Using Biosynthesized Zinc Oxide Nanoparticles to Alleviate the Toxicity on Banana Parasitic-Nematode

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Research Article

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Abstract

We appraised the use of zinc oxide nanoparticles, (ZnO-NPs) and zinc oxide bulk (ZnO-bulk) or zinc acetate, as a natural nematocide, alone or in combination with oxamyl *in vitro* and *in vivo* trials in order to improve systems for root-knot nematode (RKNs) control in banana plants. Especially, ZnO-NPs were biosynthesized from the alga, *Ulva fasciata*. In general, all applications of ZnO-NPs were more effective to control RKNs than ZnO-bulk as well oxamyl alone (chemical control). In *in vitro* conditions, ZnO-NPs with oxamyl showed 98.91% second stage juveniles2 (J2s) mortality of *Meloidogyne incognita* after 72 hrs, while 72.86% mortality was observed at the same NPs treatment without oxamyl at the same exposure time. The same treatment was the most effective in diminution of J2s community (82.77%) in soil and galls number (81.87%) in roots under *in vivo* conditions. In contrast, the highest weight and height of the shoot was observed in Zn-bulk treatment in combination with oxamyl as well oxamyl only (nematocides check). Scanning electron microscopy (SEM) reports displayed the distributions and accumulations of ZnO-NPs on the nematode (J2s) body under direct exposure, which might be the reason of NP-mediated toxicity and disruption for *M. incognita*.

Highlights

- Zno-NPs biosynthesis by macrogreen alga *Ulva fasciata* and give nanorode particles.
- Biogenic ZnO-NPs were used as nematocidal against root-knot nematodes (RKNs) solely and in combination with oxamly *in vitro*and *in vivo.*
- ZnO-NPs exhibited high nematocidal activity *in vitro*and *in vivo*.
- Banana plants infected with RKNs and treated with ZnO-NPs in combination with oxamly showed high growth.

Statement Of Novelty

This study offers importance of green technology for the development of biosynthesis ZnO-NPs, and in combinations with oxamyl in control RKNs (*in vivo* and *in vitro*). Moreover, it is possible using biosynthesis ZnO-NPs in alternative to pesticide and also to enhancement the plant yields.

Introduction

Nanoparticles (NPs) are widely employed in the environment due to their unique optical, electronic and magnetic characteristics [1,2,3]. Among the NPs, Zinc oxide nanoparticles (ZnO-NPs) are used in several industries, medicine and agriculture [4,5], due to their unique characteristics which differ from their particular bulk counterparts of the same synthesis [6,7], such as morphology, size and distribution [8]. The NPs are also used in the gene delivery [9](Manna and Bandyopadhyay, 2019) or as a synthetic drug [10,11,12]. The optical characteristics of ZnO-NPs can be controlled by producing these particles in combination with suitable elements. The use of NPs in agriculture has become increasingly popular over

the few past decades [13,14]. The optical characters of ZnO-NPs can be controlled by producing these particles in combination with suitable elements. The NPs can be synthesized through several ways and the bio-synthesis of NPs using marine algae has been under manipulated. Bio-synthesis of NPs using marine algae has become of great interest [15]. Microalgae and macroalgae are being utilized as bio-factories for the production of NPs. Significantly, little information are available regarding the biosynthesis of NPs using microalgae, which are being utilized as a bio-factory for the production of NPs [16]. Furthermore, the microalgae have a renewable origin; it could be effectually studied in the biosynthesis of nanoparticles [17]. ZnO-NPs have also unique toxic properties which might be ascribed to the production of reactive oxygen species (ROS) in living cells [18,19]. ROS formation can occur in protein, membrane and DNA of host cells, and it may cause damage in biological systems **[3,20,21]**. The plant-parasitic nematodes (PPNs) are among the major factors which affect the growth and development of several crops [22, 23]. Owing to the intense cultivation of crops, the pests increase at an alarming rate leading to crops yield reduction. Thus, there is an urgent need to manage the pests to boost crops growth **[24]**. A chemical nematocide is an artificial compound used to inhibit PPNs. The use of these artificial nematocides to manage PPNs can result in extreme hazards in the environment and also cause mammalian health risks [25, 26]. Thus, more environment friendly techniques are required to control pests in an appropriate way. Accordingly, the use of NPs for the purpose of controlling plant pathogens is a novel method **[13]** that may be effective in the future through the progress of nanotechnology applications **[27, 28, 29]**. ZnO-NPs may affect (i) the creatures causing physical damage a result of direct interaction, (ii) the zinc ions dissolution and (iii) the ROS production [30]. The other mechanism of ZnO-NPs toxicity in eukaryotic living cells with pinocytosis, is through allowing ZnO nanoparticles to pass through the host cells and unite with the cell organelles, leading to the cells being damage [31].

It was hypothesized that ZnO-NPs—as a biosynthesis nematicides— ould be a novel and safe technology to constrain the usage of chemical hazards in the environment like artificial oxamyl. Besides this, ZnO-NPs may help in depressing RKNs, *M. incognita* and may cause beneficial effects on banana health. The present research has demonstrated the impact of ZnO-NPs and their particular bulk (Zn acetate or ZnO-bulk) treatments alone or in combination with oxamyl against RKNs, *M. incognita* in potted banana plantlets.

