Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

IN VITRO EVALUATION OF THE TOXICITY OF DIFFERENT EXTRACTS OF SOME MARINE ALGAE AGAINST ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*)

[a]Ahmad S. M. El-Nuby, [b]Ashraf I. B. Afia, [c]Eman. A. S. A.H. Alam*

[a] Plant Protection Department, Desert Research Center, Cairo, Egypt.
[b] Zoology and Agricultural Nematology Department, Faculty of Agriculture, Cairo University, Cairo, Egypt.
[c] Botany and Microbiology Department, Faculty of Science, Girls Branch, Al-Azhar University, Cairo, Egypt.

A B S T R A C T

Ten marine algal extracts (MAE) viz. *Amphiroa sp*., *Caulerpa racemosa*, *Dictyota sp*., *Halimeda discoidea*, *Jania sp., Laurencia obtusa*, *Padina pavonia*, *Sargassum latifolium*, *Sargassum polycystum,* and *Hormophysa cuneiformis;* obtained by three solvents (water, methanol, and ethanol) were evaluated for their nematotoxicity against root-knot nematode (RKN) *in vitro*. Freshly hatched larvae of *Meloidogyne incognita* were exposed to these extracts. Results revealed that; all examined MAE possessed nematotoxic properties with varing degrees. The highest mortality percentage (100 %) was detected in *L. obtusa*, *Halimeda discoidea,* and *Padina pavonia*, followed by *Amphiroa sp*. which recorded 95.00% mortality and not significantly different from *Sargassum polycystum,* and *Dictyota sp.* (89.00 and 89.30 %, respectively). Generally, methanol extract followed by ethanol was better than water regarding their nematicidal potential. Water was the less effective solvent to release anti-nematodal compounds from these studied algae, with the exception of *Laurencia obtuse* that able to kill 70.00 % of subjected juveniles. Preliminary phytochemical screening on all studied algal extracts revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids, anthraquinones, steroids and/triterpenoids, sublimable substances, saponins, alkaloids and/or nitrogenous bases, cardiac glycosides, coumarins, chlorides, sulphates, and iridoids in all samples. In this regard, results of preliminary phytochemical screening showed that the richest extracts with these studied phytochemicals are belonging to methanolic extracts of these algae. The nematicidal action was related to the phytochemical structures of various marine algae and the extracting solvent. Phytochemical contents are positively correlated with antinematodal activity. These results offer promised bio-nematicide for combating phytonematodes by utilizing untapped marine algae. Evaluation of tested MAE under *in vivo* conditions will perform in the future to estimate their nematode-suppression, proper mode of application, and the significant dose. Future researches will focus on the preparation of some formulations for field application as biocides and growth promoters.

Keywords: Phytochemicals, Marine macroalgae, Nematotoxicity**,** *Meloidogyne incognita.*

INTRODUCTION

Nematodes are the amplest metazoan animals on the globe, padding all nutritional levels in the soil food cycle (van den Hoogen *et al.,* 2019). Phytonematodes (PN) are destructive pathogens and considered a great threat to crop production universally, causing severe yield loss annually up to 125 billion dollars worldwide (Mesa-Valle *et al.,* 2020). Nowadays, PN becomes a huge challenge to food production in an overpopulated world. They are listed as a serious plant pathogen in the globe, largely because of their wider allocation and their capability to attack practically all crops (Atolani and Fabiyi, 2020). The most dangerous and yield-limiting genus is *Meloidogyne spp*. (Root-knot nematodes; RKN). Individuals of this genus are considered to be a serious threat to global food security, they are widespread and easily parasitize on a vast range of plants. More than 5,000 plant species are liable to infection endo-

Submitted: May 04, 2021
Revised: May 24, 2021
Accepted for Publication: May 31, 2021
* Corresponding Author:
Email: aalam.eman@gmail.com

parasitically. Also cause great losses or reduction in either crop quantity or quality, epically in tropical and subtropical regions. The annual losses were estimated at 110 billion dollars globally (Jones *et al.,* 2013; Bebber *et al.,* 2014; Forghani & Hajihassani, 2020; El-Nuby & Alam, 2020 and Ibrahim *et al.,* 2021).

The need for eco-friendly options has become imperious. These choices included agricultural strategies, physical approaches, induction of plant resistance, addition of amino acids, yeast application, using resistance genotypes, microbial-pesticides and antagonistic plants or plant extracts (El-Nuby, 2014; Renčo *et al*., 2014; Alam & El-Nuby, 2019; El-Nuby & Bayomi, 2019; Afia & El-Nuby, 2020; Atolani & Fabiyi 2020; El-Nuby& Alam, 2020; and El-Nuby *et al*., 2020).

