Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 25(5): 721 – 738 (2021)
www.ejabf.journals.ekb.eg

Larvicidal activity and ultrastructural abnormalities in the ovaries of the housefly "*Musca domestica*" induced by the soft coral "*Ovabunda macrospiculata*" synthesized ZnO nanoparticles

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ARTICLE INFO

Article History:
Received: Sept. 25, 2021
Accepted: Oct. 29, 2021
Online: Nov. 19, 2021

Keywords:,
Ovabunda macrospiculata,
ZnO-NPs,
Larvicidal activity
Musca domestica,
Ovarian development,
Soft coral.

ABSTRACT

Compared to micro and macro particles, nanoparticles possess distinctive properties. Soft corals are appreciated marine sources with promising biological and chemical diversity. This study aimed to evaluate the effect of eco-friendly synthesis of Zinc oxide nanoparticles using *Ovabunda macrospiculata* (*Om*-ZnO-NPs) as insecticidal agent. In this trial, the ultrastructural abnormalities induced in the ovaries of the housefly, *Musca domestica* adult females were investigated. Synthesized zinc oxide nanoparticles (ZnO-NPs) were characterized using transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier transform infra-red (FTIR) spectroscopy. Morphologically, ZnO-NPs were semi-spherical in shape with particles diameter ranging from 20.4 to 21.3 nm. Structurally, XRD data showed that ZnO-NPs were crystalline in nature. The existence of chemicals required for the reduction of zinc oxide was confirmed by FTIR spectral analysis. Data obtained showed promising larvicidal activity, where synthesized *Om*-ZnO-NPs showed 2-3 folds higher larval mortality percentages than the crude extract at almost all concentrations tested. The recorded LC_{50} values were 49.061 and 22.595 ppm, while the LC_{90} values were 91.093 and 43.393 ppm for the crude extract and *Om*-ZnO-NPs, respectively. Additionally, *Om*-ZnO-NPs severely reduced the fecundity and egg-hatchability of treated females compared to those treated with the crude extract or the untreated females. Ultrastructural investigations revealed that crude extract induced abnormalities in tested ovaries and this effect was much more pronounced in those females treated with *Om*-ZnO-NPs. Generally, the obtained results suggest that coral-mediated synthesis of ZnO-NPs is more feasible than the eco-friendly synthesis of ZnO-NPs with improved properties.

INTRODUCTION

Nanoparticles (NPs) have gained wide interest over the last few decades due to their distinctive capability to refine the compatibility, bioavailability, and effectiveness of many artificial medications, and natural products within the management of chronic disorders **(Rao *et al.*, 2016; Klebowski *et al.*, 2018)**. These particles showed privileged and completely different chemical, optical, magnetic, mechanical, and biological

properties to their larger material counterparts due to their tiny materials (size ranges from 1 to 100 nm) and high surface in relation to volume magnitude **(Hemlata *et al.*, 2020)**. A promising interface between nanotechnology and applied insects management opened new ways to control vector and pest populations **(Azarudeen *et al.*, 2016; Benelli & Lukehart, 2017; Shehabeldine *et al.*, 2021)**. Though the toxic effect of nanoparticles such as ZnO has been proved on fungi, bacteria, and biological cells **(Donaldson *et al.*, 2009; Meng *et al.*, 2009)** Yet, limited studies have been conducted to address that effect on insects.

In terms of biological and chemical diversity, marine environments are among the richest and most complex ecosystems (**Farrag *et al.*, 2019; Mona *et al.*, 2019**). Several groups of marine organisms are rich in nutritionally important substances, and they use an arsenal of chemical defenses and chemical repellents to protect themselves (**Hasaballah & El-Naggar, 2017; El-Damhougy *et al.*, 2017**). Bioactive compounds isolated from marine organisms showed that they possess anti-cancer, anti-microbial, anti-fungal, anti-inflammatory, and other pharmacological activities (**Cui *et al.*, 2020**). Marine organisms act as an important source of new bioactive molecules; thus, efforts have been exerted by the scientific community worldwide to isolate and characterizethe biologically active natural products **(Attia *et al.*, 2021**). Nowadays, several chemical compounds have been either isolated from marine organisms or are still under investigation (**Ibrahim *et al.*, 2017**). Many marine products haven't yet been identified; they may be the key to the development of new types of drugs and secondary metabolites, and may, in the coming century, help solving various health problems and environmental issues (**El-Naggar & Hasaballah, 2018**).

