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Nematicidal Properties of Methanolic Extracts of Two Marine Algae against Tomato Root-Knot Nematode, *Meloidogyne incognita*

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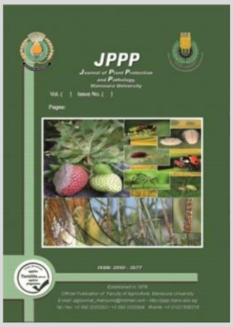

ABSTRACT

Red algae *Phacelocarpus tristichus* J. Agardh and brown algae, *Turbinaria ornata* J. Agardh at three concentrations (0.5, 1.0, and 2.0%) were evaluated for the management the infection of *Meloidogyne incognita* for tomato plants in controled conditions.The methanolic extracts of the two species improved growth parameters of the infected and uninfected plants. Both *P. tristichus* and *T. ornata* methanolic extracts pronounced nematicidal activities against *M. incognita* infecting tomato. Accordingly, *P. tristichus* (2.0%) shows significant suppression for root galling, the number of developmental stages, females, egg masses, eggs/ egg mass and total nematode population. However, *T. ornata* at different concentrations surpassed *P. tristichus* and significantly induced chemical constitutions in terms of NPK, crude proteins and carbohydrates in dried leaves of infected tomato with *M. incognita*. It could be concluded that, *P. tristichus* and *T. ornata* were recommended as biofertilizers and a promising tool in the integrated management of *M. incognita* infecting tomato.

Keywords: brown algae, management, *Meloidogyne incognita*, *Phacelocarpus tristichus*, tomato

INTRODUCTION

Root-knot nematodes are important group of plant-parasitic nematodes which have economic imporatnce worldwide, that pose a serious threat to global food security (Bernard *et al*., 2017). Root-knot nematodes control presents a major global challenge (Shukla *et. al.*, 2017). The top 10 list emerging from the survey is composed of: (1) root-knot nematodes (*Meloidogyne* spp.); (2) cyst nematodes (*Heterodera* and *Globodera* spp.); (3) root lesion nematodes (*Pratylenchus* spp.); (4) the burrowing nematode *Radopholus similis*; (5) *Ditylenchus dipsaci*; (6) the pine wilt nematode *Bur-saphelenchus xylophilus*; (7) the reniform nematode *Rotylenchulus reniformis*; (8) *Xiphinema index* (the only virus vector nematode to make the list); (9) *Nacobbus aberrans*; and (10) *Aphelenchoides besseyi* (Jones *et al*., 2013). It well known that tomato is an important vegetable crop globaly and this plant infected with the *Meloidogyne* spp. which causes disaster in the crop yield. Application of pesticides reulsted in soil and environmental pollution,so, the use of eco-friendly products is more considerable for clean and healthy environment.

Recently, the nematicidal properties of algae have been demonstrated by many researchers (Khan *et al*., 2016; Nour El-Deen and Issa 2016; El-Eslamboly *et al*., 2019; Ghareeb *et al*., 2019). Phlorotannins, are phenolic compounds are intracellular component inside the algal cells; recently Phlorotannins have been used as bioactive materials for different themes. Seaweeds (brown algae) serve as an important source of bioactive natural substances (Rajkumar and Bhavan, 2017). Several studies have measured the total phenolic compounds in extracts from various species, but the little characterization of individual phlorotannin components has been demonstrated (Cotas *et al*., 2020). It was observed that; Alkaloids, amino acids, steroids, flavonoids, tannins, phenols and phenolics are existed in the algal methanol extract. While, phenol, glycoside, protein, terpenoids and flavonoids are obtainable in the aqueous extract (Janakiraman and Janakiraman, 2019). Few studies were undertaken to evaluate the potential role of both brown and red algae as nematicides (Khan *et al*., 2016; Ghareeb *et al*., 2019). Consequently, this investigation has been performed to evaluate the nematicidal activity of the red algae *P. tristichus* and brown algae, *T. ornata* against *M. incognita* infecting tomato as a new report for their efficacy.

MATERIALS AND METHODS

Morphological of the selected algae

The studied algal species, *P. tristichus* and *T. ornata* found in the inter-tidal region of Red Sea shores between Quseir and Marsa-Alam, Egypt are rarely grown free-floating, but instead are fixed firmly at their bases and remain stationary through their life. On surfied areas, it was observed that the algae are basically sticky to rocky places, which protect the algae a resistance to wave shock. Algal species *P. tristichus* and *T. ornata* were identified according to (Bouck, 1965; Bold , 1978).

Preparation and Extraction of selected algal species

Algae were collected from seashores, brought to the lab, and washed several times thoroughly by seawater to remove sand and any adhering substance than by distilled water many times to eradicate both the committed epiphytes and salts. The algae were dried in the shade. Algal species

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were mixed electrically till they became powder, stored and preserved in dark until be used for extraction.

One hundred grams (100 g) of the dry weight of each algal species were soaked in 1000 ml of the methanolic solvent for 48 h, then filtered and concentrated under reduced pressure by using a rotary evaporator and the crude extracts were weighted.