Materials And Methods

Biosynthesis of ZnO-NPs by *Ulva fasciata* and their characterization

Ten mL of *U. fasciata* extracts (1 g of dry alga in 100 mL distilled water, 1 hr. boiling and then filtered) were added to 40 mL of distilled water containing 0.02 M Zn acetate dehydrates by constant stirring. After 10 minutes of stirring, 2.0 M NaOH was added and then stirred for 2 hrs. The pale white precipitation was obtained and then filtrated and washed two times with distilled water and one time by absolute ethanol and then dried in electric oven at 60 °C overnight [32]. Chemical structure of the green synthesis of ZnO-NPs were determined by Energy Dispersive X-ray spectroscopy (EDX). The morphology and particle size of NPs was determined by Transmission Electron Microscope (TEM).

Nematode inoculation.

Eggs of the root-knot nematode (RKN), *Meloidogyne incognita*, were extracted from tomato (*Lycopersicon esculentum* cv. Castle Rock) roots infested with the RKN using 0.5% sodium hypochlorite (NaOCl) solution [33]. Juveniles2 (J2s) were stored daily from the eggs at 15 °C. The J2s used in the trials were less than 5 days old.

Treatments

There were total six treatments including zinc oxide nanoparticles {ZnO-NPs}; Zinc oxide bulk or Zinc acetate dehydrates {Zn $(O_2CCH_3)_2$ or ZnO-bulk}; {ZnO-NPs + Oxamyl}; {ZnO-bulk + Oxamyl}; {nematocides check (Oxamyl only, Vydate 24%L)}; {Nematode check}, {Check free}.

The in vitro treatment

A volume of 1 mL active RKNs suspension containing about 100 J2s of *M. incognita* was placed in sterilized Petri-dishes (9 cm diameter) and mixed with constant volume of each treatment to prepare (ZnO-NPs and ZnO-bulk in concentration 0.5 mg/mL); (ZnO-NPs and ZnO-bulk in concentration 0.25 mg/mL mixed with 12.5 µl oxamyl and complete to 4 mL by distilled water, replicated five times of the previous treatments. Also, five inoculated petri-dishes were treated with 25 µl of oxamyl only (nematocides check). The five remaining injected petri-dishes served as nematode only without treatment (nematode check). Distilled water was used as a check. All applications were incubated at 27 °C. The number of active and inactive J2s was counted after 24, 48 and 72 hrs. Abbott formula [34] was used to determine the percentage of mortality in comparison with control whereas **X= ME-MC / 100-MC X 100***.

*(**ME**: The percentage of mortality in each treatment; **MC**: The percentages of mortality in check; **X**: The percentage of mortality in comparison with check).

The physical and chemical properties of the used soil

The structure of the artificial soil was prepared in laboratory conditions (containing 4.5 Kg/pot) of sterilized soil mixture (clay/sand 1:2) with pH 7.26. It included sand (25.33%), silt (35.30%), and clay (39.37%). The other physical and chemical characteristics of the soil texture are recorded in Table 1. Micro and macroelements were measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Thermo Scientific).

Thein vivo treatment

The banana plantlets cv. Grande-Naine (2 months-old) were received from the Tissue Culture Lab. of the Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat University, Egypt. Plantlets were placed in pots (30 cm diameter) already filled with 4.5 kg of artificially sterilized soil (1:2; clay: sand ratio) with a final pH of 7.0. The temperature of the soil was maintained at 28±2 °C during the whole experimental period. 30 pots were inoculated with 2,000 active J2s per pot at the time of seedling

transplantation. Five days later, 10 pots were injected with 2g per pot of each previously treatment (replicated five times) after preparation, with ZnO-NPs and ZnO-bulk. In addition, the same numbers were inserted with 1g per pot of the previous treatments followed by 0.5mL oxamyl. Five numbers of infested pots were treated with 1 mL oxamyl per pot (nematocides check). The remaining five inoculated pots were used as untreated nematode control (nematode check). Moreover, five pots were determined as untreated and non-inoculated control (check free). All tested applications were applied to the soil in 3 holes around the plant and followed by the application of 50 mL of water. All the pots were placed in a completely randomized design under greenhouse conditions. Plants were harvested after two months of inoculation. The roots were washed carefully with tap water to remove the soil particles and stained with artificial "Phloxine B" solution (3.5g in 750 mL distilled water + 250 mL acetic acid 5%) for 5 min to facilitate the counting of all nematode stages in the root. Nematode variables were observed per root system including galls, females and females with egg-masses. The second stage J2s per 250 g soil was extracted and counted according to Cobb's sieving and decanting method, using sieves (20 meshes, 60 meshes and 325 meshes). The banana growth variables such as shoot length, shoot weight, rhizome length and weight and corm weight were measured and recorded.

