**.**

Nirmaladevi, S. andTikoo, S.K (1992). Studies of the reaction of certain tomato genotypes and their F1 to combined infection by *Meloidogyne incognita* and *Pseudomonas solanacearum*. Indian J Genetics and PI. Breeding. 52: 118-125

Okada, H. and Harada, H. (2007). Effects of tillage and fertilizer on nematode communities in a Japanese soybean field. Appl. Soil Ecol., 35(3), 582-598.

Rizvi, M.A. and Shameel, M. (2006). *In vitro* nematicidal activities seaweed extracts from Karachi coast, *Pakistan J. Bot.*, 38:1245-1248.

Roberts, P.A., Matthews, J.R. and Ehler, J.D. (2005). Root-knot nematode resistant cowpea cover crops in tomato production systems. Agron. J., 97: 1626-1635.

Rosario, J. C. and Josephine, R. M. (2015). Mineral profile of edible algae *Spirulina platensis*. Int. J.Curr. Microbiol.App.Sci (2015) 4(1): 478-483. ISSN: 2319-7706

Rouxel, C., Bonnabeze, E., Daniel, A., Jerome, M., Etienne, M. and Fleurence, J. (2001). Identification by SDS PAGE of green seaweeds (Ulva and Enteromorpha) used in the food industry. Journal of Applied Phycology, 13:215-218.

Schlegel, N. T. Doan, N. D. and Smith, G. D. (1998). Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. Journal of Applied Phycology. vol. 10(5): 471-479.

Sikora RA. and Fernandez E, (2005). Nematode Parasites of Vegetables. In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Luc M, Sikora RA, Bridge J (Editors) 2nd Edition, CABI Wallingford, UK. pp 319-392.

Sikora, R.A., Schafer, K. and Dababat, A.A. (2007). Modes of action associated with microbially induced in planta suppression of plant-parasitic nematodes. Australasian Plant Pathol.,36:124-134.

Silva, M., Vieira, L., Almeida, A.P. and Kijjoa, A. (2013). The marine macro algae of the genus Ulva: chemistry, biological activities and potential applications. Oceanography 1:101. doi:10.4172/ocn.1000101.

Sivakumar, K. Kannappan, S. Dineshkumar, M. and Patil, P. K. (2014). Evaluation of marine macro alga, Ulva fasciata against bio-luminescent causing Vibrio harveyi during Penaeus monodon larviculture. Afr. J. Microbiol. Res., vol.8 (8): 803- .813.

Statistics yearbook.2013. Annual Bulletin of Crops Areas and Plant Production, Agricultural Statistics, CAPMAS.Ref.No.71\22122.

Williamson, V. M. and Kumar, (2006). A nematode resistance in plants: The battle underground. Trends Genet. 22(7):396-403.

Wu, Y., Jenkins, T., Blunden, G., Whapham, C., and Hankins, S.D., (1997). The role of betaines in alkaline extracts of Ascophyllum nodosum in the reduction of Meloidogyne javanica and M. incognita infestation of tomato plants. Fundamental and Applied Nematology.20: 99-102.

تقييم النشاط النيماتودى لبعض الطحالب البحرية على نيماتودا تعقد الجذور

منى عبد النبى عبد الرسول

قسم وقاية النبات - كلية الزراعه – جامعة دمنهور

اجريت هذه الدراسة لتقييم النشاط النيماتودى لاربعة طحالب بحرية وهى *Ulva fasciata* (طحلب اخضر) وطحالب حمراء مثل *Corallina mediterane* و *Corallina officinalis* وطحلب اخضر مزرق مثل *Spirulina platensis* على نيماتودا تعقد الجذور وتم ذلك معمليا وداخل الصوبة على نبات الطماطم (صنف اليسا). اوضحت التجارب المعملية، ان كل الطحالب البحرية المختبرة تسببت فى موت الطور اليرقى الثانى لنيماتودا تعقد الجذور (J2). كما اظهرت النتائج انخفاض معنوى واضح فى نسب فقس البيض. و لقد أظهرت ايضا نتائج التجارب المعملية ان المستخلص الايثانولى لكل الطحالب قد اظهر تاثير نيماتودى قوى مقارنة بالمستخلصات المائية. ولقد تم اختبار المستخلص الايثانولى لكل الطحالب داخل الصوبة واظهرت نتائج التجربة حدوث انخفاض معنوى كبير في أعداد العقد الجذرية ، وكتل البيض وتعداد النيماتودا النهائى فى التربة لنبات الطماطم باستخدام تركيز 1000 ملجم / كجم تربة مقارنة بالكنترول المعامل. ولوحظ ان المعاملة بالمستخلص الايثانولى للطحلب الاخضر *U. fasciata* قد تسببت في أكبر انخفاض معنوى في أعداد العقد الجذرية و كتل البيض وتعداد النيماتودا النهائى فى التربة مقارنة بأي طحلب آخر باستثناء المبيد الكارباماتى الاوكساميل . وتشير النتائج أن كل من المعاملات التي تم اختبارها قد احدثت زيادة معنوية بدرجات مختلفة فى قياسات نمو النبات مع تقليل الإصابة بينماتودا تعقد الجذور. وقد اظهرت النتائج انه من بين كل الطحالب المختبرة كان *U. fasciata* ويلية *C. mediteranea* و *C. officinalis* اكثر المعاملات فعالية للزيادة فى اوزان واطوال المجموع الجذرى والخضرى. هذا و لم تظهر أي من المركبات التى تم اختبارها اى سمية نباتية. وبدراسة التركيب الكيماوى للطحلب الاخضر *U. fasciata* بواسطة جهاز GC-MS وجد عديد من المركبات كان اكثرهم bis(2-ethylhexyl) phthalate بنسبة 63.75 % ويلية مركب diethyl phthalate بنسبة 18.46%. وبناءا على النتائج المتحصل عليها وجد أن الطحالب البحرية ذات نشاط نيماتودى قوى في كلا من التجارب المعملية وتجارب الصوبة ويمكن الاستفادة منها كمركبات نيماتودية طبيعية.

كلمات البحث: الطحالب البحرية ، النشاط النيماتودى ، نيماتودا تعقد الجذور.