Nematicidal properties of some algal aqueous extracts against root-knot nematode, *Meloidogyne incognita in vitro*

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Abstract

The effectiveness of aqueous extracts derived from nine algal species at different concentrations on egg hatching and mortality of *Meloidogyne incognita* (Kofoid and White) Chitwood juveniles after various exposure times were determined *in vitro*. Results indicated that *Enteromorpha flexuosa* at the concentration of 80% was the best treatment for suppressing the egg hatching with value of 2 % after 5 days of exposure, followed by *Dilsea carnosa* extract (3%) and *Codium fragile* (4%) at the same concentration and exposure time. Likewise, application of *C. fragile*, *D. carnosa* , *E. flexuosa* and *Cystoseira myrica* extracts at the concentrations of 80 and 60% were highly toxic to the nematodes, killing more than 90 % of nematode larva after 72 hours of exposure while the others gave quite low mortalities. The characteristic appearances in shape of the nematodes killed by *C. fragile*, *D. carnosa* , *C. myrica*, *E. flexuosa* and *Sargassum muticum* was sigmoid (∑-shape) with some curved shape; whereas, the nematodes killed by other algal species mostly followed straight or bent shapes. The present study proved that four species of algae *C. fragile*, *D. carnosa*, *C. myrica* and *E. flexuosa* could be used for the bio-control of root-knot nematodes.

Keywords: Algae, *In vitro*, *Meloidogyne incognita*, Nematicidal activity

Introduction

Plant parasitic nematodes caused significant damage and losses to most agricultural crops in the tropical and sub-tropics **(Luc *et al*., 2005).** The root-knot nematodes, *Meloidogyne* Göldi (Rhabditida: Meloidogynidae) are the most economically important plant parasitic nematodes group that cause serious damage to most agricultural crops worldwide **(Sasser *et al*., 1983).** *Meloidogyne incognita* is the most common species of root-knot nematodes and infects almost all cultivated plants, which makes it perhaps the most damaging of pathogens **(Sasser and Freckman, 1987).**

For several decades, the use of chemical nematicides is one of the primary means of control for root-knot nematodes. Nowadays, chemical nematicides are

loosing their popularity among farmers for protecting their crops from nematode infestations because of their harmful effects and environmental pollution that led to an urgent need for safe and more effective options **(Zuckerman and Esnard, 1994)**. Biological control promises to be such an option. The recently one of the biological control practices attempted is the study of suppression effects of cyanobacteria (blue–green algae) on plant-parasitic nematodes. Cyanobacteria are little explored even today, so it may possess the novel metabolites which may not screen yet. Microalgal metabolites have attracted attention, because they are a resource for toxins, and potential new drugs **(Shimizu, 2003).** A large number of microalgal extracts and/or extracellular products exhibit antimicrobial activity. Methanolic extract of *Nostoc* was antifungal, herbicidal, nematicidal, and insecticidal **(Kumar, 2014).** Culture filtrates of the *Microcoleus vaginatus* (Vaucher) inhibited hatching of *M. incognita* eggs and killed second stage juveniles **(Khan *et al*., 1997).**