Scanning electron microscope (SEM) analysis of juveniles2

The juveniles2 (J2s) of *M. incognita* treated with ZnO-NPs within 24 hrs. obtained from the laboratory assays study were fixed in a 2% glutaraldehyde with 2% paraformaldehyde solution in a (0.1 M) sodium phosphate buffer (pH 7.4) for 4 hrs at 4^{o}C. After 3 washes in the same buffer (0.1 M) with (0.1 M) sucrose, the J2s were dehydrated in a graded ethanol series, at the following:

- dehydrate 2 x 15 min: 50 % ethanol (in distilled water).
- contrast overnight using 70 % acetone + 0.5 % uranyl acetate + 1 % phosphotungstic acid at 4° C.
- 2 x 15 min. 80 % ethanol. - 2 x 15 min. 90 % ethanol.
- 2 x 15 min. 96 % ethanol. - 3 x 20 min. 100 % ethanol.

Finally, the specimens were placed and covered with gold-palladium membranes and were noticed in a Jeol JSM-6510 L.V SEM. The microscope was functioned at 30 KV at electron microscope (EM) Unit, Mansoura Univ., Egypt.

Statistical analysis

All data were subjected to analysis of variance (ANOVA). Significance of the variable mean differences was prescribed using Duncan's multiple range tests ($p \leq 0.05$). All data analyses were conducted using SPSS 16 software [35].

Result

ZnO nanoparticles characterization

The results obtained from the Transmission Electron Microscopy (TEM) image demonstrated that biosynthesis of ZnO-NPs had a nanorod shape **(Fig. 1).** The major composition of the biosynthesized nanoparticles contains carbon, oxygen and zinc as depicted from the energy dispersive X-ray spectrophotometry analysis **(Fig. 2).**

Evaluation of the *in vitro* trial

Influence of ZnO-NPs and their bulk alone or in combination with oxamyl on mortality of second stage J2s in *in vitro* experiment, was observed and the results are reported in figure 3. Apparently, the percentage of nematode mortality was increased with the increase of exposure time (minimum at 24 hrs. and maximum at 72 hrs.). In general, ZnO-NPs with oxamyl showed 98.91% J2s mortality at 72 hrs., while the same treatment without oxamyl recorded 72.86% mortality at the same exposure time. At 24 hrs. exposure, ZnO-NPs with oxamyl provided the greatest nematode mortality (90.51%) while nematode control gave a minimal of 2.74%. Similarly, oxamyl alone as a chemical control showed 91.76% and 100% nematode mortality at 24 hrs. and at 72 hrs., respectively.

Evaluation of the *in vivo* trial

Nematocidal evaluation

The influence of the current applications on the RKN, *M.incognita* contaminating banana plantlets caused a marked depression of the nematode community in *in vivo* experiment (Table 2). All the tested materials were compared with oxamyl only (nematocides check) and the results indicated a significant inhibition in number of galls, females and females with egg-masses per rhizome system; and number of J2s in soil **(Table 1).** Notable, the treatment of ZnO-NPs with oxamyl was the most effective in reducing J2s community (82.77%) in soil and galls number (81.87%) in roots. In contrast, ZnO-bulk with oxamyl reduced the number of females (82.09%) and females with egg-masses (79.53%) in roots **(Fig. 4).**

Plant health measurements

Growth criteria of banana plants, such as length and weight of shoots and rhizomes, and weight of corms, are documented in table 3. All the applications exhibited enhancement in plant health. On the contrary, no notable increase in corm weight was established.

The highest outcome for weight and height shoot was stipulated by bulk-ZnO with oxamyl than nematode check **(Table 3).**

Morphological analysis of ZnO-NPs and nematode

Figure 5 displays the SEM pictures, the *in vitro* investigations have been developed with a relatively high disruption of the nematode body, in addition ZnO-NPs distributions on the body of treated nematode comparing nematode control. These finding demonstrated that NPs had obviously disrupted in the

nematode body after treatment *in vitro* after one day **(Fig. 5 C&D).** The nano-ZnO are clearly pictured as spherical nano-organized particles on the nematode body **(Fig. 5 E&F).**

Discussion

ZnO nanoparticles characterization.

The results obtained from the Transmission Electron Microscopy (TEM) image demonstrated that biosynthesis of ZnO-NPs had a nanorod shape (Fig. 1). A nanorode shape of green ZnO-NPs that synthesis by using green alga like, *Chlorella vulgaris* [36]*, Chlamydomonas reinhardtii* [37] and *Santalum album* [38]. The major composition of the biosynthesized nanoparticles contains carbon, oxygen and zinc as depicted from the energy dispersive X-ray spectrophotometry analysis (Fig. 2). Whereas, the weight of Zn and oxygen was over 50% of the total constitute [39].

Effect of the ZnO-NPs on nematode community in Petri-plates

In the current study, nano-ZnO can produce toxic effects in the RKN under different exposure times in *in vitro* conditions. Using different ZnO-NPs applications gave high toxicity compared to ZnO-bulk for *M. incognita* through 72 hrs. Toxicity assay of synthesized nano-ZnO were produced using the free-living nematode, *Caenorhabdities elegans*, which has been applied commonly in ecotoxicological researches [6,40,41]. Generally, ZnO-NPs in combination with oxamyl presented 98.91% nematodes J2s mortality at 72 hrs, while the same treatment without oxamyl recorded 72.86% mortality at the same exposure time. *In vitro* assays also showed the nematocidal action of bio-synthesized nanoparticles that may provide an alternative method to avoid high-risk of artificial nematocides [13,42].

Effect of nano-ZnO and bulk-ZnO on nematode community in soil and roots.