One of the most colourful and diverse group of invertebrates found on coral reefs is soft coral (**Darweesh et al., 2021**). Soft corals are widespread throughout the tropical Indo-Pacific area including the Red Sea (**Fabricius & Alderslade, 2001**). Soft corals are potential sources that contain various bioactive compounds. Diterpenes, sesquiterpenes, furanoditerpenes, terpenoids, and steroids are major secondary metabolites in soft corals which have shown to display a pharmacological activity (**Metwally *et al.*, 2020**). In addition, they are known for producing their bioactive compounds as a chemical/defensive weapon in maintaining or winning spatial competition at benthic environment (**Changyun *et al.*, 2008; Sotka *et al.*, 2009**). The amount of those bioactive compounds was suggested as a predictor for soft coral invasiveness potential in a coral reef environment (**Lages *et al.*, 2006**). The soft coral Sarcophyton contain high amount of cytotoxic sarcophytoxide (**Iswani *et al.*, 2014**). Therefore, if a particular soft coral species dominates a reef area, it may suggest that this organism potentially contains an interesting chemical bioactive compound.

For its well-known public health importance, dipterous flies are considered the most important family of insects due to its significant role as vector of many pathogens of tropical and subtropical diseases. Those maladies include Zika virus, filarial nematodes, malaria, dengue fever virus, different types of encephalitis, and yellow fever virus as well

as bacteria (**Mehlhorn *et al.*, 2012; Benelli, 2015**). Among insects with public health importance, the housefly, *Musca domestica* is recognized, which is the prominent highly concerned pest that plays a critical role as mechanical vector of many pathogens, such as pathogens of diarrhea and dysentery (**Levine & Levine, 1991**), and pathogenic bacteria including *Staphylococcus aureus* (MRSA), *Bacillus* spp., *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella* sp. and *Clostridium difficile* (**Davies *et al.*, 2016**).

The current study focused on the development of a clean, eco-friendly, and effective approach to synthesize ZnO-NPs using the soft coral, *Ovabunda macrospiculata* extract. The experiment was organized to investigate the larvicidal and insecticidal activities of the synthesized *O. macrospiculata* ZnO-NPs. Simultaneously, the potential inhibitory effect of the synthesized *O. macrospiculata* ZnO-NPs on the ovarian development of the housefly, *Musca domestica*, was evaluated.

MATERIALS AND METHODS

1. Collection, Preservation and Identification of Soft Coral Specimens

Soft coralspecimens were collected during spring 2019 from Abu Gallum protected area, Aqaba Gulf, South Sinai. Sampling was performed using SCUBA diving at a depth of 3 m. The specimens were collected carefully for identification and were photographed by Cannon power shot underwater camera. Immediately upon collection, the specimens were cleaned by sea water, washed by distilled water, and preserved in ice box containing ice cubes until investigations. A specimen bit was fixed in 4% formalin in seawater for 24 h and then preserved in ethanol (70%) for further identification. The collected soft corals were identified according to Xeniidae of Red Sea **(Gohar, 1940)**, Des Roten Meeres **(Veron, 2000**), and Soft corals and sea fans **(Fabricius & De'ath, 2002; Ezz-Alarab, 2009)**.

2. Preparation of Soft Coral crude extract

In the lab, the frozen specimens were defrosted and chopped into small bits. 100 g of specimen tissues were extracted by soaking in 200 ml of absolute ethanol for 24 h at room temperature. The extraction was repeated thrice to ensure complete extraction. The extract was filtered through Whatman No. 1 filter paper and dried at 40^{o}C using a rotary evaporator. Chemicals used for the extraction were of pure grade purchased from well-known chemical suppliers.