Preparation of *Meloidogyne incognita* inocula

From the roots of coleus plants (*Coleus blumei* L.) a single egg mass was isolated (*M. incognita*), nematodes were inoculated in nursery coleus plants (susceptible host) for 3 months under greenhouse conditions, at Nematological Research Unit, Faculty of Agriculture, Mansoura University, Mansoura, Egypt. Collection of the egg masses was performed according to (Hussey and Barker, 1973).

Soil analysis

Both of physical and chemical analyses of the soil were examined earlier transplanting (Ryan and Rashid, 2006). The soil was a loamy clay (1.7% coarse sand, 26.3% fine sand, 32.4% silt, and 39.6% clay, with a pH of 8.1, EC of 0.93 $d.sm^{-1}$, 2.7% $CaCo^3$, and 0.83% OM). The soil nutrient analysis (ppm) was 54.6 of N, 4.8 of P, and 325.0 of K.

Greenhouse experiments

Two greenhouse experiments (25±2˚C) were conducted using clay loamy soil to estimate the influence of two species of brown and red algae, *T. ornata* and *P. tristichus* on the *M. incognita* and the effect on plant growth parameters of tomato cv. GS12. Induced Resistance (IR) of the examined bio-agents was tested via the examination of the bioactove chemical composition. Three concentrations of methanolic extracts of brown and red algae were used as follows: 0.5% (0.25 g/50 ml water), 1.0% (0.5 g/50 ml), and 2.0% (1.0 g/50 ml).

One hundred and fifteen plastic pots (15cm) containing 800g steam-sterilized soil were planted with one seedling/pot of tomato cv. GS12 and irrigated with water as needed. After three days, about 2000 and 4000 viable eggs of root-knot nematode, were inoculated into the plant seedlings. Three days later, plants were treated with 10 ml of the methanolic extracts of *P. tristichus* and *T. ornata* as a soil drench and at the previously mentioned concentrations. Five pots were treated with Oxamyl (10 % G) as a standard nematicide of the rate of 0.3 g / pot. However, five pots were left free of nematode infection and any treatment to serve as a control (Ck1). Another five pots were received with nematode alone and served as control (2000 eggs) (Ck2) and five pots were received with nematode alone and served as control (4000 eggs) (Ck3). Pots organized randomly in complete block in a greenhouse 25 ±2ºC, with five replicates and irrigated with water according to Ghareeb *et al*., (2020) with slightly modification.

Plants were harvested 45 days after nematode inoculation and roots were washed free from adhering soil. Shoot, and root length, were recorded. Nematodes were extracted from soil using sieving and modified Baermann technique (Goodey, 1957). Roots were stained in 0.01 acid fuchsin (Byrd *et al*., 1983) and examined for the developmental stages, females, galls, and egg masses under the stereomicroscope. This experiment was repeated twice to confirm the results.

Chemical analysis

For each treatment, one gram of the plant shoot dry weight was exposed to determined concentrations of nitrogen (N), phosphorus (P), potassium (K), total nitrogen, crude protein, total carbohydrate and total phenol according to (Jackson, 1967; John, 1970).

Data analysis

The data were subjected to analysis of variance (ANOVA) in a completely randomized design with three replicates and the means were compared by L.S.D. test at 0.05 levels, using Costat program (Costat Statistical Software, 1990).

RESULTS AND DISCUSSION

Results

Greenhouse experiments

The influence of the methanolic extracts of *P. tristichus* and *T. ornata* at three tested concentrations (0.5, 1.0, and 2.0 %) singly and concomitantly with nematode at two inocula levels (2000 and 4000 eggs of *M. incognita* on the plant growth response of tomato (cv. GS12) is shown in Tables (1 and 2). Although, the two bio-control agents improved growth parameters of uninfected tomato plants, some of which phytotoxic effects had. However, plants treated with *P. tristichus* and *T. ornata* at all concentrations decreased tomato shoot length relative to those of plants free of nematode (Table 1). A similar trend was noticed with shoot weight except *P. tristichus* at lower concentration (0.5%) which showed a percentage of increase reached 2%. On the other hand, *P. tristichus* (2.0%) surpassed *T. ornata* at the same concentration and significantly improved (P>0.01) growth of tomato plants inoculated with 4000 eggs/ plant in terms of fresh weight (77.4 vs 49.7%) as compared to nematode alone (Table 1). Comparatively, the standard nematicide treatment of oxamyl showed temperate improvement in the above criteria with percentage increasement up to 17.9 and 35.0 % respectively at inoculum level 2000 eggs/ plant and 12.4 and 35.2 at inoculum level 4000 eggs/ plant (Table 2).

Table (2) verifies that introduction of *P. tristichus* and *T. ornata* at three concentrations to tomato seedlings infected with *M. incognita* were significantly effective in reducing nematode population within root and soil as well as gall formation and the number of egg masses and the subsequently calculated rates of reproduction factor (RF) compared with those of the inoculated untreated check. Irrespective of tested concentrations, the results exposed that a significantly suppressed in the total nematode population was observed with all examined treatments, in addition, reproduction factor (RF) was ranged from 0.59 to 2.38 and reduction % ranged from 11.9 to 76.9. It was interesting to notice that *P. tristichus* (2.0%) application was the ultimate efficacious treatment performing crucial and significant reduction in the number of galls, developmental stages, females, number of eggs/pot and total nematode population at two levels of nematode inoculation. Meanwhile, *T. ornata* (0.5%) has been ranked second in reducing nematode parameters and overwhelming other treatments. Nevertheless, oxamyl has exceeded all treatments of two algae and significantly suppressed all reproduction parameters of *M. incognita*. This evidence showed that each value presented the mean of the experiment repeated twice to confirm the results.