The use of marine algae as control agent against plant-parasitic nematodes has been studied by many workers **(Paracer *et al*., 1987).** Different seaweeds exhibited very significant nematicidal activities **(Zaki *et al*., 2005). Wu *et al*. (1997)** reported the role of betaines in alkaline extracts of the marine brown algae *Ascophyllum nodosum* (Linnaeus) Le Jolis in suppression of the fecundity of tomato-knot nematodes *Meloidogyne javanica* and *M. incognita.* In Pakistan, **Rizvi and Shameel (2006)** collected twenty two species of seaweeds and tested the nematicidal activity of their methanolic extracts against the larvae of *M. javanica*. They recorded that *Stoechospermum polypodioides* appeared to be the most active seaweed as it caused 80 % mortality of the nematode larvae after 72 h exposure to its extract. **Ibrahim *et al*. (2007)** stated that treating sunflower plants with marine algae, *Botryocladia cabillaceae* caused reduction in root galls and egg masses of *M. incognita* as well as increased growth parameters. Certain marine algae from different localities have been reported to exhibit mosquito larvicidal, nematicidal, cytotoxic, antifouling activities **(Manilal *et al*., 2011). Nour El-Deen *et al*. (2013)** reported that application of seaweed extract of the brown algae, *A. nodosum* with cabbage leaves plus arabic gum gave the best results in terms of reducing galls and egg-masses of *M. incognita* as well as increased basil plant fresh weight to the highest percentage (85.8%). **Khan *et al*. (2015)** evaluated *in vitro* the nematicidal activity of 32 seaweeds on *M. javanica* egg hatching and larval mortality and recorded that *Sargasssum tenerrimum*, *Padina tetrastromatica* and *Melanothamnus afaqhusainii* gave maximum egg hatching (96%) and larval mortality (99%) and (100%), in water and methanol extract at 10% concentration after 72h exposure time respectively. Moreover, the use of seaweeds is positive sign against harmful microorganisms which are responsible for considerable losses in agriculture yield. Studies on the nematicidal activity of algae still unexplored except for a few reports. Therefore, the aim of this research paper was to evaluate the nematicidal potential of nine algal species on *Meloidogyne incognita* egg hatching and (J_2s) mortality *in vitro*.

Materials and Methods

In vitro study was carried out in order to determine the effectiveness of aqueous extracts derived from nine algal species isolated from Jeddah and Taif governorates, Saudi Arabia on egg hatching and mortality of *M. incognita* juveniles.

Nematode source and inocula:

The root-knot nematode *M. incognita* eggs were extracted from infected coleus (*Coleus blumei*) roots using 0.5 % NaOCl solution and shaking for 2 minutes **(Hussey and Barker, 1973),** while second-stage juveniles (J_2) were extracted from infected roots by hatching. These eggs and J_2s were obtained from a pure culture established from single egg masses of *M. incognita* that previously identified according to the characteristics of its perineal pattern **(Taylor and Sasser, 1978)** and reared on tomato plants grown in a greenhouse of Biology Department, Faculty of Science, Taif University, KSA.

Algal extracts preparation:

Nine species of algae were isolated from red sea (Jeddah) and soil in Taif (KSA) was tested for their nematicidal activity. From which, seven marine algae were *Enteromorpha flexuosa* (Wulfen) J. Agardh*, Ulva lactuca* (Linnaeus) and *Codium fragile* (Suringar) Hariot for green algae, *Cystoseira myrica* (S.G. Gmelin) C. Agardh and *Sargassum muticum* (Yendo) Fensholt for brown algae and *Dilsea carnosa* (Schimidel) O. Kuntze and *Laurencia nidifica* J. Agardh for red algae, two species were isolated from soil namely*, Scenedesmus obliquus* (Turpin) Kützing (green) and *Cyanosarcina fontana* Kovácik (blue-green algae). Macroalgae were classified into functional groups according to **Rubal *et al*., (2011)** and taxonomic classification followed Algaebase **(Guiry and Guiry, 2013).** Each species of marine ones was field collected and transported to the laboratory where it was freeze-dried. Aqueous extracts of seven algal species were handpicked and washed thoroughly with seawater to remove debris, sand particles and epiphytes. It was kept in an icebox containing slush ice, transported to the laboratory and washed thoroughly with tap water to get rid of the salts from the surface of the samples. The water was drained off and the algal material was spread on blotting paper to remove excess water. After completely drying, the different seaweed materials were ground to a fine powder using Electrical blender. Forty grams of powdered seaweeds were extracted successively with 200 ml of dist. water in Shaker for 3 h. The extracts were filtered and stored in a refrigerator for future use. On the other hand, *S. obliquus* and *C. fontana* were cultivated in synthetic medium for 7 days and harvested by centrifugations, then the algal belts (1g /100 ml) was extracted in dist. water as mentioned above **(Issa *et al*., 2015).**

Nematicidal activity test:

Egg hatching

Twenty seven wells of 24-well tissue culture plates were filled with one milliliter of one hundred of nematode eggs and extracts of different algal species at concentrations of 80, 60, 40, 20, 10 and 5 %, each of which was replicated three times. Three wells, which received only distilled water, were used as control. The number of malforming eggs and (died or alive) hatched juveniles were recorded using Hawksely counting slide after 5, 10 and 15 days.