3. Synthesis of Ovabunda macrospiculata Zinc oxide Nano Particles (ZnO-NPs)

Ovabunda macrospiculata were collected from Abu Gallum protected area, Aqaba Gulf, South Sinai, Egypt and were used for the synthesis of Zinc oxide nanoparticles (ZnO-NPs). *O. macrospiculata* (20% by weight) was prepared following the procedure of **Zare *et al.* (2017)**. Liquid extract was prepared by mixing 50 g of dried *O. macrospiculata* with 500 ml of deionized water by constant stirring on a magnetic stirrer (**Minjas & Sarda, 1986**). The suspension of *O. macrospiculata* dried in water was left for 3 h, filtered through Whatman filter paper No. 1. Then, the filtrate was stored in an

amber-coloured air-tight bottle at 20°C and was used within a week. ZnO-NPs were synthesized according to the technique of**Gutiérrez-Ramírez *et al.* (2021)**. Precursor solutions of Zn $(NO_3)_2$, and $6H_2O$ were prepared (0.028 M) and heated separately in a water bath at 70°C for 1 h. The solution was decanted, and 12 ml of this stock was added to 88 ml of 1 mM aqueous ZnO solution till the resulting solution became brown in colour. The colour intensity was measured at 421 nm for various intervals (1, 2, 3, 4, and 5 h, respectively). Finally, a drop of ZnO-NPs, covered with a carbon support film, was added and left to dry at room temperature and analyzed using a Bruker Quantax 400 instrument. Fourier-Transform Infrared Spectroscopy (FT-IR) analysis of ZnO-NPs was performed using a FT-IR-8400S spectrophotometer (IR Prestige-21, IR Affinity-1, Shimadzu, Japan) to determine ZnO-NPs possible biomolecules.

4. Characterization of synthesized ZnO-NPs

The morphology, structure, and size of the synthesized ZnO-NPs were determined by JEOL 1010 transmission electron microscopy (TEM) according to the study of **Mir *et al.* (2020)**. X-ray diffraction (XRD) was performed operating at 45 kV. Patterns were recorded between 10° and 90° angles, with speed of 5 deg/ min. The average crystallite size 'D' of the ZnO-NPs was calculated following **Sohail (2017)**. FTIR of NPs was obtained according to **Mir *et al.* (2020)**. Infrared spectroscopy was applied to determine diverse functional groups furthermore as adsorbed species and reaction intermediates on the NP surface.

5. Bioassay

5.1. Maintenance of the House fly colony

The housefly, *Musca domestica*, L. larvae were from the Research Institute of Medical Entomology, Dokki, Giza, Egypt, from laboratory susceptible strain colonized for several generations. Fifty adults from both sexes were placed in wooden cages (30 × 30 × 25 cm) containing autoclaved cotton pieces soaked in milk powder (10% w/v) as food for adults, which at the same time were used for oviposition. Newly hatched eggs were transferred to a set of 500 ml-capacity glass beakers containing larval diet for hatching and larval development purposes. The housefly larvae were reared on a mixture of 38 g of sterilized bran, 2 g og milk powder and 60 ml of water. Colony conditions were maintained at a temperature of 27 ± 2°C and 70 ± 10% humidity for 12 h photoperiod in the Insectary of Medical Entomology, Animal House, Faculty of Science, Al-Azhar University, Cairo, Egypt (**Sawicki 1964**).

5.2. Larvicidal activity

Larvicidal activity of the tested extracts was evaluated for the house fly, *Musca domestica*, using food-media technique as a standard method for testing new insecticides according to the WHO protocol (Wright 1971). Range of concentrations of (20 – 100 ppm) and (8 – 40 ppm) for the crude and *O. macrospiculata* ZnO-NPs extracts were tested, respectively. From the established colony and for each concentration, ten third instar larvae were picked up and transferred to a 500 ml glass beaker provided with larval diet (2 g coarse wheat bran) mixed with previously determined concentrations and

maintained at laboratory conditions. The control group was established by replacing the same amount of extract with distilled water to the diet. Treatments were observed daily to record mortalities, and results were calculated as mean ± standard deviation (SD) of three replicates for each treatment.

5.3. **Fecundity and egg-hatchability**

Equal numbers of both male and female house fly adults that survived after treatment with different tested concentrations alongside the control were transferred to 500 ml glass beakers covered with mesh net. Glass beakers were supplied with cotton pieces soaked in milk powder (10% w/v) to provide food for adults and petri dishes contain larval diet (25 g coarse wheat bran) acting as a substrate for egg deposition. Deposited eggs were carefully collected on daily basis. Fecundity was then calculated by counting the total number of eggs laid divided by the number of females that mated and survived till the end of the experiment. Mean egg-hatchability was calculated by dividing total no. of hatched eggs by no. of females succeeded to deposit eggs, while egg-hatchability percentages were calculated as follows: Egg-hatchability (%) = A/B × 100, where (A) no. of hatched eggs (B) no. of laid eggs. Results were calculated as Mean ± SE of three replicates.