Phytochemicals (in higher plants, algae, and plant-like organisms) are various substances that possess many antagonistic activities towards various organisms like bacteria, fungi, insects, and nematodes besides many therapeutic properties like antioxidant (Shreya *et al*., 2015, Afia & El-Nuby, 2016, Alam & El-Nuby, 2019, El-Nuby and Alam, 2020). Marine macroalgae (Seaweeds), a renewable natural resource, are photosynthetic organisms living in seas and oceans. Seaweeds are necessitous producers of the total mass of living matter in the marine ecosystem and they have a considerable economic significance as they generate a broad assortment of active metabolites in their ecosystems. Marine macroalgae are classified into three major pigmentation groups; brown algae "Phaeophyta", green algae "Chlorophyta", and red algae "Rhodophyta" as mentioned by Mouritsen, (2013). Marine algae have been used for diverse purposes; in industry and medicine also as an innovative food. Furthermore, they are known to have several benefits and are recognized as a rich source of several natural bioactive compounds with antibacterial, antifungal, antiviral, antioxidant, *etc*. In Egypt, they found growing in large quantities along the red sea coast. (Sharma *et al*., 2019 and Rashad & El-Chaghaby, 2020). Marine algae have a great impact on beneficial changes of soil properties (Hamed *et al*., 2018). Various genera of marine algae, seaweeds, have been used as biocontrol agents against phytonematodes (Abid *et al*., 2005; Afia & El-Nuby, 2016; and Ghareeb *et al*., 2019) also the commercial product of algae are used as anti-nematodes (Radwan *et al*., 2012). This study was aimed to evaluate the toxic effects of different extracts of certain marine macroalgae (seaweeds) collected from Red Sea (Hurghada - Egypt) against root-knot nematodes *(Meloidogyne incognita)* under *in vitro* conditions.

MATERIALS AND METHODS

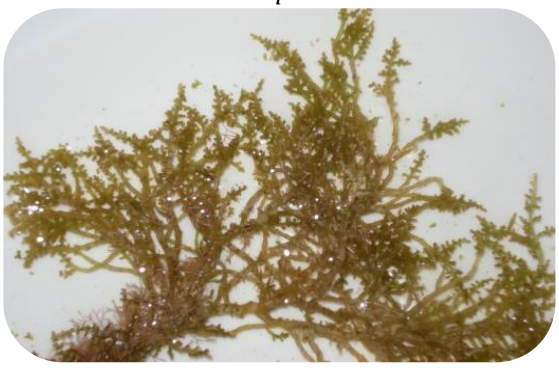
Sample collection: Ten Marine algae were handpicked from the shallow water *beside the shore of the Red Sea, in the* Hurghada *region (Egypt) in September 2019.* Collected *samples were primarly washed* thoroughly *with seawater to remove planktons,* debris*,* epiphytes, *and soil particles then* kept 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*. Secondary washing was carried out using tab water then dried in air at room temperature.* The water was drained off and the algal material was spread on blotting paper to remove excess water. *Samples were identified with the aid of the National Institute of Oceanography and Fisheries (NIOF),* Hurghada*, Egypt.*

Dictyota sp.

Amphiroa sp.

Padina pavonia

Jania sp.



Laurencia obtusa

Halimeda discoidea

Sargassum platycarpum

Sargassum latifolium

Hormophysa cuneiformis

Caulerapa racemosa

Figure 1. Marine macroalgae (seaweeds) used in the study:

Figure 2. Naturally growing seaweeds (Macro-algae), flushing at the Red Sea Shore

Preparation of algal (water, methanol, ethanol) extracts: Air-dried various seaweed materials viz. *Amphiroa sp*., *Caulerpa racemosa*, *Dictyota sp*., *Halimeda discoidea*, *Jania sp., Laurencia obtusa*, *Padina pavonia*, *Sargassum latifolium*, *Sargassum polycystum,* and *Hormophysa cuneiformis* were ground to a fine powder using electrical blender. 10 grams of powdered seaweeds were extracted successively with 200 ml of distilled water in a shaker for 3 hours. The extracts were filtered and stored in a refrigerator for future use. The same procedures are followed using methanol, and ethanol to prepare ethanol, and methanol algal extracts by using a discending successive extraction method according to the polarity gradients of the solvents.These extracts (1 ml of each extract = 50 mg Dry Weight of the sample) were evaluated for their nematicidal activity (Alam, 2019).

Preliminary phytochemical screening on the studied algal extracts: The Preliminary phytochemical screening was carried out on water, ethanol and methanol algal extracts of *Amphiroa sp*., *Caulerpa racemosa*, *Dictyota sp*., *Halimeda discoidea*, *Jania sp., Laurencia obtusa*, *Padina pavonia*, *Sargassum latifolium*, *Sargassum polycystum,* and *Hormophysa cuneiformis* to detect the presence of carbohydrates and/or glycosides, tannins, flavonoids, anthraquinones, steroids and/or triterpenoids, sublimable substances, saponins, alkaloids and/or nitrogenous bases, cardiac glycosides, coumarins, chlorides, sulphates, and iridoids in all samples using the following indexed methods:

1.1. Carbohydrates and/ or Glycosides (Stank *et al.,* 1963).
1.2. Saponins (Hungund and Pathak, 1971).
1.3. Tannins (Trease and Evans, 1978).
1.4. Unsaturated sterols and/or Triterpenes (Claus, 1967).
1.5. Alkaloids and/or Nitrogenous bases (Shellard, 1957).
1.6. Cardiac glycosides (Balbaa *et al*., 1981).
1.7. Flavonoids (Mabry *et al*., 1970).
1.8. Anthraquinones (Farnsworth *et al.,* 1969).
1.9. Coumarins (Feigl, 1960).
1.10. Iridoids (Weiffering, 1966).
1.11. Chlorides and Sulphates (Islam *et al*., 1993).
1.12. Sublimation (Afifi, 1972).

