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RESEARCH ARTICLE

Nematicidal efficacy of *Spirulina platensis* on egg hatching and second stage juveniles of *Meloidogyne incognita in vitro*

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Abstract

This study was conducted at the Pesticides Laboratory of Plant Protection Department, Faculty of Agriculture, South Valley University, Qena, to assess the effect of the blue green alga, *Spirulina platensis* on the egg hatching process and second stage juveniles (J_2s) mortality of the root-knot nematode, *Meloidogyne incognita* at five different concentrations: 1000, 500, 250, 125 and 62.5 ppm *in vitro*. Effects were recorded after 2, 4 and 7 days of exposure for egg hatching, while J_2s mortality was determined after 24, 48, and 72 hours of application, compared to a control. The obtained results revealed that all tested concentrations of *S. platensis* inhibited egg hatching and caused high mortality of *M. incognita* J_2s compared to control. Regarding the toxic effects of *S. platensis* on *M. incognita* egg hatching, the maximum inhibition (99.45%) was recorded at a concentration of 1000 ppm, while the minimum inhibition (59.88%) was observed at 62.5 ppm. Meanwhile, the maximum J_2s mortality (93.65%) was observed at a concentration of 1000 ppm after 72 hours of application, with an LC_{50} value of 0.23 ppm. The minimum mortality (43.81%) was recorded at a concentration of 62.5 ppm after 24 hours, with an LC_{50} value of 0.69 ppm. It can be concluded that *S. platensis* has potential effect in controlling *M. incognita* and could be a possible replacement for chemical nematicides.

***Keywords*:** Biocontrol, *Meloidogyne incognita*, *Spirulina platensis*, egg hatching, *in vitro*

1. Introduction

Phyto-nematodes or Plant-parasitic nematodes (PPNs) are considered hidden dangerous pests infecting economic and noneconomic plants all over the world (Bakr *et al*., 2020). It can attack fruits and vegetables causing losses ranging from mild to severe, depending on their population density, the host plant species, and the nematode genus. PPNs cause a 12.3% annual loss of 40 main crops all over the world. Annually, losses are higher in countries that are developing (14.6%) than in developed nations (8.8%). An estimated US$ 173 billion has been lost in economic crop yields as a result of PPNs in key crops (Kumar *et al.*, 2020.; Muhammad *et al.*, 2024). Root-knot nematode, *Meloidogyne* spp., are obligatory, sedentary endoparasites of different plant species (Khan and Ahmad, 2000), these considered the most important nematodes due to their potential host range, which includes over 5000 plant species from different families (Basyony *et al.*, 2020.; Mostafa *et al.*, 2023) and are ranked among the most difficult agricultural pests to control due to their short life cycles, high reproduction rate and wide host range (Archidona-Yuste *et al.*, 2018). *Meloidogyne javanica*, *M. incognita*, *M. hapla*, *and M.*

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arenaria are the four most frequent species of *Meloidogyne*. Infected plants suffer deformation roots and galls formation, which negatively impacts their ability to absorb water and nutrients, ultimately affecting plant growth and production (Kepenekci *et al.*, 2016). Chemical nematicides have important role for controlling strategies of *M. incognita* (Sikora *et al.*, 2018). However, excessive use of them can lead to soil and water contamination and effect on human health. Recently, limitations on the use of some chemical pesticides have been obligatory therefore, there are many studies to screen and develop eco-friendly, greener, chemical-free and sustainable alternatives for controlling nematodes associated with many plant species (Westerdahl, 2021). Nematicidal effect of *Spirulina platensis* is one of the biological control practices that applied on PPNs. In addition to its role in reducing nematode infection and increasing plant productivity, *S. platensis* extracts have been shown to prevent nematode hatching and to cause immobility and mortality of plant parasites (Holajjer *et al.*, 2013). Sharaf *et al.* (2016) stated that *S. platensis* have a strong inhibitory effect on *Meloidogyne* spp., and an effective stimulant effect on the growth of plants. El-Ansary and Al-Saman (2018) said that blue green algae, *S. platensis* have the ability to fix nitrogen in the atmosphere as effective biofertilizers due to its content of certain mineral nutrient components. From the previous points of view, the aim of the present study is to evaluate the toxic effect *in vitro* of *S. platensis* on egg hatching process and J_2s mortality of *M. incognita*.