6. **Transmission electron microscopy (TEM)**

Following the same procedures used in the evaluation of larvicidal activity, groups of ten larvae were treated with the LC_{50} and LC_{90} concentrations calculated from the larval mortality bioassay to detect ovarian abnormalities and further TEM investigations. Control group was run alongside the tested extracts. For each treatment, two replicates were used. After 72 h of emergence, adult females from each treatment in addition to the control were collected to dissect their reproductive region for TEM examination. Survived females were examined ultrastructurally using TEM technique; specimens were prepared for TEM (using JEOL 1010 Transmission Electron Microscope) to investigate the well-prepared grids uploaded with different samples at the Regional Center of Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

7. **Statistical analysis**

Data entry was done using Excel 365. Descriptive statistics including mean and standard deviation (SD) was calculated for each category. Larval mortality was subjected to *probit* analysis to calculate regression equation, LC_{50}, and LC_{90} at 95% confidence limits. One-Way analysis of variance, lower and upper confidence limits, and chi-square values were done using SPSS ver. 25. Holm Sidak post hoc method was used for pairwise comparisons. Data presented as Mean ± SD. The *P*-value was considered significant at < 0.05.

RESULTS

1. Characterization of ZnO-NPs

In the current study, ZnO-NPs were characterized in terms of morphology, size and structure. The TEM images exhibited that ZnO nanoparticles were approximately

crystalline, semi-spherical with diameter ranging from 20.4 to 21.3 nm (Fig. 1A). The X-ray diffraction of ZnO-NPs demonstrated sharp peaks with high intensity and greater crystal size of ZnO (Fig. 1B). XRD pattern of ZnO-NPs annealed at 400^{o}C for 2 hours. The diffraction peaks were depicted in the spectrum at 2θ positions of 32.7°, 35.42°, 37.14°, 46.16°, 52.59°, 59.02°, 64.03°, and 68.21^{o} corresponding to (100), (002), (101), (102), (110), (103), (112), and (202), respectively, which belong to a hexagonal crystalline structure. In Fourier-transform infrared spectroscopy (FTIR), the peaks observed at 3620 and 3220 may be due to O-H bond and asymmetric stretching, respectively. Peaks at 1618 and 1450 may be due to O-H bending, while peaks at 820 and 560 may be due to the stretching mode of ZnO (Fig. 1C).