Juveniles mortality

Approximately thirty freshly hatched second stage juveniles of the root-knot nematode, *M. incognita* in one ml distilled water was poured into wells of 24-well tissue culture plates over one ml of the tested concentrations. The plates were then covered with the lid and kept in an incubator at 25ºC. A 2ml of distilled water containing nematode larvae served as control. Each treatment was replicated three times. Dead nematodes were counted and recorded after 24, 48 and 72h using Hawksely counting slide. Mobility was confirmed by touching the nematode with a fine needle. Nematodes that appeared no realistic movement were considered as dead. Percentages of the nematode mortality were then calculated and recorded for each concentration. Mortality percentages were transformed to arcsin **(Bliss, 1937)** values just before statistical analysis. Characteristic shapes of the dead nematodes were studied under the microscope.

Statistical analysis:

Statistically, the obtained data were subjected to analysis of variance (ANOVA) as a factorial in complete block design **(Gomez and Gomez, 1984)** followed by Duncan's multiple range test to compare means **(Duncan, 1955).**

Results

The aqueous extracts of nine algal species and concentrations (80, 60, 40, 20, 10 and 5%) in comparison distilled water on egg hatching after 5, 10 and 15 days exposure time as well as mortality percentage of newly hatched juveniles of *M. incognita* after 24, 48 and 72 h exposure period are shown in Tables (1) and (2), respectively. In general, egg hatching significantly decreased with increase in algal extract concentrations tested after all days of exposure duration, whereas, larval mortality percentages increased with increase in algal extract concentrations tested after the three of exposure durations tested.

Egg hatching:

Data in Table (1) revealed that among treatments of the aqueous extracts of algae affect on *M. incognita* egg hatching, *E. flexuosa, D. carnosa* and *C. fragile*

applications significantly gave the lowest values that were amounted to 15.9, 20.6 and 22.7%, respectively. On the other hand, the highest percentage of egg hatching was observed by aqueous extract of *C. fontana* with value averaged 66.2% as compared with control and all other treatments. Moreover, 55.4 and 45.5% of eggs were hatched in *S. obliquus* and *C. myrica* extracts, respectively. Treatments of *S. muticum, U. lactuca* and *L. nidifica* in aqueous extracts gave a considerable egg hatching percentages with values of 40.7, 38.3 and 32.6%, respectively.

Table (1): Effect of aqueous extract derived from nine species of algae on *Meloidogyne incognita* egg hatching *in vitro*.