Table 1. Influence of methanolic extracts of the two marine algae *P. tristichus* and *T. ornata* on plant growth parameters of tomato cv. GS12 uninfected and infected with *Meloidogyne incognita* under greenhouse conditions (25 ±2ºC)

Treatments	*Plant growth parameters								
	Plant length (cm)					Plant fresh Wt. (g)			%Inc. ±
	Con%	Shoot	%Inc.±	Root	%Inc. ±	Shoot	Root	Total plant	
P. tritichus	0.5	66.0 c-f	-13.8	23.0 ab	6.5	22.6 b-f	6.0 d-h	28.6	-4.0
	1	72.0 a-c	-6.0	20.0 a-c	-7.4	24.8 ab	5.4 e-h	30.2	1.3
	2	63.0 d-f	-17.8	21.0 a-c	-2.8	24.2 b-d	5.5 d-h	29.7	-0.34
T. ornate	0.5	75.0 ab	-2.1	24.0 a	11.1	29.1 a	5.9d-g	35.0	17.4
	1	68.0 b-e	-11.2	23.0 ab	6.5	23.4 b-e	6.3 d-g	29.7	-0.34
	2	60.0 fg	-21.7	22.0 ab	1.9	24.6 ab	8.6 bc	29.5	-1.0
CK		76.6 a	0.0	21.6 a-c	0.0	25.3 ab	4.5 gh	29.8	0.0
P. tritichus+ N1	0.5	68.6 b-e	14.3	22.6 ab	9.7	21.9b-g	6.7 d-g	28.6	24.9
	1	74.3 ab	23.8	21.0abc	1.9	24.5 ab	4.3 gh	28.8	25.8
	2	69.6 a-d	16.0	22.3 ab	8.3	18.1 fg	9.5 b	27.7	20.96
P. tritichus+ N2	0.5	73.0 a-c	35.2	19.0 a-c	15.2	25.1 ab	7.0 c-e	32.2	48.4
	1	72.3 a-c	33.9	18.6 bc	12.7	25.1 ab	7.8 b-d	33.0	52.2
	2	71.0 a-c	31.5	18.3 bc	10.9	25.1 ab	13.4 a	38.5	77.4
T. ornata+ N1	0.5	72.3 a-c	20.5	23.3 ab	13.1	24.4 a-c	9.2 bc	33.6	46.7
	1	61.3 d-f	1.7	18.6 ab	-9.7	18.6 fg	4.6 f-h	23.2	1.3
	2	62.3 def	3.8	20.0 abc	-2.9	19.8 b-g	5.6 d-h	25.4	10.9
T. ornata + N2	0.5	67.6 b-f	25.2	19.3 a-c	17.0	19.3 e-g	7.0 c-e	26.3	21.2
	1	69.3 a-d	28.3	20.6 a-c	24.8	21.8 b-g	6.3 d-g	28.2	29.95
	2	72.3 a-c	33.9	21.0 a-c	27.3	22.8 b-f	9.6 b	32.5	49.7
Oxamyl+ N1		71.3 a-c	18.8	20.6 a-c	0.0	22.2 b-g	4.8 e-h	27.0	17.9
Oxamyl+ N2		69.0 a-e	27.8	19.3 a-c	17.0	19.5 d-g	4.9 e-h	24.4	12.4
N1 alone		60.0 fg	0.0	20.6 a-c	0.0	19.2 e-g	3.7 h	22.9	0.0
N2 alone		54.0 g	0.0	16.5 c	0.0	17.9 g	3.8 h	21.7	0.0
L.S.D 5%		4.15		2.97		2.55	1.39	3.25	
F		14.86		3.18		10.28	20.90	12.24	
P		.0000		.0001		.0000	.0000	.0000	

Each value presented the Mean of five replicates. N1= 2000 eggs/plant, N2 = 4000 eggs/plant
Means in each column followed by the same letter(s) significantly are not different (P ≤0.0001) by Duncan's multiple range tests

Table 2. Influence of methanolic extracts of the two marine algae *P. tristichus* and *T. ornata* on the reproduction of *M. incognita* infecting tomato cv. GS12 under greenhouse conditions at 25 ± 2 ˚C.