Results established that NPs successfully controlled the RKNs counts in soil samples and plant-roots by using ZnO-NPs and its bulk, alone or in combination with oxamyl which presented nematocidal action to a variety of PPNs that may extremely harm the banana plants [43]. Evaluation of ZnO-NPs trial *in vivo* and bio-synthesized NPs by algae confirmed its beneficial role for RKN control and nematode community reduction in soil and roots. Currently, there were several studies that examined the toxicity of nano-ZnO towards various pests, especially nematodes [44,45, 46]. The field assessment revealed its benefits for modifying injury caused by RKN in plants [47,48,49]. Overall, all treatments of ZnO-NPs compared to bulk-ZnO as well oxamyl alone (chemical check) were more effective to RKN control due to their defensive power. ZnO-NPs also revealed superior high toxicity than its bulk due to the smaller particles of ZnO-NPs having a larger surface area/ unit mass [50]. The toxic effects of nano-ZnO were examined in the case of several animals utilized in bio-monitoring, for instance nematode, *C. elegans* [6]. In general, the treatment of ZnO-NPs with oxamyl was the most effective in the reduction of *M. incognita* community J2s (82.77%) in soil and count of galls (81.87%) on roots.

Effect of the ZnO-NPs and its bulk on banana growth

All applications of the tested treatments as well oxamyl caused relatively an incredible increase in banana growth than nematode check. In addition, the highest outcome of the weight and height of the banana shoot was achieved by bulk-ZnO with oxamyl than nematode check. The Zn-bulk enhanced the vegetative growth of plants [51], for this reason it has been mainly applied in lettuce plant that obtained the maximum weight [52]. Zinc ion is the main component of several proteins and it is playing a crucial role in organizing domain fixation of plant protein through association with the plant DNA [32]. Consequently, Zn deficiency can affect the plant growth [54] and creation of the plant proteins [55, 56].

Effect of the ZnO-NPs distribution on nematode body

The SEM alterations observed in the laboratory experiments demonstrated the occurrence of relations between both the ZnO-NPs and some J2s structures, especially the nematode cuticle, which has a crucial role in parasitic-nematode functions. Generally, these laboratory investigations have been developed with a relatively high disruption of the nematode body and occurred of nano-ZnO particles after one day with nematodes. These similarities showed that both nanoparticles and their bulk ZnO, Al_2O_3 and TiO_2 equals were toxic and inhibited the development of the parasitic-nematode in *in vitro* trial [57]. For instance, the 24 hrs LC50 for nano-ZnO (2.3 mg/L) and ZnO-bulk were not significantly ($p < 0.05$) different among nano-ZnO, ZnO-bulk and $ZnCl_2$. Similarly, the 24 h LC50 for both Al^{3+} and Zn^{2+} to free living nematode, *C. elegans* in Petri-dishes were recorded as 79 and 202 mg/L, respectively [58].

Nematocidal activity and toxicity mechanism of ZnO-NPs

In the published research, a novel management strategy was discovered for controlling PPNs based on biosynthesis of ZnO-NPs by microalga [16, 59]. The successful applications of bio-ZnO NPs discovered from laboratory experiments were utilized to a continuous banana-potting to study its effects on nematodes control and the RKNs community both in soil and roots. Many potential RKNs depression approaches have revealed exceptional effects *in vitro* and *in vivo* experiments; notwithstanding, these methods are costly because they used artificial ZnO-NPs to mainly inhibit pests [60]. Therefore, there is now excessive stimulation to create bio-nanopesticides, which are less injurious to the environment and costs little compared to synthesis formulations [13]. The several mechanisms of nanoparticles against pests are frequently related with their high surface to particle size ratio [61] and with their unique physical and chemical properties [8]. In general, we can summarize the different mechanisms of antibacterial action of nanoparticles according to the literature as follows: (i) reactions between nano-ZnO and bacterial cell walls causing cell walls destruction, whereas liberation of Zn^{+2} ions occurs by accumulating Zn nanoparticles in bacteria cells [62] (ii) reactive oxygen species (ROS) are formed [63]. (iii) creation of free radicals[64, 65], that stimulate cell membrane injury and reaction with deoxyribonucleic acid (DNA) happens resulting in bacterial cell death [66]. Hence, this research used ZnO nanoparticles activity and its bulk alone or in combination with oxamyl against plant-nematodes infestation in *in vitro* and *in vivo* conditions as a novel method.

Conclusions

This study presented an alternative method for controlling RKNs, *M. incognita* in banana which might be applied to several crops. We have also described a new way to biosynthesis nematocidal of ZnO-NPs using microgreen alga, *U. fasciata*. The toxicity of nano-ZnO and its bulk against parasitic-nematodes is mainly determined by the exposure time in *in vitro* conditions. Also, our results of the *in vivo* experiment confirmed that all treatments were effectual approaches to reduce the nematode community and to promote banana health. Electron microscopy (SEM) studies showed that the distributions and accumulations of nano-ZnO on the body of nematode J2s under direct exposure, which aim to assess the influence of NPs to the toxicity for *M. incognita*.