2. Materials and Methods

This study was conducted at the Pesticides Laboratory of the Plant Protection Department, Faculty of Agriculture, South Valley University, Qena Governorate, Egypt.

2.1. Tested bio- agent

An aqueous extract of the blue-green alga, *Spirulina platensis*, was obtained from the Algal Department, Soils, Water and Environment Research Institute, Agriculture Research Center, Giza, Egypt. In this study, efficacy of *S. platensis* against egg hatching and J_2s mortality of *M. incognita* was determined *in vitro*. Randomized complete design (RCD) was used in the study with five concentrations (1000, 500, 250, 125, 62.5 ppm). Each concentration was replicated three times.

2.2. Preparation of egg masses and J_2s of M. incognita

2.2.1. *Collecting egg masses*

Egg masses of *M. incognita* were collected from infected grape roots. Egg masses were selected by hand from the galled root with help of forceps. The picked egg masses were kept in petri dishes with a diameter of 3 cm containing 2 ml distilled water.

2.2.2. *Preparing suspension of M. incognita J_2s*

Suspension of J_2s was prepared as follows: egg masses were selected by hand from galls of grape roots that infected with *M. incognita*. Then, they were put in distilled water and incubated for 7 days at 28±2 °C (Misiha *et al.*, 2013). Every day, hatched juveniles (J_2s) were collected and kept in refrigeration at 5°C until use. After obtaining suspension of J_2s, counting the juveniles was carried out to calculate the numbers of J_2s in 1 cm^3 of the suspension. Counting of J_2s in 1 cm^3 was repeated three times after which the average number of J_2s per 1 cm^3 of the suspension was calculated under a stereo microscope **(**Poveda *et al.*, 2020).

2.3. Effect of *S. platensis* on egg hatching of *M. incognita*

To determine the effect of *S. platensis* on egg masses, three egg masses of *M. incognita* almost equal sizes were poured into petri dishes (3 egg masses/ petri dish) which containing 5 ml of

each concentration of *S. platensis*. Egg masses put into distilled water only were treated as control. All petri dishes were incubated at 28 ± 2 °C for seven days. The number of hatched J_2s were recorded at 2, 4 and 7 days of exposure under a stereo microscope. Inhibition % in egg hatching was determined as follows:

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

C = no. of hatched juveniles in control after 2, 4 and 7 days.

T = no. of hatched juveniles in each concentration after 2, 4 and 7 days.

2.4. Mortality test for J_2s of *M. incognita*

Two ml of hatched J_2s suspension of *M. incognita* composed of 150±10 juveniles/ml were poured into tubes which contained 2 ml of each concentration of *S. platensis*. The test tubes with nematodes and without any concentration were treated as controls. All test tubes were incubated at 28±2 °C for 3 days and the numbers of live and dead J_2s were recorded at 24, 48 and 72 h for mortality under a stereo microscope. The dead juveniles attained the shape of straight line. Mortality percentage was calculated by equation:

Mortality (%) = (C/T) × 100

C = no. of dead J_2s in each concentration.

T = total number of J_2s in each concentration.

2.5. Data analysis

Data were adjusted using Abbott's formula (1925). Concentration-mortality regression lines were explored across LdP line (modified computer program) from Finney (1971) to assess the LC_{50}, the confidence limits and the slopes.

3. Results

3.1. Effect of *S. platensis* on egg hatching of *M. incognita*

3.1.1. Egg hatching after 2 days

The obtained results in Table (1) showed that the minimum number of hatched juveniles (0.00) was observed with concentration 1000 ppm followed by concentration 500 ppm (8.67), concentration 250 ppm (23.33), concentration 125 ppm (49.33) and concentration 62.5 ppm (192.00), respectively while, the maximum numbers of hatched juveniles (354.00) were observed in control.