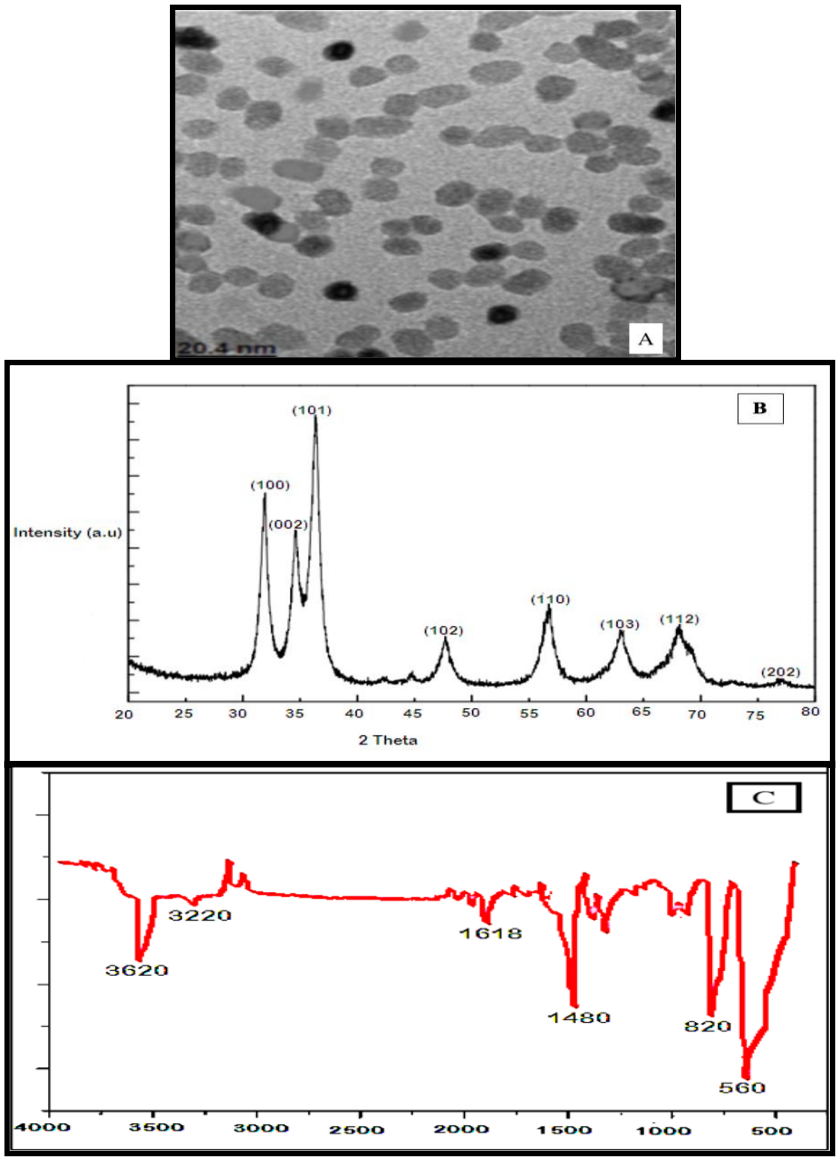

Fig. 1. (A)Transmission electron microscope showing semi-spherical shape of ZnO-NPs , (B) X-ray Diffraction (XRD) pattern of ZnO powder , (C) Fourier-transform infrared spectroscopy peaks of ZnO-NPs

2. Identification and description of the soft coral, *Ovabunda macrospiculata*

The collected sample of soft coral was identified as shown in Fig. (2). The collected specimens were 18 - 22 mm high form stalk base to polyp apex. Most stalks' width ranged from 10 - 12 mm at the base. Each stalk split into three branches with varying widths. It was observed that, the polyp width reached up to 2 mm, and the tentacle width reacheda value of 5 mm. The polyp is pinnate having pulsating tentacles bearing two or three rows of pinnules. The polyp's pinnules are 1 mm long and 0.2 mm wide, and there are many pinnules (14-18) in the outermost row on each side of the tentacle. The sclerites are Ovabunda-type with both regular and irregular pear-shaped. The collected specimens were whitish in colour. They are distributed along Red Sea and the Gulf of Aqaba.

Kingdom: **Animalia**
Phylum: **Cnidaria**
Class: **Anthozoa**
Subclass: **Octocorallia**
Order: **Alcyonacea**
Family: **Xeniidae**
Ovabunda macrospiculata
(Gohar, 1940)

Fig. 2. Scientific identification and photograph of the soft coral sample (*Ovabunda macrospiculata*), Synonymized name: *Xenia macrospiculata*, Gohar, 1940

3. Insecticidal activity

3.1. Larvicidal activity

The larvicidal activity of the crude and zinc oxide nanoparticles (ZnO-NPs) of the soft coral,*Ovabunda macrospiculata*, was evaluated against the last instar larvae of the housefly, *Musca domestica*. To explore the lethal activity of the tested extracts and consequently the survival potential of *M. domestica*, the newly moulted last instar larvae of the housefly, *M. domestica*, were treated with five concentrations from the crude extract (20, 40, 60, 80, and 100 ppm) and synthesized *O. macrospiculata*-ZnO-NPs (Om-ZnO-NPs), (8, 16, 24, 32, and 40 ppm). The LC50 and LC90 values at ($P < 0.05$) of both tested extracts are shown in Table (1). The obtained data revealed that synthesized Om-ZnO-NPs extract showed 2-3 folds higher larval mortality percentages than crude extract at almost all concentrations tested. As expected, larval mortality percentages increased in a concentration-dependent manner in both tested extracts, and it was much more pronounced in Om-ZnO-NPs compared to those in the crude extract. It reached about

96.67 ± 3.08% for larvae treated with 100 ppm of the crude extract and 86.67 ± 3.55% for those treated with Om-ZnO-NPs at 40 ppm.