Abbreviations

PPNs: Plant-parasitic nematodes; **RKNs**: root-knot nematodes; **ZnO NPs**: Zinc oxide nanoparticles; **Zinc acetate, ZnO-bulk**: zinc oxide bulk; **J2s**: juveniles2; **SEM**: Scanning electron microscopy.

Declarations

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Authors' contributions

M.S.M.E. carries out of the experiment, which related by nematode control and SEM, interpreted the data and contributed substantially to the writing and revising the manuscript. R.A.H. design the experiment performed the statistical analysis, analyzed and interpreted the data and contributed substantially to the writing and revising of the manuscript. M.E provided some necessary tools, participated in ZnO-NPs characterizations.

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Availability of data and materials

All data are available in the manuscript and the materials used in this work are of high quality.
The data and materials of this study have been presented in the manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Conflict of interest

The authors declare that has no conflict of interest

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Tables

Table 1 The physiochemical properties of the tested soil

Parameter	Value
pH	7.26
EC (dS m1)	580.3
Total dissolved salts (ppm)	383
Chloride (ppm)	167
Carbonate (ppm)	271
Sodium adsorption ratio (SAR)	167
Micro and macro-elements (ppm)	
Na^{+}	40.05
Mg^{2+}	19.43
K^{+}	27.65
Ca^{2+}	2.67
Mn^{+2}	13.39
Fe^{+2}	0.43
Zn^{+2}	13.39
Cu^{+2}	3.28
Cd^{+2}	1.53
Co^{+2}	1.71

Table 2 Effect of ZnO-NPs and ZnO-bulk alone or in combination with oxamyl used for nematocidal actions

Treatments	Number per root			Number per 250 g. soil
	Galls	Females	Females with egg - masses	Juveniles2 (J2s)
ZnO-NPs	319.14 b	482.2 b	391.21 b	1846.5 b
ZnO-bulk	397.67 b	469.02 b	393.55 b	2635.75 b
ZnO-NPs + oxamyl	175.27 a	249.73 ab	234.27 ab	1098.75 a
ZnO-bulk + oxamyl	211.54 a	257.98 ab	176.01 ab	1354 ab
Nematocides check (Oxamyl only)	153.45 a	186.79 a	122.51 a	767.25 a
Nematode check	966.86 c	1193.08 c	860.22 c	6377.75 c
L.S.D	0.000	0.000	0.000	0.000

Means followed by the same letter(s) within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

Table 3 Effect of ZnO-NPs and ZnO-bulk alone or in combination with oxamyl used for enhancing plant health

Treatments	Shoot		Rhizome		Corm
	height	weight	height	weight	Weight
ZnO-NPs	40 ^{ab}	31.96 ^{ab}	25.5 ^{b}	18.47 ^{bc}	12.37 ^{a}
ZnO-bulk	39.25 ^{ab}	27.07 ^{ab}	23 ^{b}	10.97 ^{ab}	8.27 ^{a}
ZnO-NPs + oxamyl	35.25 ^{a}	24.35 ^{a}	12.25 ^{a}	5.05 ^{a}	5.48 ^{a}
ZnO-bulk+ oxamyl	45.25 ^{bc}	30.63 ^{ab}	17.25 ^{ab}	11.08 ^{ab}	14.08 ^{a}
nematocides check (Oxamyl only)	40.25 ^{ab}	39.62 ^{bc}	16 ^{ab}	8.64 ^{ab}	11.96 ^{a}
Nematode check	39.5 ^{ab}	19.25 a	18.5 ^{ab}	16.14 ^{b}	11.29 ^{a}
Check	50 ^{c}	50.26 ^{c}	20.25 ^{ab}	27.19 ^{c}	43.08 ^{b}
L.S.D	0.033	0.001	0.09	0.002	0.002

Means followed by the same letter(s) within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