Results in Table (2) and Fig. (1) revealed that all tested concentrations (1000, 500, 250, 125 and 62.5 ppm) of *S. platensis* were significantly reduced egg hatching process of *M. incognita* over control. Inhibition percentages were as follows: concentration 1000 ppm (100%), concentration 500 ppm (97.55%), concentration 250 ppm (93.41%), concentration 125 ppm (86.06%) and concentration 62.5 ppm (45.76%), respectively.

3.1.2. Egg hatching after 4 days

The least numbers of hatched juveniles (4.67) were found at concentration 1000 ppm, followed by concentration 500 ppm (18.67), concentration 250 ppm (43.33), concentration 125 ppm (84.67) and concentration 62.5 ppm (316.00), respectively whereas, the maximum numbers of hatched juveniles (849.00) were observed in control Table (1). The obtained results in Table (2) and Fig. (1) indicated that all tested concentrations (1000, 500, 250, 125 and 62.5 ppm) of *S. platensis* were significantly reduced egg hatching process of *M. incognita* compare to control. Inhibition percentages were as follows: concentration 1000 ppm (99.45%), concentration 500 ppm (97.80%), concentration 250 ppm (94.90%), concentration 125 ppm (90.03%) and concentration 62.5 ppm (62.78%), respectively.

3.1.3. Egg hatching after 7 days

Data presented in Table (1) show that the least numbers of hatched juveniles (13.33) were found at concentration 1000 ppm, followed by concentration 500 ppm (23.33), concentration 250 ppm (70.00), concentration 125 ppm

(135.33) and concentration 62.5 ppm (348.67), respectively while, the maximum numbers of hatched juveniles (1206.67) were recorded in control. The obtained data in Table (1) reported that there were significant differences in the numbers of hatched eggs among all tested concentrations LSD 5% = 20.02. The results in Table (2) and Fig. (1) indicated that all tested concentrations (1000, 500, 250, 125 and 62.5 ppm) of *S. platensis* were significantly reduced egg hatching process of *M. incognita* over control. Inhibition percentages were as follows: concentration 1000 ppm (98.90%), concentration 500 ppm (98.07%), concentration 250 ppm (94.20%), concentration 125 ppm (88.78%) and concentration 62.5 ppm (71.10%), respectively. As shown in Table (2) the egg hatch inhibition rate was increased with increasing the concentration. Concentration 1000 ppm (99.45%) was found to be the most effective, followed by concentration 500 ppm (97.81%), concentration 250 ppm (94.17%) and concentration 125 ppm (88.29%) whereas, concentration 62.5 ppm (59.88%) occupied the least effective compare to control.

Table 1. Average numbers of hatched eggs/ 3 egg masses of *M. incognita* treated with five concentrations of *S. platensis* after 2, 4 and 7 days of exposure *in vitro*

Concentration (ppm)	Average no. of hatched eggs/ 3 egg masses			Grand mean
	2 days	4 days	7 days	
1000	0.00	4.67	13.33	6.00 [e]
500	8.67	18.67	23.33	16.89 [e]
250	23.33	43.33	70.00	45.55 [d]
125	49.33	84.67	135.33	89.78 [c]
62.5	192.00	316.00	348.67	285.56 [b]
Control	354.00	849.00	1206.67	803.22 [a]
LSD 5%				20.02

means in column or row followed by different letter (s) are significantly different at 5% level

Table 2. Inhibition percentages of *M. incognita* egg hatching treated with five concentrations of *S. platensis* after 2, 4 and 7 days of exposure *in vitro*.