Table 1. Larvicidal activity of *Ovabunda macrospiculata* crude and zinc oxide nanoparticles of the housefly, *Musca domestica*

Treatment	Concentration (ppm)	Larval mortality (%) Mean ± SD	Regression equation	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	χ^2
Crude extract	Control	0.0 ± 0.0a	Y = 0.9583X + 2.8333	49.061 (28.966 - 67.156)	91.093 (74.497 - 112.341)	4.216 n.s.
	20	23.33 ± 1.78b				
	40	36.67 ± 2.54b				
	60	63.33 ± 1.41c				
	80	81.67 ± 3.58cd				
	100	96.67 ± 3.08d				
Om-ZnO-NPs *Extract*	Control	0.0 ± 0.0a	Y = 1.9583X + 5.6667	22.595 (16.002 - 28.189)	43.393 (28.683 - 63.496)	1.958 n.s.
	8	26.67 ± 1.52ab				
	16	33.33 ± 1.81ac				
	24	46.51 ± 2.11bc				
	32	70.0 ± 3.32cf				
	40	86.67 ± 3.55df				

Mortalities are presented as Mean ± SD of three replicates; means with different letters are significantly different $P < 0.05$. (LC_{50}) concentration that kills 50% of population, (LC_{90}) concentration that kills 90% of population, (LCL) lower confidence limit, (UCL) upper confidence limit, χ^2 Chi-square, (n. s.) not significant at Alpha= 0.05, *Om*-ZnO-NPs= *Ovabunda macrospiculata* zinc oxide Nanoparticles.

3.2. Fecundity and egg hatchability

Fecundity and egg-hatchability of the adult female housefly, *Musca domestica*, treated with different concentrations of *O. macrospiculata* crude and ZnO-NPs were investigated and presented in Table (2). For the crude extract, concentrations below 60 ppm non-significantly (P<0.05) affected the fecundity of treated females, while concentrations of 60 - 100 ppm reduced the fecundity significantly (P<0.05) to reach about 29.0 eggs/♀ at the highest concentration applied of 100 ppm versus 64.0 eggs/♀ for the untreated females. For the *Om*-ZnO-NPs extract, all tested concentrations significantly (P<0.05) reduced the fecundity of treated females, and this effect was concentration-dependent. Fecundity of females treated with *Om*-ZnO-NPs extract was 3-4 folds lower than those treated with the crude extract. The lowest fecundity was obtained at 40 ppm with a value of 14.33 eggs/♀. In other words, *Om*-ZnO-NPs extract severely reduced the fecundity of treated females compared to the females treated with the crude or the untreated females.

The egg-hatchability percentages of *M. domestica* females treated with different concentrations of *O. macrospiculata* crude and ZnO-NPs extracts compared to the untreated females are given in Table (2). Data obtained revealed that tested concentrations severely reduced the hatchability percentages in both tested extracts, and

this reduction was concentration-dependent. Tested concentrations reduced the egg-hatchability percentages in the crude extract to 30 % for females treated with the highest concentration applied (100 ppm) and this effect was much more pronounced in *Om*-ZnO-NPs extract treated females, where egg-hatchability percentages were reduced to 5 % for females treated with 40 ppm.

Table 2. Fecundity and egg hatchability of the adult female housefly, *Musca domestica*, treated with different concentrations of *Ovabunda macrospiculata* crude and zinc oxide nanoparticles

Treatment	Concentration (ppm)	Fecundity (eggs/♀)		Egg-Hatchability	
		n	Mean ± SD	Mean ± SD	Hatchability %
Crude extract	Control	22	64.0 ± 1.73a	50.0 ± 2.0a	94.96
	20	18	59.33 ± 2.08a	30.33 ± 2.51b	90.97
	40	13	55.67 ± 4.04a	19.0 ± 2.67c	80.09
	60	10	41.33 ± 2.51b	11.0 ± 1.73cd	66.12
	80	4	40.67 ± 1.15bc	13.0 ± 2.64c	61.64
	100	1	29.0 ± 0.0d	10.0 ± 0.0d	30.95
	Statistic	DF= 5, F= 64.827, P = 0.000		DF= 5, F= 83.56, P = 0.00	
Om-ZnO-NPs *Extract*	Control	23	62.67 ± 2.52a	51.33 ± 1.15	97.63
	8	15	52.67 ± 2.52b	19.67 ± 2.52	59.02
	16	14	40.33 ± 3.16c	13.33 ± 1.52	56.24
	24	11	32.67 ± 2.52c	6.67 ± 1.15	43.59
	32	5	18.33 ± 3.51d	4.67 ± 0.58	28.11
	40	3	14.33 ± 3.06d	4.0 ± 1.0	5.63
	Statistic	DF= 5, F= 121.172, P = 0.000		DF= 5, F= 313.646, P = 0.000	