Nematode culture: Pure culture of *Meloidogyne incognita,* previously identified, was maintained on tomato cv. Castel Rock roots in 20 cm pots in the greenhouse. Forty days before commencing the study 3-weekold tomato seedlings were transplanted in pots, contains sterilized soil mixture (3 sand: 1 clay, v/v). Ten days later plants were inoculated with about one thousand second-stage juveniles of *M. incognita.* Plants were checked for producing egg masses, allowed to hatch for collecting freshly hatched juveniles, which were used in bioassay experiments.

Nematotoxicity of marine algae extracts: Two ml of each marine algae extract (MAE) extract was transferred to sterilized Petri dishes, 5 cm diameter, then 100 freshly hatched infective-stage larvae, in100µ volume, of *M. incognita* were added to each petri dish. Larvae transferred to sterile distilled water serves as a control

treatment, all plats were incubated at 27 ºC ±2. One day later, mobile and static nematode larvae in each dish were observed with stereo microscope (60x), also light microscope at 150x was used for ultra-investigation of larval mobility detailed morphology and. The mortality of larvae was based on their mobility i.e. the lack of nematode movement when touched with a fine probe consider dead (Abbasi *et al.,* 2008). To ensure nematode death or temporal paralyzed, nematodes were put in sterilized distilled water for 24 hours. Only larvae that still static after the recovery test was counted as killed ones (permanent death). Five replicates for such treatment were used and the test was conducted twice under the same conditions. Percentage of nematode mortality was estimated depending on this formula; Mortality percentage (M %) = [(No. of killed larvae) / (No. of killed+ live larvae)] x 100.

STATISTICAL ANALYSIS

Data were subject to analysis of variance (ANOVA) test, and the differences between means were tested using Duncan's Multiple ranged test at the 5% significance level (Duncan's, 1955).

RESULTS AND DISCUSSION

Nematotoxicity of some marine macroalgae against *M. incognita:* Varied nematotoxicity of tested marine algal extracts (MAE) was observed in (Table. 1). The complete death (100%) was achieved by methanolic extract of *Halimeda discoidea* and *Padina pavonia,* ethanolic extract of *Laurencia obtusa*. Water extracts showed the minimum toxicity against nematodes. All aqueous MAE recorded mortality less than 10% except *Laurencia obtusa* (70%). Methanol solvent was released more antinematodal compounds that resulted in killing high numbers of juveniles. More than 50% of infective juveniles in most tested seaweeds were dead by methanolic MAE, only two (*Laurencia obtusa* and *Jania sp.*) possessed low nematicidal effect and killed 10 and 14.3 individuals, respectively. Ethanolic extracts were ranked after methanol in increasing MAE toxicity towards nematode. The rate of death in four algae was more than 50% (*Laurencia obtuse, Amphiroa sp., Caulerpa racemosa,* and *Dictyota sp.*). Obviously, all tested marine algae showed varied nematotoxicity based on the used solvent and the type of algae, also all the extracts of *Halimeda discoidea, Padina pavonia,* and *Laurencia obtusa* appeared to be the most potent nematicides, as they caused 100% mortality of juveniles *in vitro*.

Table 1. Effects of some algal extracts on mortality of *Meloidogyne incognita* juveniles after 24hours of exposure.

Algae group	Marine macroalgae(Sea weeds)	Water extract	Methanolic extract	Ethanolic extract
Rhodophyta	*Amphiroa sp.*	4.3 cd	50.3 e	95.0 b
Chlorophyta	*Caulerapa racemosa*	5.3 bc	66.7 d	65.3 c
Phaeophyta	*Dictyota sp.*	6.3 b	89.3 b	63.7 c
Chlorophyta	*Halimeda discoidea*	1.0 ef	100.0 a	40.0 e
Phaeophyta	*Hormophysa cuneiformis*	4.7 bc	50.3 e	14.7 f
Rhodophyta	*Jania sp.*	4.7 bc	14.3 f	31.0 f
Rhodophyta	*Laurencia obtusa*	70.0 a	10.0 g	100.0 a
Phaeophyta	*Padina pavonia*	4.3 cd	100.0 a	18.3 g
Phaeophyta	*Sargassum latifolium*	2.7 df	75.7 c	47.0 d
Phaeophyta	*Sargassum polycystum*	4.7 bc	89.0 b	32.0 f
Control (distilled water)		0.0 f	0.0 h	0.0 i

Rhodophyta = Red Algae, Chlorophyta =Green algae, Phaeophyta= Brown algae

No difference with means of the same letter (Duncan's multiple range test, P≥5%).

Preliminary phytochemical screening on some (water, methanol, ethanol) extracts of marine macroalgae: Data in Table 2 represents the results of preliminary phytochemical screening on water extract of the investigated ten algae (alkaloids, anthraquinones, carbohydrates (and/or glycosides), cardiac glycosides, chlorides, flavonoids, iridoids, saponins, sublimable substances, sulphates, tannins and unsaturated sterols and/or triterpenoids), it was noticed that except saponins, amount extracted was similar to other extracted from the rest seaweeds, only *Laurencia obtuse* extract gave the higher quantities of chemical compounds. The seaweeds *Halimeda discoidea, Caulerpa racemos, Dictyota sp.,* and *Sargassum polycystum* extracts were found to be devoid of saponins.