Concentration (ppm)	Inhibition (%) in egg hatching			Grand mean
	2 days	4 days	7 days	
1000	100.00	99.45	98.90	99.45
500	97.55	97.80	98.07	97.81
250	93.41	94.90	94.20	94.17
125	86.06	90.03	88.78	88.29
62.5	45.76	62.78	71.10	59.88
Control	0.00	0.00	0.00	0.00

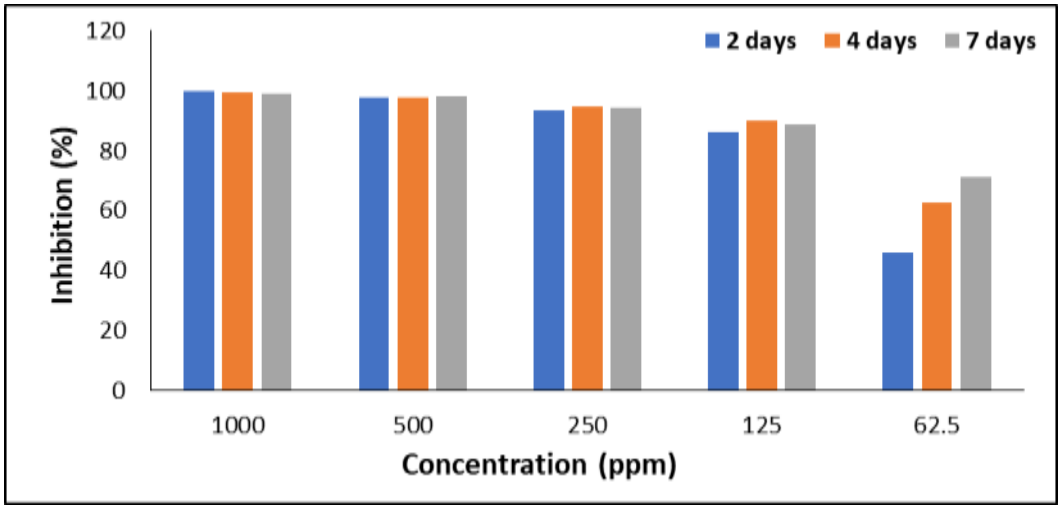

Figure 1. Inhibition percentages of *M. incognita* egg hatching after 2, 4 and 7 days at different concentrations of *S. platensis in vitro*.

3.2. Mortality of M. incognita J_2s

3.2.1. Mortality of J_2s after 24 hours

Data presented in Tables (3&4) and Figs. (2&3) showed that *S. platensis* at concentration 1000 ppm resulted the highest mortality as 85.32% tracked by concentration 500 ppm as 80.59%, concentration 250 ppm of 70.00% and concentration 125 ppm of 63.24%. While, the lowest mortality 43.81% was observed at concentration 62.5 ppm compare to control. LC_{50} and LC_{90} values were 0.69 and 14.75 ppm, respectively, on the other hands, χ^2 value was 4.17.

3.2.2. Mortality of J_2s after 48 hours

Data presented in Tables (3&4) and Figs. (2&3) indicated that *S. platensis* at concentration 1000 ppm resulted the highest mortality of 90.12% tracked by concentration 500 ppm as 85.51%, concentration 250 ppm as 77.98% and concentration 125 ppm as 70.83%. While, the lowest mortality 47.27% was observed at the concentration 62.5 ppm compare to control. LC_{50} and LC_{90} values were 0.57 and 7.76 ppm, respectively, on the other hands, χ^2 value was 5.84.

3.2.3. Mortality of J_2s after 72 hours

Data presented in Tables (3&4) and Figs. (2&3) show that *S. platensis* at 1000 ppm concentration recorded the highest mortality of J_2s as 93.65%, followed by 500 ppm concentration as 89.63%, concentration 250 ppm as 80.83%, and concentration 125 ppm as 77.14%. While, the lowest mortality was recorded at concentration 62.5 ppm as 61.59% compare to control. LC_{50} and LC_{90} values were 0.23 and 7.69 ppm, respectively, on the other hand, χ^2 value was 6.40. As shown in Table (3) and Fig. (2) mortality percentages in J_2s were increased with the increase of *S. platensis* concentration as well as the exposure period. The maximum mortality in J_2s was observed at concentration of 1000 ppm after 72 h of application (93.65**%**), while the minimum mortality was recorded at concentration 62.5 ppm after 24 h (43.81**%**).