Treatments [a]	Time (Days)	Hatchability % [b] Concentration (%)						Tr. Mean
		80	60	40	20	10	5	
Enteromorpha flexuosa	5	2 xy	7 v	16 s-u	19 st	21 r	24 q	15.9 j
	10	3 x	8 vw	17 s-u	20 r-t	22 rs	26 p-r	
	15	3 x	9.7 uv	18 s	21 r	22 rs	27 p	
Laurencia nidifica	5	20.7 r	25 qr	28 pq	35 n-p	38.7 m	39 m	32.6 g
	10	22.7 q-s	26 p-r	30 o	37 no	40 m-o	41 m-o	
	15	23.7 q	27 p	31 o-q	38 m	42 mn	42.7 mn	
Sargassum muticum	5	33 op	35 n-p	37 no	39.7 m-o	40 m-o	48 k	40.7 e
	10	35 n-p	36.3 no	38 m	40.7 m-o	44 l	49 k-m	
	15	37 no	36.3 no	39 m	42 mn	46.3 lm	51 j	
Ulva lactuca	5	50 kl	47 lm	44 l	40 m-o	23.3 q	16.7 s-u	38.3 f
	10	51 j	48 k	45.7 l-n	41 m-o	26 p-r	19 st	
	15	53 i	50 kl	46.3 lm	41 m-o	27 p	20.7 r	
Scenedesmus obliquus	5	66 h-j	62 h	54 i-k	53 i	46.7 lm	43 mn	55.4 c
	10	68 g	64 hi	55 ij	54 i-k	48 k	45 l-n	
	15	69 g	64 hi	55 ij	55 ij	50 kl	45 l-n	
Cyanosarcina fontana	5	86 e	73 f	70 gh	62 h	50 kl	45 l-n	66.2 b
	10	89 d	74.3 fg	71 gh	64.7 hi	52 jk	48 k	
	15	90.7 cd	76.3 f-h	72 g-i	66 h-j	53 i	49 k-m	
Dilsea carnosa	5	3 x	7 v	20.7 r	25 qr	28 pq	30 o	20.6 i
	10	4.3 wx	11 u	22 rs	27 p	30 o	32 o-q	
	15	5 w-y	11 u	24 q	28 pq	30 o	33 op	
Codium fragile	5	4 wx	9 u-w	24 q	28 pq	28 pq	30.7 o-q	22.7 h
	10	5.3 w	10 uv	27 p	28 pq	30 o	34 n	
	15	7 v	12 t	30 o	31.3 o-q	33 op	38 m	
Cystoseira myrica	5	5 w-y	12 t	46.3 lm	50 kl	68 g	79 e	45.4 d
	10	7.7 vw	13 t-v	48 k	52 jk	72 g-i	81.7 ef	
	15	10 uv	14 tu	48.3 k-m	54 i-k	74 fg	83 e-g	
N alone (Ck) [c]	5				92.7 c			96.3 a
	10				96.3 b			
	15				100 a			
Conc. Mean		38.1 e	38.6 d	44.9 c	46 b	46 b	47 a	

[a] Each figure represents the mean of three replicates.

[b] Means in each column followed by the same letter did not differ at P< 0.05 according to Duncan's multiple range tests.

[c] N= 100 *M. incognita* eggs.

The interaction between the three factors tested showed that *E. flexuosa* at the concentration of 80 % was the best treatment for suppressing the egg hatching with value of 2 % after 5 days of exposure, followed by *D. carnosa* extract (3%) and *C. fragile* (4%) at the same concentration and exposure time. The highest percentage of egg hatching (100%) was obtained from distilled water (control)

treatment after 15 days, followed by *C. fontana* extract at 80 % (90.7%), then *C. myrica* extract at 5% (83%).

Juveniles mortality:

Data presented in Table (2) showed the efficacy of algal species at six concentrations on *M. incognita* mortality percentages. Likewise, a similar trend was observed concerning larval mortality percentages that were increased as the concentrations and exposure durations increased. Aqueous extract of *C. fragile* gave the highest percentage of *M. incognita* mortality with value of 86.7%, followed by *D. carnosa* (82.9%), then *C. myrica* (73.3%); whereas, *S. obliquus* gave the least value of larval mortality (1.7%). Moderate mortality percentage values of J_2 were obtained when exposed to *C. fontana*, *U. lactuca*, *L. nidifica* and *S. muticum* that averaged 42.4, 35.7, 31.5 and 29.3 %, respectively.

Table (2): Effect of aqueous extract derived from nine species of algae on *Meloidogyne incognita* second stage juvenile (J_2) mortality *in vitro*.