Figures

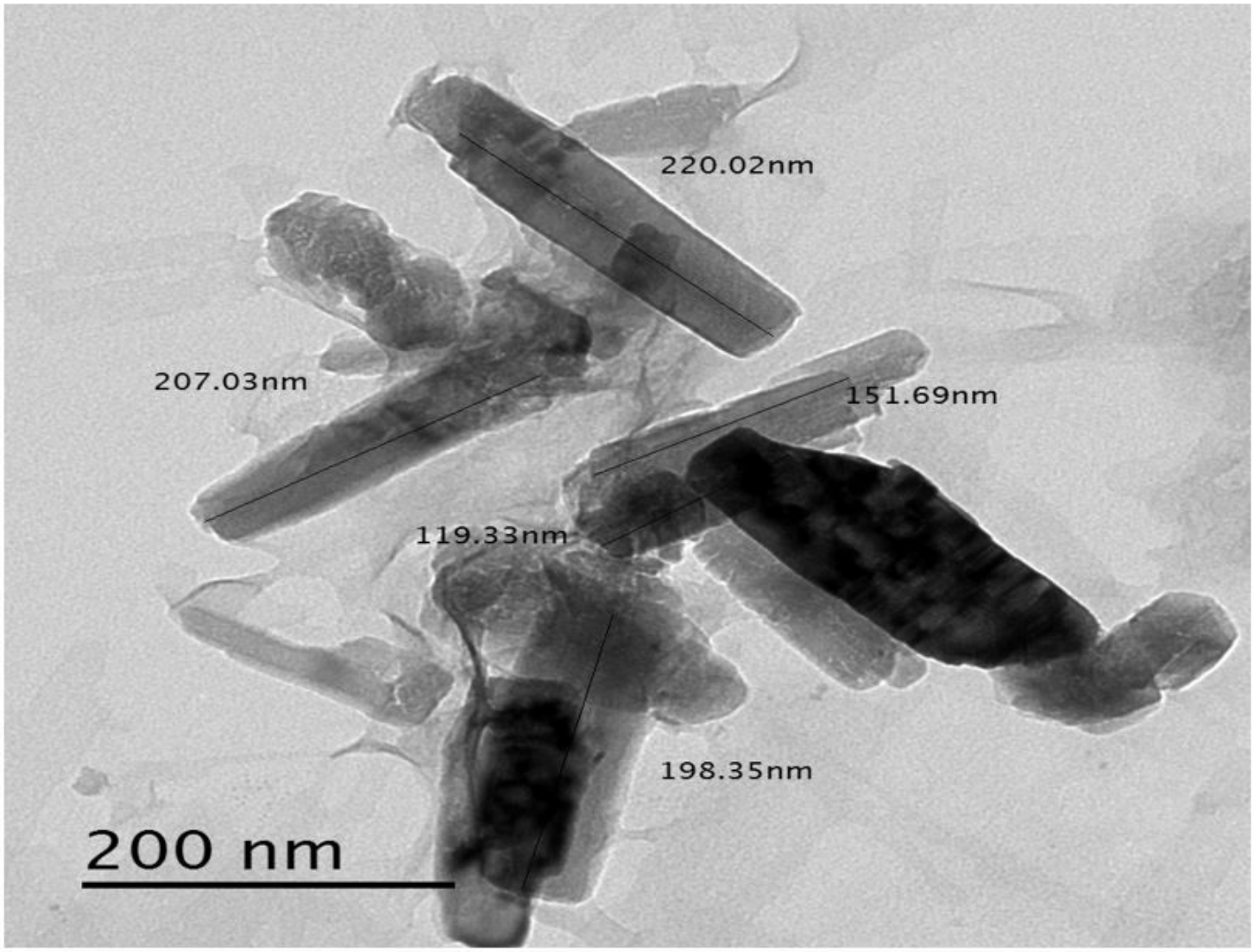

Figure 1

TEM image of the ZnO-NPs synthesized by the green alga, Ulva fasciata.