Treatments	Conc.%	Nematode population in Root			N. of eggs/ pot	Total population	RF*	Dec.%	GI**
		Soil	D. S	Females					
P. tritichus+ N1	0.5	0.0 d	4.6 ab	173.9 cd	8876.16 c-e	2644.1 e-g	1.32	54.3	3.0
	1	0.0 d	0.0 d	165.5 c-e	33464.32 b	2779.9 e-g	1.38	51.9	3.3
	2	0 .0d	10.8 ab	201.5 b-d	32371.2 bc	1337.2 gh	0.66	76.9	2.6
P. tritichus+ N2	0.5	184.0 c	3.6 ab	97.4 de	14837.7 c-e	2704.2e-g	1.35	69.7	3.0
	1	192.0 c	0.0 d	140.8 c-e	142889.5c-e	7853.3 bc	1.96	11.9	3.0
	2	168.0 c	20.1 a	347.1 ab	73256.0 d-f	2366.7 fg	0.59	73.5	3.0
T. ornata + N1	0.5	0 .0d	3.5 ab	165.8 c-e	109326.4 cd	3825.7 d-f	1.91	33.8	2.3
	1	0.0 d	4.8 ab	185.2 b-d	290400.0 c	4190.0 d-f	2.09	27.5	3.0
	2	0.0 d	5.1 ab	177.7 cd	261331.2 c	4650.0 de	2.32	19.6	3.3
T. ornata + N2	0.5	288.0 c	3.7 ab	237.6 b-d	9115.2 ef	3061.9 e-g	0.76	65.7	3.0
	1	266.0 d	4.6 ab	291.9 bc	0.0 f	4234.2 d-f	1.05	52.5	4.0
	2	0.0 d	7.0 ab	279.1 bc	132080.8c-e	5794.2 a	2.38	35.0	3.0
N1 alone		3696.0 b	11.1 ab	284.1 bc	131677.8c-e	5780.5 cd	2.89	0.0	4.0
N2 alone		4160.0 a	15.5 ab	487.3 a	840134.4 a	8918.8 ab	2.22	0.0	4.6
Oxamyl+ N1		0.0 d	0.0 d	0.0 e	0.0 f	0.0 h	0.0	0.0	0.0
Oxamyl+ N2		0.0 d	0.0 d	0.0 e	0.0 f	0.0 h	0.0	0.0	0.0
LSD 5 %		90.95	10.73	89.63	943.67	946.97			
F		1693.3	2.51	14.90	43.76	63.50			
P		.0000	.0056	.0000	.0000	.0000			

N1= 2000 eggs, N2= 4000 eggs of *M. incognita*, D.S = Development stages, each value is the mean of five replicates. Means in each followed by the same letter(s) significantly are not different (P ≤0.0001) by Duncan's multiple range test. *Root gall index (RGI) was determined according to the scale given by Taylor& Sasser (1978) , as follows: 0= no galls, 1= 1-2 , 2= 3-10, 3= 11-30, 4= 31-100 and 5= more than 100 galls.

Chemical constituent changes

The statistical differences between infected and uninfected treatments were reported. However, notable that no significant differences were shown with the three mentioned concentrations of *P. tristichus* and *T. ornate*. Meanwhile, it was noticed that significant differences between the two tested leaves of *M. incognita* infected tomato plants in their chemical constituent (Table 3) and (Fig. 1, 2 & 3).

NPK contents

In uninfected tomato plants, *T. ornata* treatment at the highest concentration (2.0%) represented the maximum percentage increase values of N (13.7%), P (12.89%) and K (12.46%) comparing to control (Table 3).

A similar trend was evident for the infected ones at 2000 and 4000 eggs/plant with the percentage increase value of N amounted to 24.6 & 28.3%, P amounted to 24.1 & 31.9% and K amounted to 25.9 & 40.9% in compared with nematode alone in respective manner. However, the plant receiving *P. tristichus* at different concentrations which represented the minimum percentage increase values of N. Oxamyl as a nematicide gave moderately values of N (20.9& 28.9%), P (23.1& 38.2%), K (23.7& 36.4%) at 2000 and 4000 eggs/plant, (Fig. 1).

Table 3. Impact of methanolic extracts of the two marine algae *P. tristichus* and *T. ornata* on the chemical changes in leaves of Tomato cv. GS12 under greenhouse conditions at 25 ± 2 ˚C.

Treatments	Conc.%	*Chemical changes in leaves % Inc. ± (I)*											
		N	*I*	P	*I*	K	*I*	Total phenol	*I*	Crude protein	*I*	Total carbohydrate	I
P. tritichus	0.5	2.71^{d}	0.37	0.382^{c}	0.53	3.56^{e}	0.85	0.539^{a}	8.89	16.93^{d}	0.36	39.70^{c}	*0.0*
	1	2.79^{cd}	3.33	0.391^{c}	2.89	3.63^{de}	2.83	0.528^{b}	6.67	17.43^{c}	3.32	40.11^{bc}	*1.03*
	2	2.84^{bcd}	5.19	0.398^{bc}	4.74	3.70^{cd}	4.81	0.513^{c}	3.64	17.75^{c}	5.22	42.10^{ab}	*6.04*
T. ornate	0.5	2.95^{abc}	9.26	0.413^{ab}	8.68	3.81^{bc}	7.93	0.474^{e}	-4.42	18.43^{b}	9.25	42.93^{a}	*8.14*
	1	3.03^{ab}	12.2	0.420^{a}	10.5	3.88^{ab}	9.92	0.465^{ef}	-6.10	18.93^{a}	12.21	43.52^{a}	*9.62*
	2	3.07^{a}	13.7	0.429^{a}	12.9	3.97^{a}	12.46	0.456^{f}	-7.88	19.18^{b}	13.69	38.51^{c}	*-2.99*
CK		2.70^{d}	0.0	0.380^{c}	0.0	3.53^{e}	0.0	0.495^{d}	0.0	16.87^{d}	0.0	39.70^{c}	*0.0*
L.S.D		0.213	--	0.0183	--	0.1107	--	0.0105	--	0.390	--	2.007	--
F		4.502	--	10.002	--	20.77	--	84.867	--	35.990	--	8.23	--
P		*.0096*	--	*.0002*	--	*.0000*	--	*.0000*	--	*.0000*	--	*.0000*	--

Means in each column followed by the same letter(s) significantly are not different (P ≤0.0001) by Duncan's multiple range test. Each value is the mean of five replicates.