Treatments [a]	Time (Days)	Mortality % [b] Concentration (%)						Tr. Mean
		80	60	40	20	10	5	
Enteromorpha flexuosa	24	95.7 b	66.7 i-k	60 k-m	0.0 y	0.0 y	0.0 y	59.5 d
	48	100 a	100 a	81 f	41 op	37.7 pq	31 q-s	
	72	100 a	100 a	83.3 ef	67.7 ij	59 l	47.7 n	
Laurencia nidifica	24	14.3 uv	3.3 w-y	2 x	0.0 y	0.0 y	0.0 y	31.5 g
	48	72.3 hi	52.3 m	40 o-q	35.7 q	27.7 r-t	29 r	
	72	81 f	64.3 jk	50 mn	37.7 pq	29 r	29 r	
Sargassum muticum	24	4.3 wx	2 x	1 xy	0.0 y	0.0 y	0.0 y	29.3 h
	48	61 kl	44.3 o	41 op	35.7 q	31 q-s	26 st	
	72	72.3 hi	53.3 l-n	50 mn	40 o-q	37.7 pq	27.7 r-t	
Ulva lactuca	24	9 vw	6.7 v-x	5.7 w	1 xy	0.0 y	0.0 y	35.7 f
	48	69 i	61 kl	54.3 lm	45.7 no	35.7 q	27.7 r-t	
	72	72.3 hi	62.3 k	60 k-m	53.3 l-n	45.7 no	33.3 qr	
Scenedesmus obliquus	24	0.0 y	0.0 y	0.0 y	0.0 y	0.0 y	0.0 y	1.7 i
	48	4.3 wx	3.3 w-y	0.0 y	0.0 y	0.0 y	0.0 y	
	72	12 u-w	10 v	0.0 y	0.0 y	0.0 y	0.0 y	
Cyanosarcina fontana	24	69 i	40 o-q	36.7 p-r	31 q-s	22.3 t	20 t-v	42.4 e
	48	76.7 gh	53.3 l-n	46.7 n-p	39 p	26.7 s	21 tu	
	72	80 fg	60 k-m	54.3 lm	39 p	26.7 s	21 tu	
Dilsea carnosa	24	95.7 b	86.7 d-f	84.3 ef	79 g	66.7 i-k	62.3 k	82.9 b
	48	100 a	93.3 bc	87.7 de	81 f	74.3 h	65.7 j	
	72	100 a	100 a	93.3 bc	82.3 e-g	74.3 h	65.7 j	
Codium fragile	24	95.7 b	93.3 bc	82.3 e-g	75.7 g-i	75.7 g-i	69 i	86.7 a
	48	100 a	95.7 b	87.7 de	84.3 ef	83.3 ef	79 g	
	72	100 a	100 a	91 cd	85.7 e	83.3 ef	81 f	
Cystoseira myrica	24	89 d	80 fg	74.3 h	62.3 k	47.7 n	41 op	73.3 c
	48	92.3 b-d	87.7 de	80 fg	66.7 i-k	60 k-m	59 l	
	72	96.7 ab	93.3 bc	84.3 ef	74.3 h	67.7 ij	62.3 k	
N alone (Ck) [c]	24			0.0 y				0.0 j
	48			0.0 y				
	72			0.0 y				
Conc. Mean		62.0 a	53.7 b	47.7 c	38.6 d	33.7 e	27.1 f	

[a] Each figure represents the mean of three replicates.

[b] Means in each column followed by the same letter did not differ at P< 0.05 according to Duncan's multiple range tests.

[c] N= 30 *M. incognita* J_2s.

Among interaction between the three factors tested, *C. fragile* and *D. carnosa* treatments at the concentrations of 80 and 60% gave 100% mortality after 72 h from exposure, whereas, *C. myrica* with the same concentrations gave 96.7 and 93.3%, respectively after the same exposure period. Moreover, these algal species with the lowest concentration (5%) killed 81, 65.7 and 62.3 % for *C. fragile*, *D. carnosa* and *C. myrica*, respectively after the same exposure time. The LC_{50} value was 40 % for *L. nidifica* and *S. muticum* extracts after 72 h of exposure.

Characteristic shapes of dead nematodes were shown in (Fig. 1). It is clear that they had either one of four very distinct shapes i.e. straight (I shape), bent (banana-shape), sigmoid (∑-shape) and curved (c-shape) after 72 h exposure to the highest concentration of the algal extract. The dead nematodes from *U. lactuca* or *S. obliquus* or *L. nidifica* or *C. fontana* groups mostly was straight (I shape) with only very few showing a bent (banana) shape. The appearance of nematodes killed by *C. fragile*, *D. carnosa*, *C. myrica*, *E. flexuosa* and *S. muticum* was sigmoid (∑-shape) with some curved shape.