Regarding Table 3, noticeably methanol could release a higher amount of phytochemicals compared with the water

extracts of the same algae. The highest phytochemical amounts were released from four algae; *Sargassum polycystum, Dictyota sp., Halimeda discoidea,* and *Padina pavoni*. On the other hand, *Laurencia obtuse* and *Jania sp*. produced the lowest chemical constituents in comparison to all examined algae. The moderated amounts of phytochemicals were extracted from *Amphiroa* sp., *Caulerpa racemos*, *Hormophysa cuneiformis,* and *Sargassum latifolium*. Ethanol was found to be an enhancer for releasing high amounts of phytochemicals from *Amphiroa* sp. and *Laurencia obtusa* (Table. 4). Moderate quantities of chemicals were produced by two seaweeds viz., *Caulerpa racemos* and *Dictyota* sp. The rest five marine algae produced fewer amounts of phytochemicals by using ethanol for extraction. Marine algae species were varied in their phytochemical structures (kinds and quantities of phytochemicals). And these are due to genetic differences and environmental factors. In general, the chemical constituents obtained from water extract were less than other organic solvents. Also, the bioactivity of such seaweed was related to the quantities of phytochemicals found in extracts according to the used solvents.

The nematicidal properties are varied according to the type of extract (solvent kind), this hypothesis is confirmed in a current investigation; methanolic extract in most cases was more lethal to nematodes. Also, the potency of the methanolic extract for the same marine algae may differ; *According to results of Zaki et al., (2005*) it is revealed that; all marine macroalgae extracts achieved high nematicidal action against *M. javanica*. Water extracts of three of them achieved maximum morality percentages of juveniles (99%) as compared to other seaweeds. However, methanolic extracts of the same seaweeds exhibited 100% mortality after 72 hours of exposure. Similarly, the results of Rizvi and Shameel (2006) showed that; nematicidal activity of methanolic extract of *Caulerpa racemosa* hardly killed 42% of RKN juveniles, but in this current study 66.7% of juveniles were killed. Other reports supported this thought (Sultana *et al*., 2008), they stated that; out of three organic solvents only methanol was able to release more nematicidal compounds that kill RKN juveniles.

Recently, Prarthana and Maruthi, (2019) found that; quantities and types of secondary metabolites like; alkaloids, flavonoids, phenolics, and essential oils, also extracted bioactive compounds are varies depending on the extraction protocols and the types of use. In many cases, the ethanolic extract was more active in killing nematode than water extract, Ghareeb *et al*., (2019) recorded that; ethanolic extract of marine algae achieved higher mortality of *M. incognita* juveniles than water extract. The additional benefit of marine algae is using their extract in green synthesis of nanoparticles (Ghareeb *et al*., 2020), which was found to be more effective against *M. javanica in vitro* and *in vivo***.**

The nematicidal activity of marine algae may be varied due to various commodes inside the marine algae; like bromophenols, phloroglucinol, tannins, and terpenoids (Abid *et al.,* 1997). Another study recorded that; marine algae contain high amounts of antinematodal and antimicrobial compounds such as antioxidants, flavonoids, polyphenols, and some enzymes (Chtourou *et al*., 2015). Seaweeds contain diverse types of secondary metabolites, like alkaloids, phenols, saponins, steroids, tannins, triterpenoids, and biocides mineral salts which include multidisciplinary biocides, also mineral salts may assist and accelerate the penetration rate of algal metabolites through a nematode cuticle and subsequently increase the nematicidal effects (Arunkumar *et al*., 2010 and El-Ansarya & Hamouda, 2014 and El-Eslamboly *et al*., 2019).

By comparing the brown marine algae with green and red seaweeds from some coastal areas, it is clear that; they have always possessed much stronger nematotoxicity against nematode larvae **(***Ara et al., 1997 and Sultana et al., 2008, Whapham et al., 1994 and Zaki et al., 2005)***.** On average, the species of brown marine macroalgae were found to be most active and those of green seaweeds are the least active, while the members of red seaweeds presented an intermediate nematicidal activity. These results are not matched in all cases; according to our results methanolic extract of *Halimeda discoidea* (green algae) achieved 100% mortality, meanwhile, ethanolic extract of *Laurencia obtuse* (red algae) showed 100% mortality.

Phytochemicals offer potent alternatives instead of chemical pesticides. Our findings is in harmony with the finding of *El-Ansarya and Hamouda, (2014).* Various marine macroalgae possessed high nematicidal activity, also mixing between seaweed and nematicides can enhance the inhibitory effect against phytonematodes *(Sultana et al., 2009 and Afia & El-Nuby, 2016).*

The quantities of most phytochemical constituents in methanolic extracts are larger than ethanolic and water extracts, so the nematicidal action was increased in methanolic extract for most tested marine algae. Another study matched with our results; Hassan and Shobier (2018) stated that; marine algae nematotoxicity is not due to one compound, but it may be achieved by a combination between some of the different compounds**.**

Table 2. Preliminary phytochemical screening on water algal extracts under investigation:

Detected phytochemicals	*Amphiroa sp.*	*Caulerapa racemos*	*Dictyota sp .*	*Halimeda discoidea*	*Hormophysa cuneiformis*	*Jania sp.*	*Laurencia obtusa*	*Padina pavoni*	*Sargassum latifolium*	*Sargassum polycystum*
Alkaloids	+	+	+	+	+	+	++	+	+	+
Anthraquinones	+	+	+	+	+	+	++	+	+	+
Carbohydrates and/or Glycosides	+	+	+	+	+	+	++	+	+	+
Cardiac Glycosides	+	+	+	+	+	+	++	+	+	+
Chlorides	+	+	+	+	+	+	++	+	+	+
Flavonoids	+	+	+	+	+	+	++	+	+	+
Iridoids	+	+	+	+	+	+	++	+	+	+
Saponins	+	-	-	-	+	+	+	+	+	-
Sublimation	+	+	+	+	+	+	++	+	+	+
Sulphates	+	+	+	+	+	+	++	+	+	+
Tannins	+	+	+	+	+	+	++	+	+	+
Unsaturated sterols and/or Triterpenoids	+	+	+	+	+	+	++	+	+	+

(+): Lower amounts of phytochemicals, (++): Moderate amounts of phytochemicals, (+++): Higher amounts of phytochemicals

Table 3. Preliminary phytochemical screening on methanol algal extracts under investigation:

Detected phytochemicals	*Amphiroa sp.*	*Caulerapa racemos*	*Dictyota sp .*	*Halimeda discoidea*	*Hormophysa cuneiformis*	*Jania sp.*	*Laurencia obtusa*	*Padina pavoni*	*Sargassum latifolium*	*Sargassum polycystum*
Alkaloids	++	++	+++	+++	++	+	+	+++	++	+++
Anthraquinones	++	++	+++	+++	++	+	+	+++	++	+++
Carbohydrates and/or Glycosides	++	++	+++	+++	++	+	+	+++	++	+++
Cardiac Glycosides	++	++	+++	+++	++	+	+	+++	++	+++
Chlorides	++	++	+++	+++	++	+	+	+++	++	+++
Flavonoids	++	++	+++	+++	++	+	+	+++	++	+++
Iridoids	++	++	+++	+++	++	+	+	+++	++	+++
Saponins	++	++	+++	+++	++	+	+	+++	++	+++
Sublimation	++	++	+++	+++	++	+	+	+++	++	+++
Sulphates	++	++	+++	+++	++	+	+	+++	++	+++
Tannins	++	++	+++	+++	++	+	+	+++	++	+++
Unsaturated sterols and/or Triterpenoids	++	++	+++	+++	++	+	+	+++	++	+++

(+): Lower amounts of phytochemicals, (++): Moderate amounts of phytochemicals, (+++): Higher amounts of phytochemicals

Table (4) Preliminary phytochemical screening on ethanol algal extracts under investigation:

Detected phytochemicals	*Amphiroa sp.*	*Caulerapa racemos*	*Dictyota sp .*	*Halimeda discoidea*	*Hormophysa cuneiformis*	*Jania sp.*	*Laurencia obtusa*	*Padina pavoni*	*Sargassum latifolium*	*Sargassum polycystum*
Alkaloids	+++	++	++	+	+	+	+++	+	+	+
Anthraquinones	+++	++	++	+	+	+	+++	+	+	+
Carbohydrates and/or Glycosides	+++	++	++	+	+	+	+++	+	+	+
Cardiac Glycosides	+++	++	++	+	+	+	+++	+	+	+
Chlorides	+++	++	++	+	+	+	+++	+	+	+
Flavonoids	+++	++	++	+	+	+	+++	+	+	+
Iridoids	+++	++	++	+	+	+	+++	+	+	+
Saponins	+++	++	++	+	+	+	+++	+	+	+
Sublimation	+++	++	++	+	+	+	+++	+	+	+
Sulphates	+++	++	++	+	+	+	+++	+	+	+
Tannins	+++	++	++	+	+	+	+++	+	+	+
Unsaturated sterols and/or Triterpenoids	+++	++	++	+	+	+	+++	+	+	+

(+): Lower amounts of phytochemicals, (++): Moderate amounts of phytochemicals, (+++): Higher amounts of phytochemicals