Data were analyzed by one-way ANOVA, followed by Holm Sidak post hoc test and presented as Mean ± SD of three replicates. For each treatment, means followed by different letters differ significantly, *P* < 0.05, *n* = sample size, *Om*-ZnO-NPs= *Ovabunda macrospiculata* zinc oxide Nanoparticles.

3.3. Transmission electron microscopy

Adult *M. domestica* females treated with the LC_{50} and LC_{90} values of the crude and *Om*-ZnO-NPs extracts and other untreated were examined ultrastructurally. The reproductive potential was investigated at the level of oogenesis to assess the impact of tested extracts on the ovarian development of tested females. As illustrated in Fig. (3), the ovarian development of the untreated females (Fig. 3A) exhibited a well-developed nurse cells, ideal vitellogenic region with intact ovarian sheath and oocytes. However, females treated with the LC_{50} concentration of the crude extract showed signs of degeneration process in the vitellogenic region (Fig. 3B), while females treated with the LC_{90} from the same extract exhibited oocytes degeneration, vacuoles, and degenerated nurse cells (Fig. 3C). On the other hand, females treated with the LC_{50} concentration of *Om*-ZnO-NPs extract showed severe signs; oocytes disruption, lysis, and damage of nurse cells (Fig. 3D), while those treated with the LC_{90} from the same extract additionally showed

disappearance of nucleus with condensed chromatin, dense vacuolations, and degenerated oocytes without the ovarian sheath (Fig. 3E).