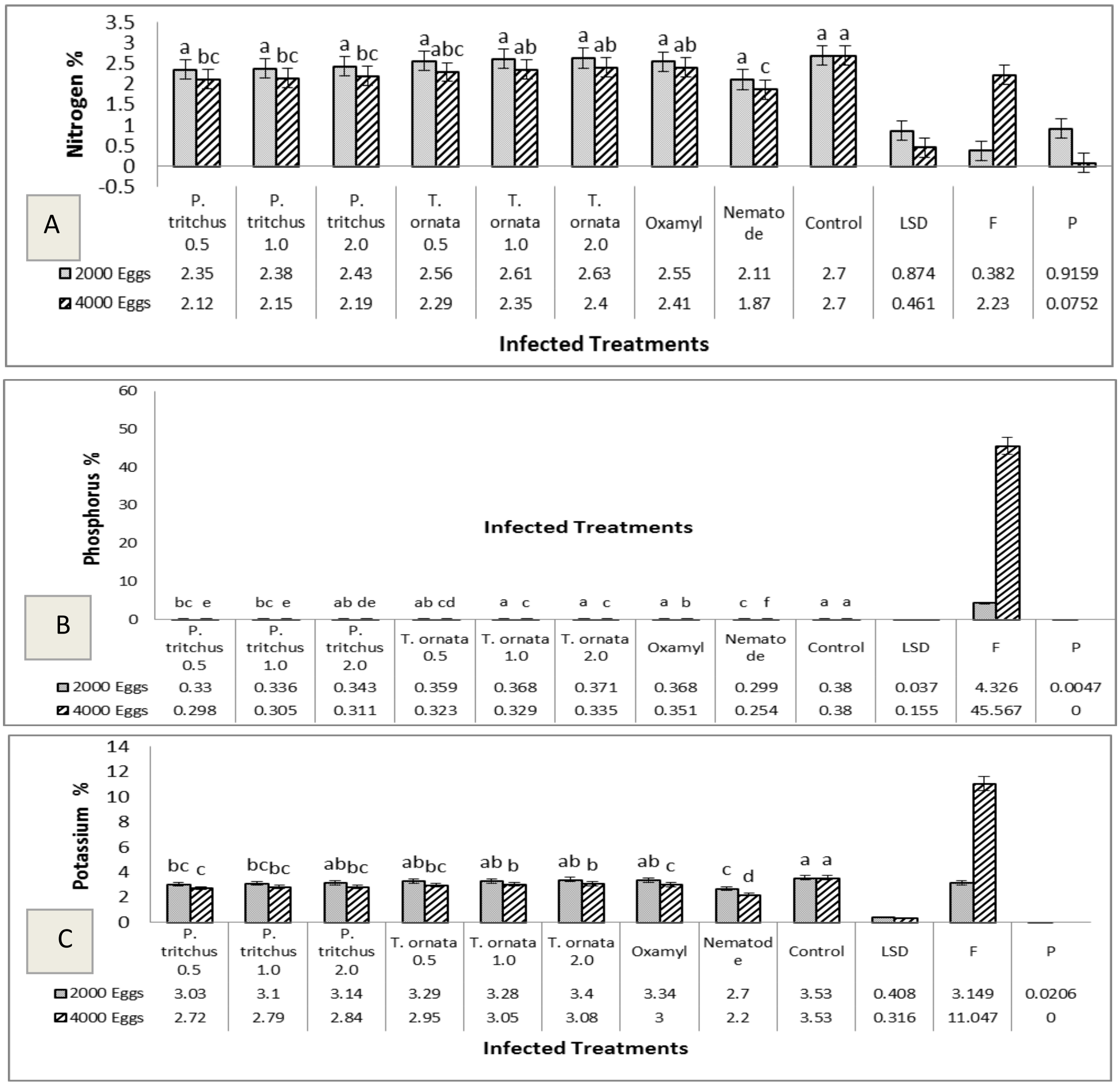

Figure 1. Percentage of A) nitrogen, B) phosphorus, and C) potassium in leaves of tomato infected with *M. incognita* as influenced by the addition of two algal species *P. tristichus* and *T. ornata*.

Phenol content

Conversely, a moderate enhancement of the total phenol existed in tomato leaves infected with *M. incognita* compared to control plants (Fig. 2). All treatments have showed a noticeable reduction in total phenol compared to untreated uninoculated plants. However, the least reduction in total phenol has been recorded with *T. ornata* (2.0%) either in 2000 eggs or 4000 eggs of *M. incognita* infection (Fig. 2).