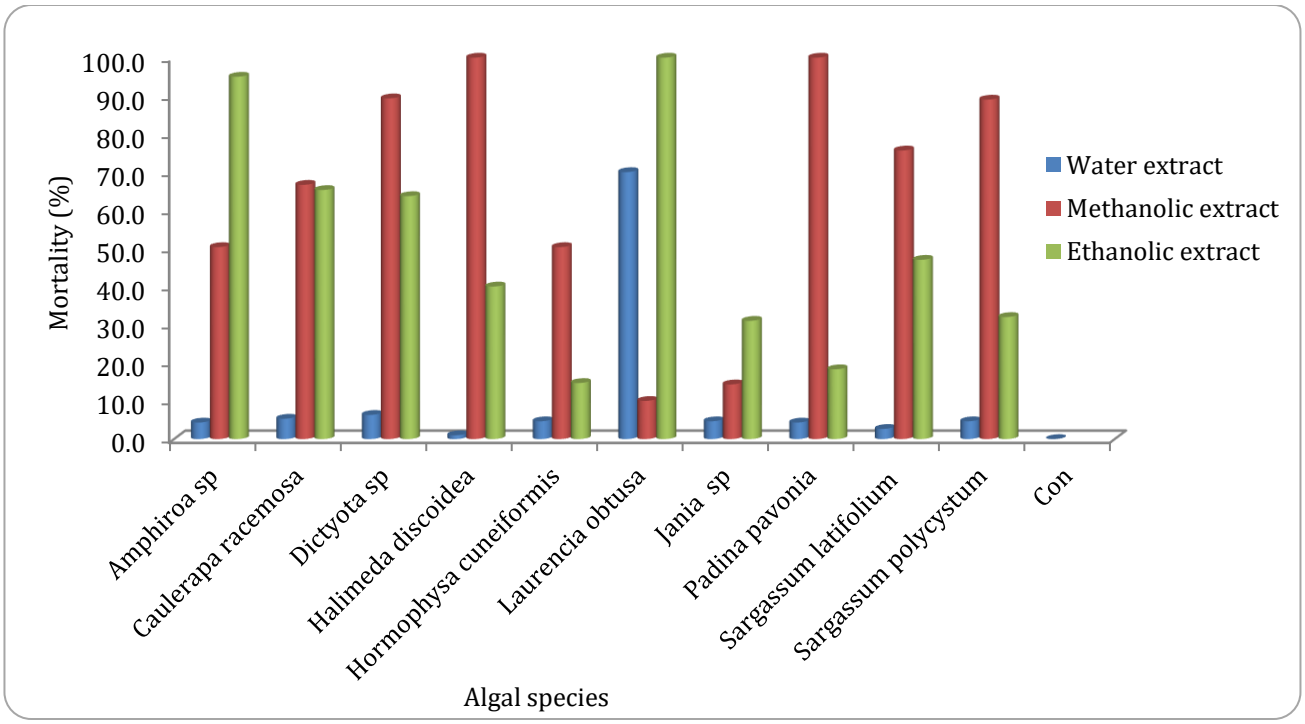


Figure 3. Mortality rate of root-knot nematode juveniles exposed to various investigated algal extracts.

Chemical constituents of such algae were varied according to the species; the algae that showed high nematicidal activity was contained some antinematodal compounds. Previous phytochemical analysis of brown marine algae, *Turbinaria ornate,* extract showed the presence of alkaloids, flavonoids, phenolics, saponins, steroids, tannins and terpenoids, also anthraquinones, carboxylic acid, coumarins, and xanthoproteins were recovered (Paul *et al*. 2013 and Rajkumar & Bhavan 2017). The nematicidal properties are mainly due to phenolic compounds especially tannins. Phenolic compounds are known to behave as reducing agents, hydrogen donators, and singlet oxygen quenchers because of redox properties responsible for their antioxidant and antinematodal activity.

In vivo antinematodal activity of some marine algae may differ from *in vitro* results; Afia and El-Nuby (2016) found that; although the lowest juvenile mortality was achieved by *Padina pavonia* water extract, it was the highest suppressor of *M. incognita* infecting tomatoes. So, greenhouse and field experiments will carry out to realize these antinematodal effects**.**

CONCLUSION

All tested algal extracts were nematotoxic for root-knot nematode juveniles showed nematicidal activity at 0.5 % concentration against *Meloidogyne incognita*. However, extracts of *Laurencia obtusa*, *Halimeda discoidea,* and *Padina pavonia* exhibited maximum nematotoxicity, whereas *Amphiroa, Dictyota,*and *Sargassum* ranked as highly nematode toxicants compared to the rest of seaweeds species. This investigation revealed that; nematicidal potential of all marine algae dependent on their species (genotypes) and extraction protocol, which released various antinematodal compounds; compounds released by methanol were more effective than ethanol and water extracts in most cases**.** Notably, the nematicidal activity is increased in methanolic, then ethanolic and water extracts successively. The present investigation pays attention to the antinematodal activity of marine algae and may offer an additional method, environment-friendly, to combat plant pathogenic nematodes. Furthers *in vivo* studies to ensure their antinematodal potential, validate their efficacy against nematode, know their effects on plant health and determine the proper dose for application are in need.

REFERENCES

Abbasi, W. M., N. Ahmed, N. Zaki and S. S. Shaukat. 2008. Effect of *Barleria acanthoides* Vahl. on root-knot nematode infection & growth of infected okra & brinjal plants. Pakistan Journal of Botany, 40: 2193-2198.

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

Abid, M., M. J. Zaik, M. Q. Khan, M. A. Maqbool and A. Sattar. 2005. Use of marine algae for the management of root-knot nematode (*Meloidogyne javanica*) in okra and tomato plants). International Journal of Phycology and Phycochemistry, 1: 187-192.

Afia, A. I. and A. S. M. El-Nuby. 2016. Seaweeds as a Managing Approach For Root-Knot Nematode Disease. Egyptian Journal of Agronematology, 15: 95-108.

Afia, A. I. and A. S. M. El-Nuby. 2020. Soybean genotypes response to root-gall nematode. Pakistan Journal of Nematology, 38: 130-138.

Afifi, M. 1972. Pharmacological studies on some genera of Polygonaceae and Cucurbitaceae grown in Egypt. Ph.D. Thesis, Pharmacology Department, Faculty of Pharmacy, Cairo University, Egypt: 68.

Alam, E. A. 2019. Natural Phytochemical Products and Our Life (Herbal Medicine from the Land to the Hand), Osiris Publisher, Cairo, Egypt.

Alam, E. A. and A. S. M. El-Nuby. 2019. Phytochemical and antinematodal screening on water extracts of some plant wastes against *Meloidogyne incognita.* International Journal of chemical and pharmaceutical sciences, 10:1-16.