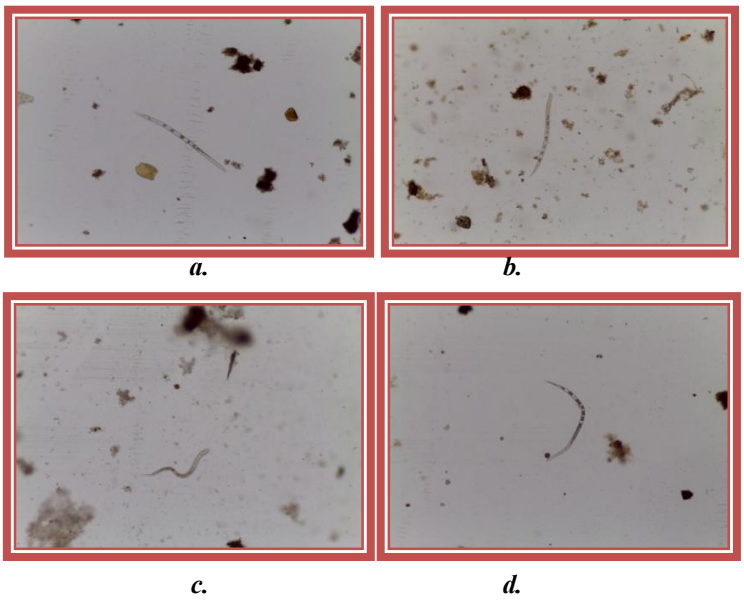

Figure (1): Characteristic shapes of dead nematodes: a. straight (I shape), b. bent (banana-shape), c. sigmoid (∑-shape), and d. curved (c-shape) after exposure to the highest concentration of the algal extract.

Discussion

Using bioagents against root-knot nematodes is a desire trend for existing a nematode management program. Of these materials, there are algae. Utilization of such natural compounds has earned much more benefits in excreting nematode **(Rizvi and Shameel, 2006; Manilal *et al*., 2011; Nour El-Deen *et al*., 2013; Kumar, 2014; Khan *et al*., 2015).**

Apparently, results from the present *in vitro* experiment indicated that tested algal species, concentrations and exposure periods directly affect hatchability of nematode eggs as well as mortality of newly hatched second stage juveniles. It is evident that the aqueous extracts of *E. flexuosa*, *D. carnosa* and *C. fragile* algae reduced the egg hatching of *M. incognita*, whereas, *C. fontana* and *S. obliquus* increased the number of hatched eggs. However, *S. muticum, U. lactuca*, *C. myrica* and *L. nidifica* extracts showed moderate nematicidal activity on egg hatching. These findings disagreed with **Khan *et al*. (2015)** in respect to *Saragassum, Ulva* and *Laurencia* algae which gave the lowest values for egg hatching and larval mortality percentages, whereas, agreed with him in respect to *Codium*. In the present work, it was clear that egg hatching decreased with increase in extract concentrations of *E. flexuosa*, *D. carnosa*, *C. fragile*, *C. myrica*, *S. muticum* and *L. nidifica*; however, increasing the concentration of *C. fontana*, *S. obliquus* and *U. lactuca* extracts increased egg hatching percentages. These results indicated that the first algal group may contain secondary metabolites inhibit egg hatching of *M. incognita*, whereas, the second group seems to be has stimulating constituents for hatching the eggs. Our results supported the findings of **Manilal *et al*. (2012)** who recorded that the bioactivity exhibited by *Lobophora variegate* might be due to the synergistic activity of seven fatty acids.

Regarding larval mortality, results of the present investigation showed that *E. flexuosa* algae gave a considerable percentage of larval mortality although it was the best treatment in suppressing egg hatching. All concentrations of *C. fragile, D. carnosa* and *C. myrica* algae were highly toxic to J_2s of *M. incognita* after 72 h exposure; however, less than 50% of larvae were killed by low concentration of *E. flexuosa*. *S. obliquus* extract seems to be non toxic to nematodes, since most of concentrations tested not able to kill larvae. These results are in accordance with **Massa (2010)** who showed that the seaweed extract has no toxic effect on the J_2 upon exposure to the product for longer period and this confirms an earlier report by **Wu *et al*. (1998)** in this respect**.** Nematode killing mechanism may be attributed to a direct effect of cyanobacterial neurotoxins. However**, Wu *et al*., (1997)** recorded that betaines of the brown alga *Ascophyllum nodosum* caused a reduction of *M. javanica* and *M. incognita* infection on tomato.