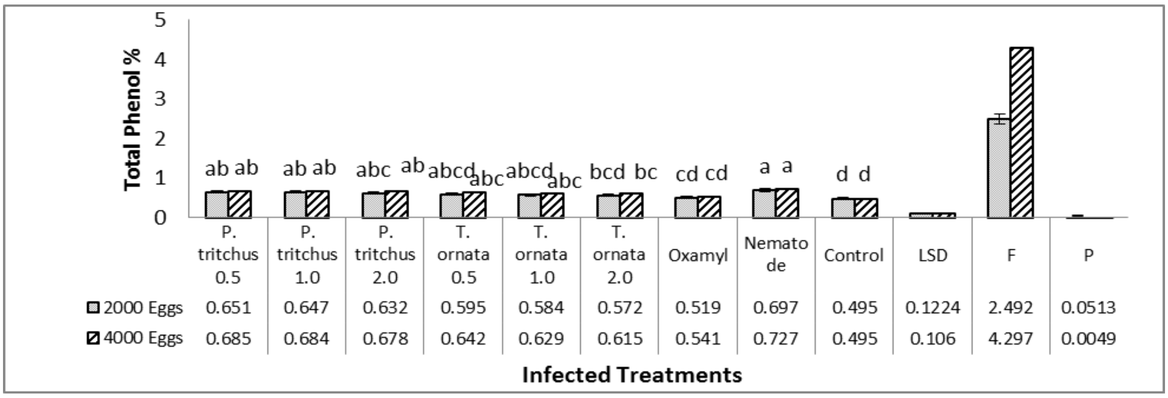

Figure 2. Total phenol content in leaves of tomato infected with *M. incognita* as influenced by the addition of two algal species, *P. tristichus*, and *T. ornata*

Crude proteins and Total carbohydrates

Untreated infected tomato with *M. incognita* displayed a significant reduction in total crude proteins as compared with untreated uninoculated plants with a reduction percentage of 34.1, 32.0% at two inocula levels, respectively (Fig. 3). It is worthy to note that the percentage increase values of crude proteins have been higher in the uninfected plants than those of the infected ones. The greatest induction in total proteins was more pronounced with *T. ornata* at the concentrate of 2.0% with values averaged 24.7 and 28.4% either in 2000 eggs or 4000 eggs of *M. incognita* infection, respectively.

Further, a significantly suppression of the total carbohydrates were obsrved due to nematode infection (Fig. 3). It was evident that the percentage of increase in total carbohydrates was moderately induced with *T. ornata* (2.0%) with values averaged 22.4 and 18.2% either in 2000 eggs or 4000 eggs of *M. incognita* infection, respectively compared with untreated inoculated plants (Table 3 & Fig. 3).

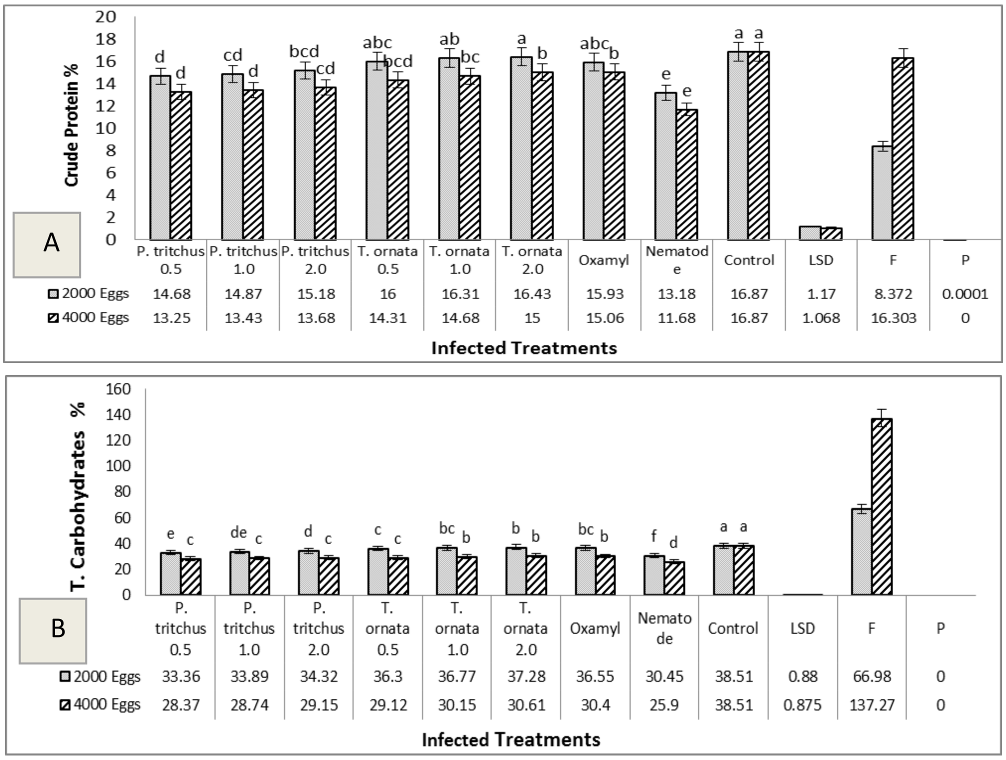

Figure 3. A) Crude protein and B) percentage of total carbohydrate in leaves of tomato infected with *M. incognita* as influenced by the addition of two algal species *P. tristichus* and *T. ornate*.