4. Discussion

The obtained results are in harmony with that conducted by Gerwick *et al.* (2001) who reported that *S. platensis*, produced a wide range of secondary metabolites, like nitrogen containing compounds, polyketides, lipopeptides, cyclic peptides and others.

Table 3. Mortality percentages of *M. incognita* J_2s after 24, 48 and 72 h treated with *S. platensis in vitro.*

Concentration (ppm)	Mortality (%)		
	24 h	48 h	72 h
1000	85.32	90.12	93.65
500	80.59	85.51	89.63
250	70.00	77.98	80.83
125	63.24	70.83	77.14
62.5	43.81	47.27	61.59
Control	0.00	0.00	0.00

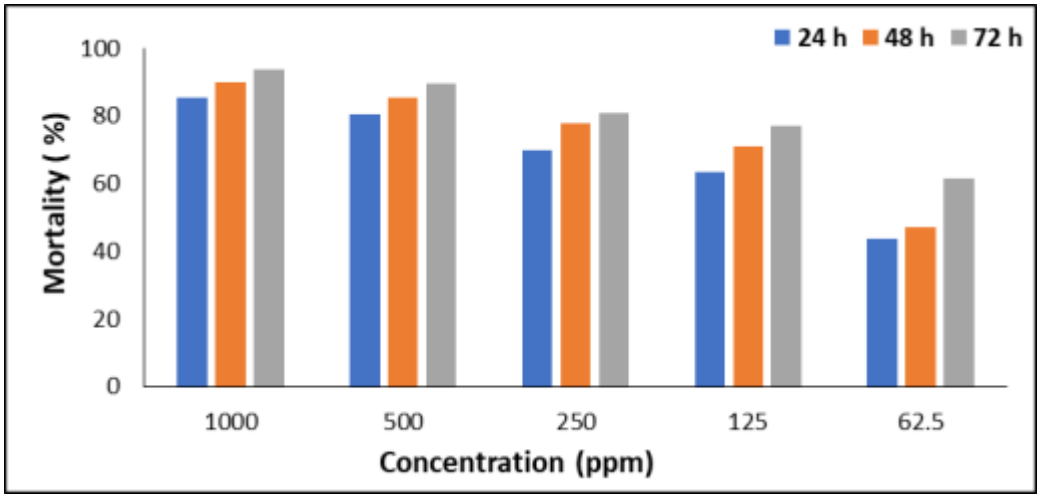

Figure 2. Mortality percentages of *M. incognita* J_2s treated with *S. platensis* after 24, 48 and 72 h exposure *in vitro*

Table 4. Toxicity of *S. platensis* on *M. incognita* J_2s after 24, 48 and 72 h *in vitro*

Time exposure	χ^2	LC_{50} (ppm)	Confidence limits of LC_{50}		LC_{90} (ppm)	Slope ± SE	T. I.
			Lower	Upper			
24 h	4.17	0.69	0.49	0.89	14.75	0.96±0.10	32.80
48 h	5.84	0.57	0.40	0.74	7.76	1.14±0.12	39.55
72 h	6.40	0.23	0.09	0.37	7.69	0.84±0.13	100.00

χ^2 = Chi-square T. I. = Toxicity Index (compared with *Spirulina platensis* after 72 h.)