Ara, J., S. Ehteshamul-Haque, V. Sultana, A. Ghaffar and R. Qasim. 1997. Use of *Sargassum* species for the control of *Meloidogye javanica* in Okra. Nematologia Mediterranea, 25: 125-128.

Arunkumar, K., S. R. Sivakumar and R. Rengasamy. 2010. Review on Bioactive Potential in Seaweeds (Marine Macroalgae): A Special Emphasis on Bioactivity of Seaweeds Against Plant Pathogens. Asian Journal of Plant Sciences, 9:227-240.

Atolani O. and O. A. Fabiyi. 2020. Plant Parasitic Nematodes Management Through Natural Products: Current Progress and Challenges. In: Ansari R., Rizvi R., Mahmood I. (eds) Management of Phytonematodes: Recent Advances and Future Challenges. Springer, Singapore, 297-315. doi.org/10.1007/978-981-15-4087-5_13

Balbaa, S. I., H. H. Sayed and Ashgan Y. Z. 1981. Medicinal plant constituent, General organization for university and school books,3rd ed. pp:190-255.

Bebber, D. P., T. Holmes, and S. J. Gurr. 2014. The global spread of crop pests and pathogens. Global Ecology and Biogeography, 23:1398-407

Chtourou, H., I. Dahmen, A. Jebali, F.Karray, I. Hassairi, S. Abdelkafi, H. Ayadi, S. Sayadi and A. Dhouib. 2015. Characterization of Amphora sp., a newly isolated diatom wild strain, potentially usable for biodiesel production. Bioprocess and Biosystems Engineering, 38:1381-1392.

Claus, E. P. (1967): Pharmacognosy, Henery Krimpton, London, 5 th ed.: 168.

Duncan's, D.B. (1955). Multiple ranged multiple F-test-Biometrics, 11:1- 47.

El-Ansarya, M. S. M. and R. A. Hamouda. 2014. Biocontrol of Root-Knot Nematode Infected Banana Plants by Some Marine Algae. Russian Journal of Marine Biology, 40:140-146

El-Eslamboly, A. A. S. A., M. M. Abd El-Wanis and A.W. Amin. 2019. Algal application as a biological control method of root-knot nematode Meloidogyne incognita on cucumber under protected culture conditions and its impact on yield and fruit quality. Egyptian Journal of Biological Pest Control, 29:18.

El-Nuby, A. S. M. 2014. Evaluation of some Resistance Inducers Against the root-knot nematode. PhD Thesis, Faculty of Agriculture, Cairo University, pp 297.

El-Nuby, A.S.M. and E. A. Alam 2020. Phytochemical and Nematicidal Activity Studies of Some Extracts of Different Plant Parts of *Leucaena leucocephala* against *Meloidogyne incognita* International Journal Of Chemical and Pharmaceutical Sciences, 11:1-17.

El-Nuby, A. S. M. and K. E. M. Bayomi. 2019. Evaluation of tomato inbred lines for resistance to root-gall nematode. Egyptian Journal of Desert Research, 69: 33-46.

El-Nuby, A. S. M., S. A. Montasser and I. A. El-Khadrawy. 2020. Control of root-knot nematodes using wild plants colonized Sinai, Egypt. Pakistan Journal of Nematolology, 38: 67-80.

Farnsworth, N. R., H. H. Fong, R. N. Blomster and F.G. Draus. 1969. Studies on Vinca major (Apocyaceae). Journal of Pharmaceutical Science, 51: 217-224.

Feigl, F. 1960. Spot tests in organic analysis. Elsevier Publishing Co, New York, 6th ed.,: 2959.

Forghani, F. and A. Hajihassani. 2020. Recent Advances in the Development of Environmentally Benign Treatments to Control Root-Knot Nematodes. Frontiers in Plant Science, 11.

Ghareeb, R. Y., I. A. Adss, S. R. Bayoumi and D. E. El-Habashy. 2019. The nematicidal potentiality of some algal extracts and their role in enhancement the tomato defense genes against root knot- nematodes. Egyptian Journal of Biological Pest Control, 29: 1-10.

Ghareeb, R. Y., H. Alfy, A. A. Fahmy, H. M. Ali and N. R. Abdelsalam. 2020. Utilization of *Cladophora glomerata* extract nanoparticles as eco-nematicide and enhancing the defense responses of tomato plants infected by *Meloidogyne javanica*. Scientific Reports, 10:1-15.

Hamed, S. M., A. A. Abd El-Rhman, N. Abdel-Raouf, I. B. M. Ibraheem. 2018. Role of marine macroalgae in plant protection & improvement for sustainable agriculture technology. Beni-Suef University Journal of Basic and Applied Sciences, 7: 104-110

Hassan, S. W. M. and A. H. Shobier. 2018. GC/MS identification and applications of bioactive seaweed extracts from Mediterranean coast of Egypt, Egyptian Journal of Aquatic Biology & Fisheries, 22:1- 21.

Hungund, B. L. and C. H. Pathak,: USDA forest, 1971. Service Research Paper, NE: 201.

Ibrahim, D. S. S., R. Y. Ghareeb, Abou El Atta D. A. and H. A. A. Azouz. 2021. Nematicidal Properties of Methanolic Extracts of Two Marine Algae against Tomato Root-Knot Nematode, *Meloidogyne incognita*. Journal of Plant Protection and Pathology, 12: 137-144.