Discussion

The current investigation was focused on the potential use of methanolic extracts of two marine algae applied each singly for controlling *Meloidogyne incognita* on the response of the tomato growth of, cv. GS12 and chemical constituents change as well. Under greenhouse conditions, soil was changed with red alga *Phacelocarpus tristichus* J. Agardh and brown alga, *Turbinaria ornata* J.

Agardh at three concentrations (0.5, 1.0, and 2.0%) 7 days before *M. incognita* inoculation at 25±2˚C. Irrespective, botanicals displayed a noticable enhancement in uninfected tomato plants with *M. incognita* and these criteria are represented in percentages increased over the control. However, the two examined treatments, the good results obtained only with lower concentrations. On the other hand, the total plant fresh weight was the highet with *T. ornata* at the concentration of 0.5% (17.4%) and (1.3%) with *P. tristichus* with concentration of 1%. Apparently, low improvement to moderate obtainable in fresh weight of uninfected tomato plants with *M. incognita* in pots treated with all concentrations of *T. ornata*. It was reported that marine algae have a timulator effect on the treated plant growth (Nabti *et al*., 2016; Hamed *et al*., 2018; Ghaderiardakani *et al*., 2019).

The root-knot nematode, *M. incognita* at two inocula levels (2000 and 4000 eggs) triggered a noticable reduction in the growth parameters of the infected tomato plants; both of shoot and root length and shoot weight acombined with drop in the percentage of the tomato fresh weight. Response of the infected tomato plant growth against *M. incognita* was higher in those plants treated with extractof the red alga *P. tristichus* than the brown alga *T. ornate*. Besides, the nematicidal activity of *P. tristichus* was signficantly against *M. incognita* and in addition it suppressed population, gall formation, and egg numbers of the examined nematode per pot treated with the three different concentrates and inoculated with 4000 eggs/ plant. These findings are in agree with many researchers (Khan *et al*., 2016; Nour El-Deen and Issa, 2016; El-Eslamboly *et al*., 2019; Ghareeb *et al*., 2019). Generally, oxamyl, showed potent actions than bio-products dealing with the reduction the number of galls/root, the amount of J_2 in soil and as well as both of the number of different instars infected the root and the number of eggs/ root. The same observation was reported by by Khalil *et al*.(2012).

The natural product of seaweed extract as an antioxidant is a common source of plant growth regulator (RGR) that exhibits multiple functions i.e. regulate plant growth and development, increase plant resistance to various environmental stress of disease, salinity, drought, a low temperature since it possesses cytokinin and auxin properties which can stimulate endogenous cytokinin activities of plants (Shukla *et al*., 2016). These findings are as per those of that (Shalaby, 2012) which showed that there was an improvement in growth paramters of the plants infected with nematode and showd resistance against biotic and abiotic stress in the presense of seaweeds extracts. According to those, different secondary metabolites biosynthesized by seaweeds, these compounds are capable to mediate a wide range of interactions intra and interspecific ecologicalrelationship between different organisms.The nematicidal activities of the tested seaweeds may be accredited in accordance with the chemical a compostion of the active materials eixsted in algae, and also their concentrations applied. The safety of such botanical nematicides and biopesticides and their low cost is one of their advantages.

On the other hand, it was found that, Seaweeds i.e. *P. tristichus* and *T. ornata* extract act as bio-fertilizers in addition to their thermostable compounds in the management of *M. incognita* as endo-parasite nematode on tomato plants can be varied from one component to another. Undoubtedly, the tested seaweed extracts as organic fertilizers may involve in the biophysiological processes of the infected plant (Shalaby, 2012). Similar results were reported with (Nour El-Deen and Eissa, 2016) concerning the effects of different nine algal aqueous extracts in range of concentrations on *M. incognita*. (Khan *et al*., 2016) also documented the effect of marine red alga *Melanothamnus afaqhusainii* against *M. incognita.* Rajkumar and Bhavan (2017) illustrated that the edible seaweed, *T. ornata* was subjected to hexane, acetonic and methanolic extractions.

It well known that the plant active compounds; alkaloids, terpenoids, flavonoids, polyphenols, saponins, tannins, and cardiac glycosides, have antimicrobial, antiparasitic and antiviral properties. In plants different chemical constituents were significantly suppressed due to nematode infection. However, a notable induction quantively in these chemical was obsrved with applying of *T. ornata* as treatment at concentration (2.0%) both in the uninfected and infected plants.

In general, *P. tristichus* and *T. ornata* methanolic extracts significantly increased percentage values of tomato leaves parameters i. e. N, P, and K contents in the uninfected and infected. It is worthy to notice that the percentage increase values of N, P, and K contents were higher in the uninfected plants than those of the infected ones. These findings are in agreement with (Fayaz *et al*., 2005) postulated that the edible seaweeds contain a significant amount of essential vital nutrients i.e. minerals (Ca, P, Na, K, S, Mg, Fe, etc.) which have brought an enhancement in tomato plant growth parameters and increment in NPK content which induce resistance towards nematodes infection. The present study supported by the results of Shalaby *et al*., (2012) indicates that seaweeds can give moderate increase values of NPK contents for the infected plants. A similar observation was reported with the uninfected plants under different applications i.e. root-dipping, foliar spraying, and their mixing.