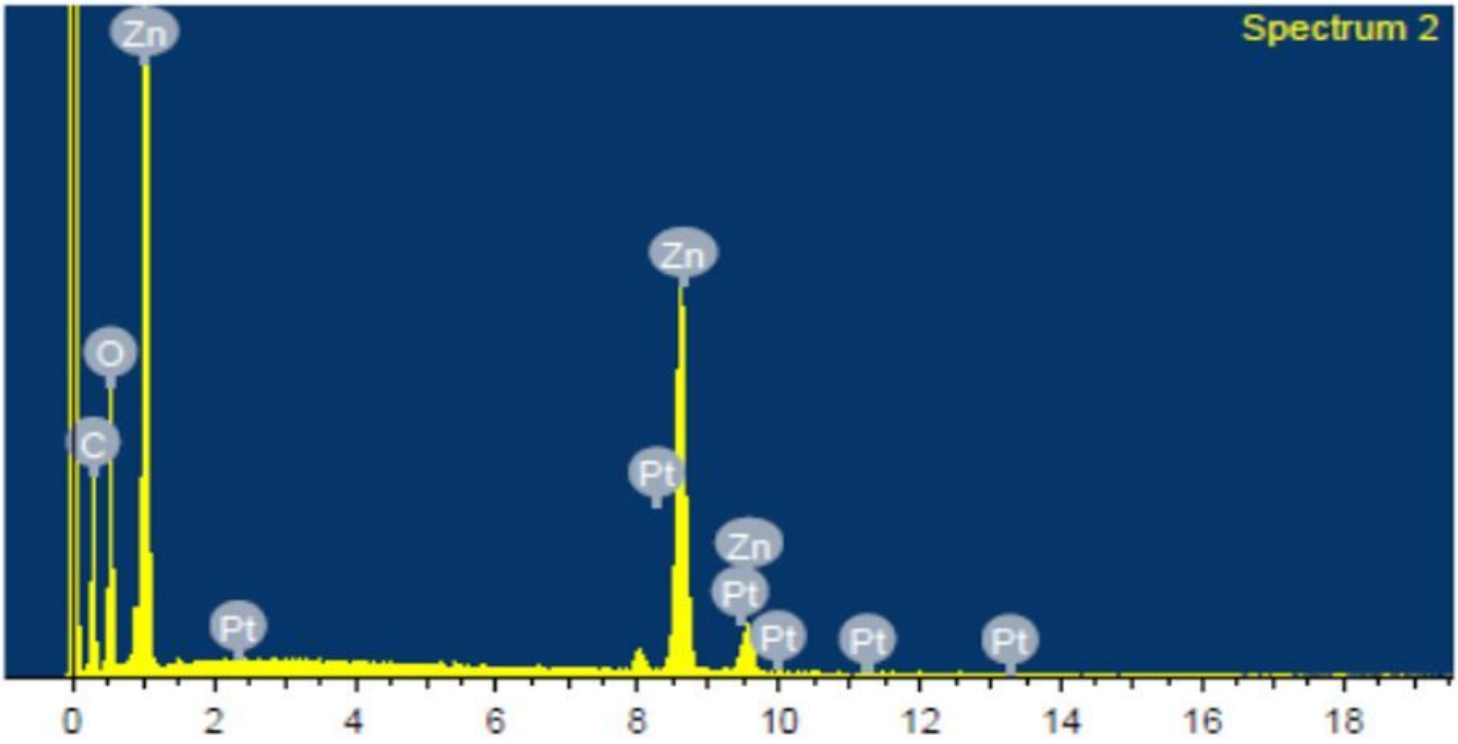

Figure 2

Energy dispersive X-ray spectrophotometry analysis of the ZnO-NPs synthesized by the green alga U. fasciata.

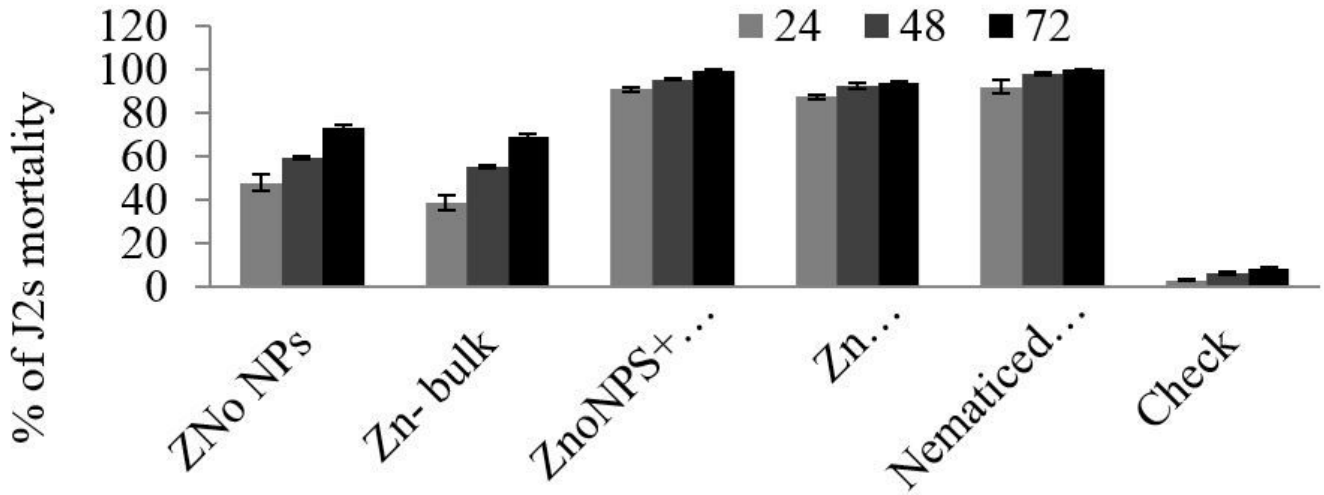

Figure 3

Effect of ZnO NPs and its bulk alone or/ in combination with oxamyl on the J2s mortality of M. incognita, at different exposure time.

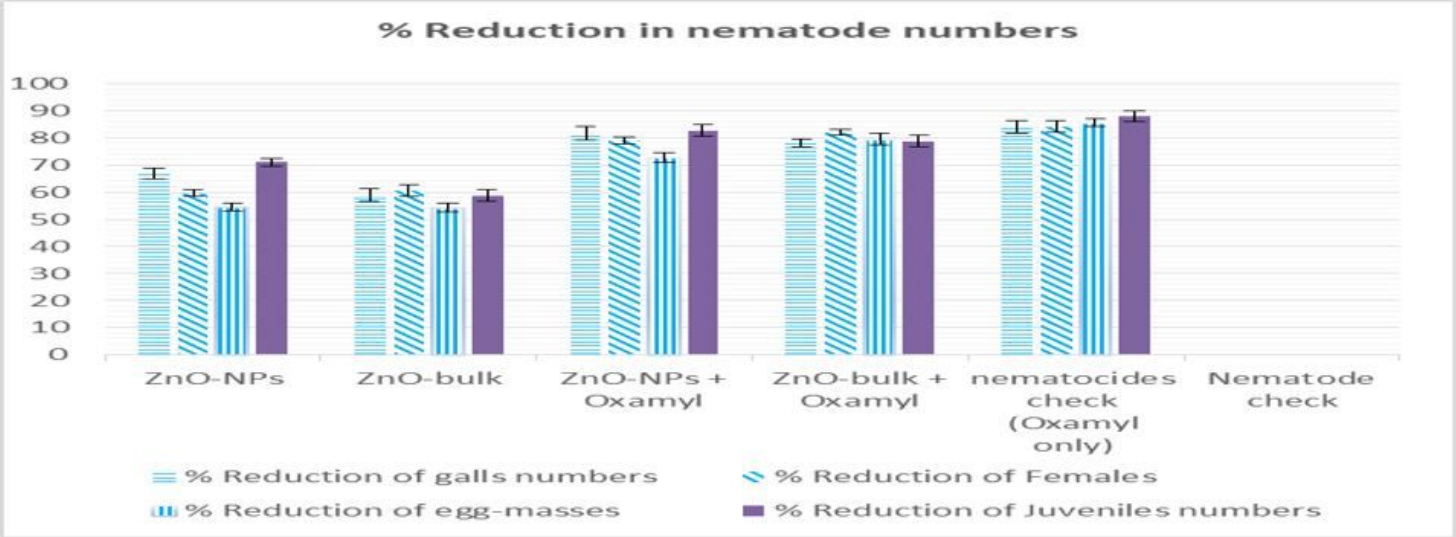

Figure 4

Incidence percentage of RKNs community after applying ZnO-NPs and ZnO-bulk alone or in combination with oxamyl