The observed characteristic differences in shape of the nematodes killed by aqueous algal extracts was an interesting finding that might be useful as an indication to analyze the major mode of toxic action of these natural biocide. The present finding showed that the nematodes killed by *C. fragile, D. carnosa*, *C. myrica*, *E. flexuosa* and *S. muticum* was sigmoid (∑-shape) with some curved shape, which was similar to those killed by the acetylcholine esterase inhibitors; whereas, the appearances of the nematodes killed by other algal species mostly followed straight or bent shapes, similar to those killed by the pyrethroid. This finding is in line with the report of **Wiratno *et al*. (2009)** who mentioned that the

shapes of the dead nematodes differed in a characteristic way, and groups of pesticides and plant extracts could clearly be distinguished based on this phenomenon. Based on the shapes of the dead nematodes, it is suggested that most of the algal extracts tested had a pyrethroid-like effect on the central nervous system of the nematodes.

Obviously, results of this investigation indicated that the nine algal extracts tested could be divided into 4 main groups based on their toxicity i.e. highly toxic (>70% mortality), consisting of *C. fragile* and *D. carnosa*; moderately toxic (41-70% mortality), consisting of *C. myrica*, *E. flexuosa* and *C. fontana*; slightly toxic (10-40 % mortality) consisting of *U. lactuca*, *L. nidifica* and *S. muticum* and not toxic (<10% mortality) consisting of *S. obliquus* extract. Finally, this initial study was conducted to isolate and identify nine algal species from Jeddah and Taif those offer nematicidal proprieties against root-knot nematode, which have not been the subject of previous studies in Saudi Arabia. So our results offer reliable base for promising nematode control method, further investigations are necessary, especially under greenhouse and field conditions.

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الملخص العربي

الخصائص الإبادية للمستخلص المائي لبعض الطحالب ضد نيماتودا تعقد الجذور (ميلويدوجين انكوجنيتا) في المعمل

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تم دراسة تأثير المستخلص المائي لتسعة أنواع من الطحالب بتركيزات مختلفة على فقس البيض وموت يرقات نيماتودا تعقد الجذور (ميلويدوجين انكوجنيتا) بعد فترات تعرض مختلفة معمليًا. أوضحت النتائج أن المستخلص المائي للطحلب ***Enteromorpha flexuosa*** بتركيز ٨٠% كان الأفضل في منع فقس البيض حيث كانت نسبة الفقس ٢% فقط بعد ٥ أيام من التعرض، يليه مستخلص ***Dilsea carnosa*** (٣%) ثم ***Codium fragile*** (٤%) بعد نفس فترة التعرض. كانت المستخلصات المائية لكل من ***C. fragile***، ***D. carnosa***، ***E. flexuosa*** و***Cystoseira myrica*** بتركيزي ٨٠ و٦٠% عالية السمية للنيماتودا حيث قتلت أكثر من ٩٠% من اليرقات بعد مدة تعرض ٧٢ ساعة، بينما أعطت الأنواع الأخرى نسب موت منخفضة. أظهرت النتائج أيضًا أن اليرقات الميتة أخذت شكل الزجزاج عند التعرض لمستخلصات كل من ***C. fragile***، ***D. carnosa***، ***C. myrica***، ***E. flexuosa*** و***Sargassum muticum*** مع ظهور بعض الأفراد الملتوية، بينما اليرقات الميتة نتيجة التعرض لباقي الطحالب أخذت شكلاً مستقيمًا أو قليل الالتواء. من هذه الدراسة نستنتج أنه يمكن استخدام أربعة أنواع من هذه الطحالب هي ***C. fragile***، ***D. carnosa***، ***C. myrica*** و***E. flexuosa*** في مكافحة نيماتودا تعقد الجذور.