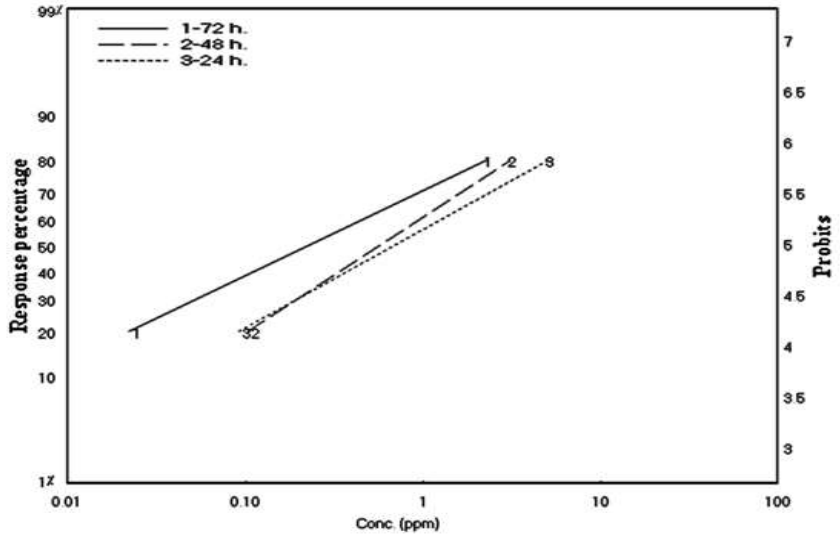

Figure 3. Toxicity of *S. platensis* on *M. incognita* J_2s after 24, 48 and 72 h of exposure *in vitro*.

In addition, Shawky *et al.* (2009) found that the highest mortality rates were observed for nematode juveniles exposed to the algal culture filtrates might be caused by the presence of certain mineral salts and phenolic chemicals that speed up the rate at which algal byproducts penetrate, hence increasing their detrimental effects. Also, the obtained results are confirmed with that of Shawky *et al.* (2014) who used extracts of *S. platensis, Anabaena azollae*, *Azolla pinnata* and *Pleurotus columbinus* in addition to olive mill waste to control *M. javanica* that infecting banana trees *in vitro*, and found that the juvenile mortality rates were high at all exposure periods of all treatments, the best results were obtained after 72 h of exposure. Furthermore, they reported that, the highest concentration of 1:10, *S. platensis*, followed by *A. azollae, A. pinnata, P. columbinus* and olive watery extract were considerably raised juvenile mortality up to 70% after 72 h (85.2, 81.4, 79.9, 73.5, 71.7, and 70.1%, respectively). Also, Abdel Rasoul (2017) assessed the toxicity effect of four marine algae species on *M. incognita*: *Ulva fasciata* Delile (UF) (green algae), *Corallina mediteranea*, *Corallina officinalis* (red algae) and *S. platensis* (blue green algae) at four concentrations (125, 250, 500 and 1000 µg/ml) in the laboratory, and found that all tested algae were significantly reduced egg hatching and caused mortality of J_2s. In addition, Annapurna *et al.* (2018) evaluated defense-related enzymatic activities of the fungal bioagents viz., *Trichoderma viride*, *T. harzianum*, *Pochonia chlamydosporia* and *Purpureocillium lilacinum* against , *M. incognita* on tomato , and revealed that all tested fungal bioagents have the ability to induce defense-related enzymatic activity against *M. incognita* which resulted in increase in plant growth and decrease in nematode multiplication in root and soil 30 and 45 DAI, Also they added that the mortality of *M. incognita* was increased with exposure time and concentration, due to the release of lytic enzymes such as chitinases, lipases, and acetic acid, which break down nematode cuticle proteins, and may be the cause of high mortality of J_2s.

5. Conclusion

All tested concentrations of *S. platensis* considerably reduced hatched eggs as well as increased mortality of *M. incognita* J_2s compare to untreated control. So that, it can be used as a biological control agent against PPNs that is both safe and friendly to the environment.

Declarations

Authors' Contributions
All authors are contributed in this research
Funding
There is no funding for this research.
Institutional Review Board Statement
All Institutional Review Board Statements are confirmed and approved.
Data Availability Statement
Data presented in this study are available on fair request from the respective author.
Ethics Approval and Consent to Participate
Not applicable
Consent for Publication
Not applicable.
Conflicts of Interest
The authors disclosed no conflict of interest.

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