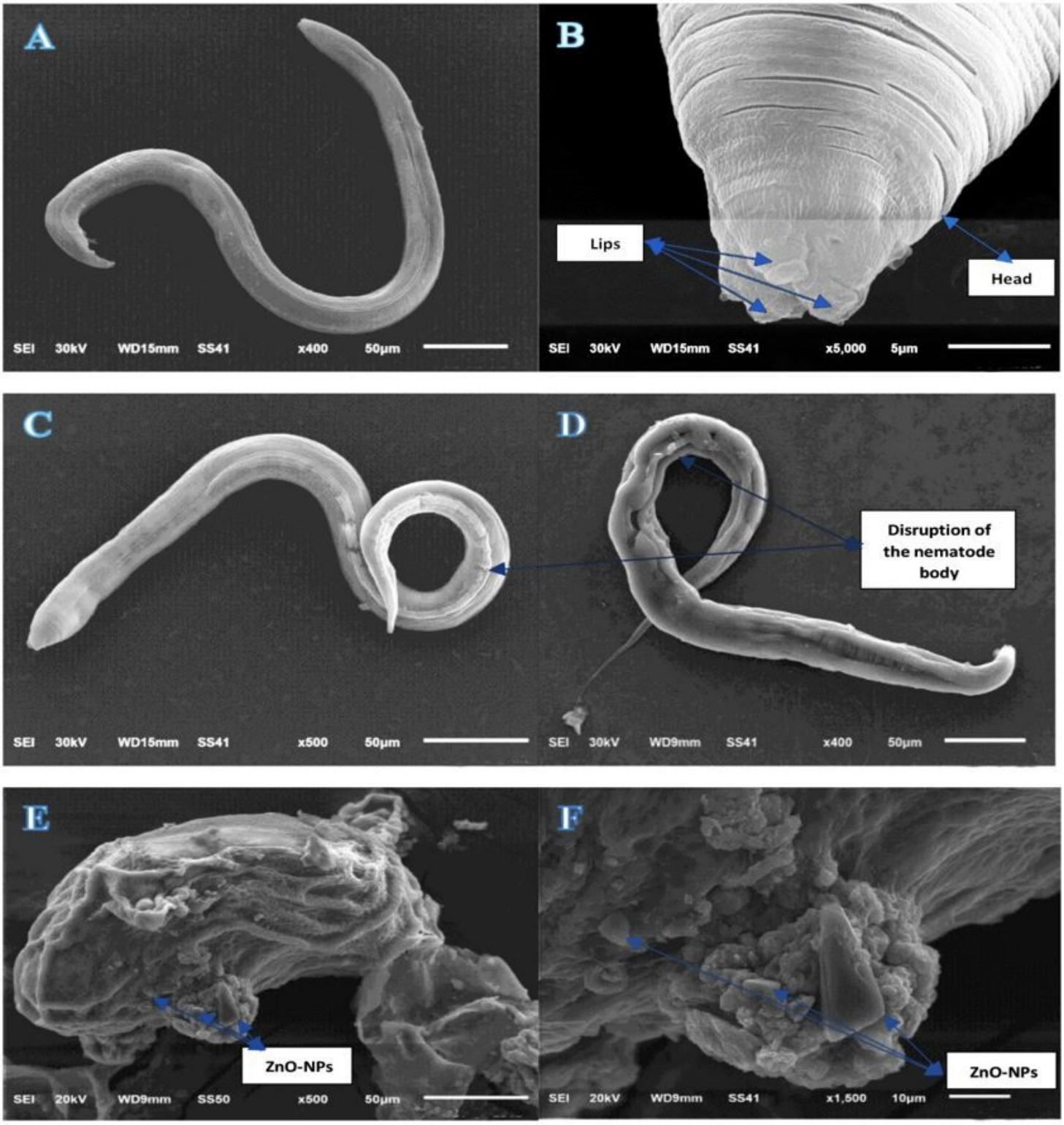

Figure 5

SEM images of ZnO-NPs on the nematode body in in vitro. A&B) Picture of the untreated nematode: a) Nematode with magnification 400x, b) Nematode with magnification 5000x. C&D) Disruption of the nematode treated with ZnO-NPs: c) Nematode body disruption with magnification 500x, d) Nematode body disruption with magnify 400x. E&F) ZnO-NPs on the nematode body: e) ZnO-NPs attached to the

nematode body with magnification 500x, f) ZnO-NPs attached to the nematode body with magnification 1500x.

Supplementary Files

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