Islam, A. M., E. A. Hassan and I. B. Hannout.1993. Manual of Practical Chemistry, Dar AlMaaref, Egypt, 2nd ed. pp. 19-39.

Jones, J. T., A. Haegeman, E. G. J. Danchin, H. S. Gaur, J. Helder, M. G. K. Jones, T. Kikuchi, R. ManzanillaLópez, J. E. Palomares-Rius, W. M. L. Wesemael and R. N. Perry. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology 14: 946-961.

Mabry, T. T., K. R. Markhan and M. B. Thomas. 1970. The systemic identification of flavonoids. Springer, Verlag, New York, pp. 46-54.

Mesa-Valle, C. M., J. A. Garrido-Cardenas, J. Cebrian-Carmona, M. Talavera and F. Manzano-Agugliaro. 2020. Global Research on Plant Nematodes. Agronomy, 10: 1148

Mouritsen, O. G. 2013. Marine macroalgae benefit people culturally, industrially, nutritionally, and ecologically. American scientist, 101: 458.

Paul, S., N. Devi and G. C. Sarma 2013. Ethnobotanical utilization of some medicinal plants by Bodo people of Manas biosphere reserve in the treatment of malaria. International Research Journal of Pharmacy, 4:102-105.

Prarthana, J. and K. R. Maruthi. 2019. Fresh Water Algae as a Potential Source of Bioactive Compounds for Aquaculture and Significance of Solvent System in Extraction of Antimicrobials. Asian Journal of Scientific Research, 12: 18-28.

Radwan, M. A., S. A. A. Farrag, M. M. Abu-Elamayem and N. S. Ahmed. 2012. Biological control of the root-knot nematode, *Meloidogyne incognita* on tomato using bioproducts of microbial origin. Applied Soil Ecology, 56: 58-62.

Rajkumar, G. and P. S. Bhavan. 2017. Phytochemical characterization of themarine brown alga *Turbinaria ornata*. Research Journal of Chemistry and Environment, 21: 54-63

Rashad, S. and A. G. El-Chaghaby. 2020. Marine Algae in Egypt: distribution, phytochemical composition and biological uses as bioactive resources (a review). Egyptian Journal of Aquatic Biology & Fisheries, 24: 147-160.

Renčo, M., N.Sasanelli and L. Maistrello. 2014. Plants as natural sources of nematicides, In book: Nematodes: Comparative genomics, Disease management and Ecological importance. Nova Science Publishers, Inc, New York, pp. 115-142.

Rizvi, M.A. and M. Shameel. 2006. In vitro nematicidal activities of seaweeds extracts from Karachi coast. Pakistan Journal of Botany., 38: 1245-1248.

Sharma, A., R. Koneri and D. K. Jha. 2019. Review of pharmacological activity of marine algae in Indian coast. International Journal of Pharmaceutical Sciences and Research, 10: 3540-3549.

Shellard, E. J. 1957. Practical plant chemistry. Pitman, Medicinal publishing Co., LTD, London, pp 53-54.

Shreya, A., D. Manisha, and J. Sonal. 2015. Phytochemical screening and antimicrobial activity of cinnamon spice against urinary tract infection and fungal pathogens. International Journal of Life science and Pharma Research, 5: 30-38.

Stank, J., M. Cerny, J. Kocoursk and J. Pacok. 1963. The monosaccharides, Publishing House of the Czechosolovak, Academy of Sciences, Prague. pp. 22-100.

Sultana, V., J. Ara, and S. Ehteshamul-Haque. 2008. Suppression of Root Rotting Fungi and Root Knot Nematode of Chili by Seaweed and *Pseudomonas aeruginosa*. Journal of Phytopathology, 156: 390-395.

Sultana, V., S. Ehteshamul-Haque, J. Ara and M. Athar. 2009. Effect of brown seaweeds and pesticides on root rotting fungi and root-knot nematode infecting tomato roots. Journal of Applied Botany and Food Quality, 83:50-53.

Trease, G. T. and W. C. Evans. 1978. Text book of Pharmacognosy 1978, Bailliere Tindall and Cox, London , 11th Ed. pp. 536-537.

Van den Hoogen, J., S. Geisen, D. Routh, H. Ferris, W. Traunspurger, D.A. Wardle. 2019. Soil nematode abundance and functional group composition at a global scale. Nature, 572: 194-198.

Weiffering, J. H. 1966. Aucubin artigeglucoside und verwandte heteroside als systematische merkmale. Phytochemistry, 5: 1053.

Whapham, C. A., T. Jenkins, G. Blunden and S. D. Hankins. 1994. The role of seaweed extracts *Ascophyllum nodosum* in the reduction in fecundity of *Meloidogyne javanica*. Fundamental & Applied Nematology, 17: 181-183.

Zaki, M. J., A. Sattar, and M. Q. Khan. 2005. Effect of seaweeds on the efficacy of *Pasteuria penetrans* in the control of root-knot nematode, *Meloidogyne javanica* in eggplant. International Journal of Phycology and Phycochemistry, 1: 65-72.

Contribution of Authors:

Ahmad S. M. El-Nuby	:	In vitro studies of the prepared algal extracts, participation in the writing process
Ashraf I. B. Afia	:	Collection and identification of algae
Eman. A. S. A.H. Alam	:	All phytochemical preparations of extracts and phytochemical examinations, participation in the writing and editing processes before and after the submission of the manuscript.