Moreover, Ananthavalli *et al*., (2019) reported an increased level of electrical conductivity, NPK, and organic carbon, C: N ratio, and organic matter contents were reduced in the seaweed of the vermicomposting with physicochemical characteristics.

The composition of seaweed and the using of them as fertilizers revealed that, they are rich in plant growth regulators (Mirparsa *et al*., 2016). Other study indicated that the isolated copounds; polysaccharides, fatty acids, phlorotannins, pigments, lectins, alkaloids, terpenoids, and halogenated compounds have from green, brown, and red algae showed apotential antimicrobial activities (Pe´rez *et al*., 2016). Conversely, the increase in total phenol percentage has been recorded with untreated infected plants which considred as a hypersensitive reaction (HR) aginst nematode infection. The obtained results are in agreement with those of (Vijayabaskar and Shiyamala, 2012) who reported the antioxidant properties of seaweed polyphenol from *T. ornata*. Also, (Rajkumar and Bhavan, 2017) illustrated the presence of moderate to luxuriant polyphenols in three solvents extracts of *T. ornata*.

Crude proteins in this study (24.7 & 28.4%) and total carbohydrates (22.4 & 18.2%) were significantly reduced in tomato plants with 2000 eggs or 4000 eggs of *M. incognita* infection, respectively with the application of *T. ornata*

(2.0%). These results support the findings of Clarke *et al*., (2014) in egg-plant and tomato that decrease may be due to some activities related to hypersensitive response. It is clear that plant growth depends on both cell division and cell expansion and that seaweed is involved in plant growth regulation through controlling the biosynthesis of hydroxyproline containing protein. Such a process is represented by some of the biological defense mechanisms in plants. These findings agree with those reported by Ponnan *et al*., (2017) who investigated that seaweeds could be used as fertilizer and pesticide. Nabanita *et al*., (2010) reported that most of the common phytochemicals are present in *T. conoides*.

As our knowledge, this study considered the first report conveys that of the red algal *P. tristichus* and the brown one *T. ornate* have nematicidal activities. Here in, it can concluded that a further studies should be carried out on either red or brown algae using them as nematicides against root-knot nematodes and the application should be performed firstly under the condition of the green house and secondly in the open field for controlling the nematode infection for economic crops. Hence, the two algal species, *P. tristichus* and *T. ornata* could be recommended as safe alternatives to chemical nematicides in controlling *M. incognita* infecting tomato and a promising tool in integrated pest management.

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تقييم الخواص الإبادية للمستخلص الإيثانولى من الطحالب البحرية ضد نيماتودا تعقد الجذور *Meloidogyne incognita*على نباتات الطماطم

دينا صلاح الدين سراج الدين إبراهيم[1] ، رحاب ياسين غريب [2] ، دعاء عبدالمقصود أبو العطا[3] و حسين عزوز [3]

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ركزت هذه الدراسة على اختبار تأثير المستخلص الإيثانولى لكلا من الطحلب الاحمر *Phacelocarpus tristichus* J. والطحلب البنى *Turbinaria ornata* بإستخدام ثلاث تركيزات (٠,٥ ,١,٠ و ٢,٠ %) فى مكافحة نيماتودا تعقد الجذور (*Meloidogyne incognita*) التي تصيب نباتات الطماطم تحت ظروف الصوبة. وقد اظهرت النتائج ان المستخلصات الميثانولية لنوعى الطحالب قد حسنت من نمو النباتات المصابة وغير المصابة على حد سواء, كما أدت هذه المعاملات إلى حدوث تأثير إبادى في أعداد النيماتودا. ونلاحظ أن النتائج أسفرت أن المعاملة *P. tristichus* بتركيز ٢ ٪ اعطت أفضل نتائج فى خفض تعداد النيماتودا ، كما أدت إلى خفض معنوي فى عدد الأطوار اليرقية ،وكذاخفض عدد الإناث ،وتقليل كتل البيض، مما أدى إلى تقليل إجمالي لتعداد النيماتودا فى التربة. بالإضافة إلى ذلك ، فقد أدت المعاملات بالمستخلص الطحلبى *T. ornata* و المعاملة بالمستخلص الطحلبى *P. tristichus* بتركيزات مختلفة إلى إحداث فروقا معنوية فى محتوى نبات الطماطم من عناصر النيتروجين والفوسفور والبوتاسيوم والبروتينات والكربوهيدرات في أوراق نباتات الطماطم المصابة بنيماتودا تعقد الجذور (*Meloidogyne incognita*). وعلية ، يمكن التوصية بـإستخدام مستخلصات كلا" من طحلب *P. tristichus* وطحلب *T. ornata* كأسمدة حيوية كونها صديقة للبيئه وأيضا أداة واعدة في المكافحة المتكاملة لنيماتودا تعقد الجذور (*Meloidogyne incognita*) التي تصيب نباتات الطماطم.