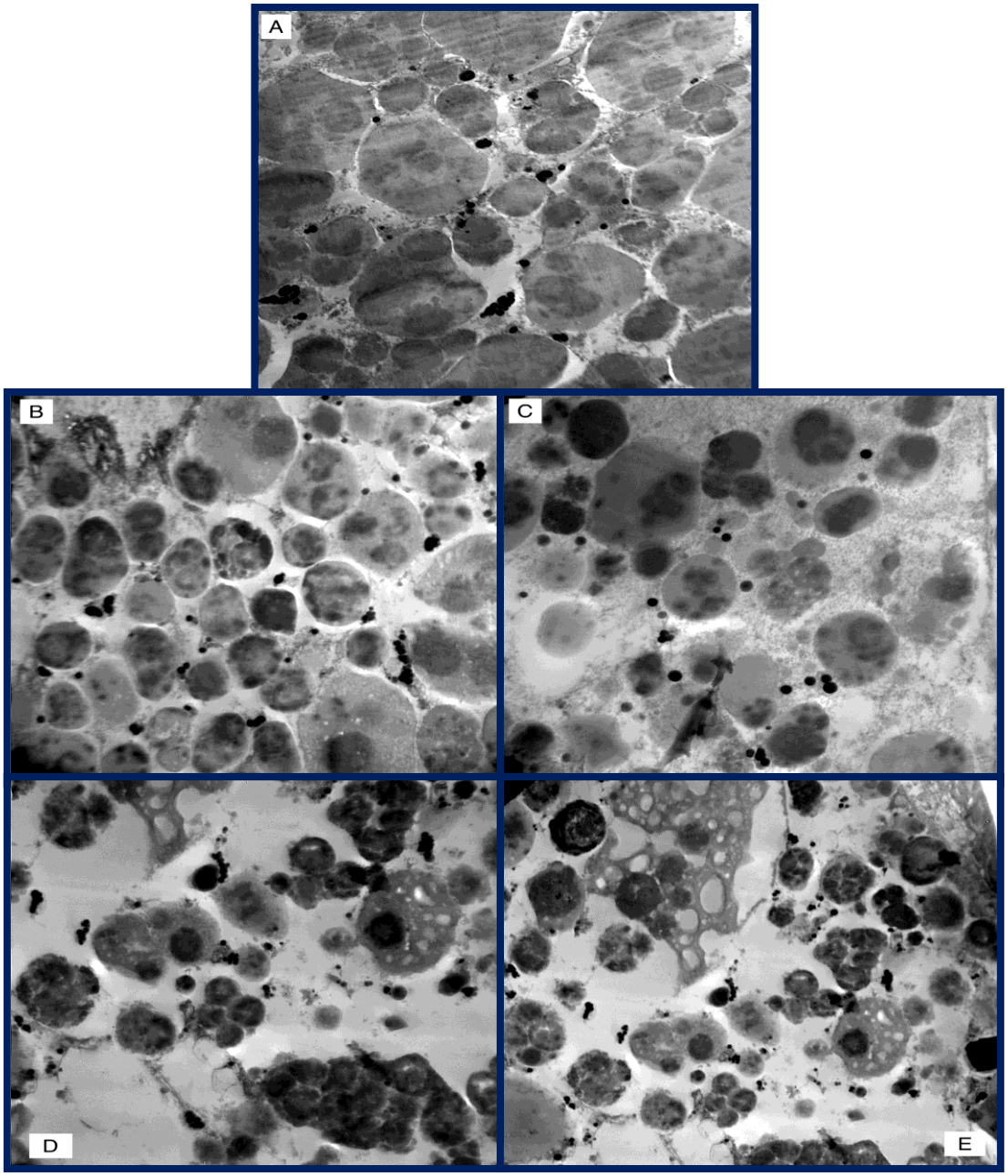

Fig. 3. Transmission Electron micrograph of the housefly, *Musca domestica* females' reproductive region treated with different concentrations of *O. macrospiculata* crude and ZnO-NPs extracts: A (control, magnification= 5000X), B (female treated with the LC_{50} of the crude extract, magnification= 5000X), C (female treated with the LC_{90} of the crude extract, magnification= 6000X), D (female treated with the LC_{50} concentration of the *Om*-ZnO-NPs extract, magnification= 5000X) and E (female treated with the LC_{50} of the *Om*-ZnO-NPs extract, magnification=4000X)

DISCUSSION

Innovation, ingenuity, and improving industrial production using cost-inexpensive materials and energy-efficient has paved the avenues of nanotechnology. The recent marine bio-nanotechnology has a considerable promise in nanomaterials synthesis to create a new technique for the future in various disciplines such as pharmaceuticals, medicines, fabric industries, foodstuff, etc. (**Ul Haq *et al.*, 2017**). The biosynthesis of metal nanoparticles using marine resources is regarded to be a "green technique" that is environmentally safe, non-toxic and clean. Scientists attempt to develop an eco-friendly and cost-effective method for biosynthesis of nanoparticles, especially from marine resources. Among a variety of nanomaterials, zinc oxide nanoparticles (ZnO-NPs) have benefits due to their exceptional chemical and physical characteristics. It is a low-cost material used in electrical devices, nano fertilizers, cosmetics, bioimaging, targeted gene delivery and medication, as well as an excellent sensor for pollution detection, toxins and environmental remediation (**Sabir *et al.*, 2014**). Soft corals have been discovered to be key sources of secondary metabolites with biologically intriguing properties (**Hegazy *et al*., 2012**).

In the present study, an attempt was made to test the activity of *Ovabunda macrospiculata* zinc oxide nanoparticles (*Om*-ZnO-NPs) as potential larvicidal and insecticidal agent. ZnO-NPs have attracted special attention over time, because of their stabilization and safe use, besides; exposure to nanoparticles has a major impact on the induction of oxidative stress and detoxification if compared to exposure to free ions **(Sang Woo *et al.*, 2009; Eleka *et al.*, 2010; Abdel-Gawad, 2018)**. No peaks for any traces were observed that further confirm the pure phase of ZnO **(Muthukumaran & Gopalakrishnan, 2012; Palacios-Padros *et al.*, 2013)**. In the current study, larvicidal activity of the crude and synthesized *Om*-ZnO-NPs was evaluated against the larvae of housefly, *Musca domestica*. Results showed that those nanoparticles could be an efficient pest control approach for the housefly. The diffraction peaks depicted in the spectrum at 2θ positions of XRD are in the same line with the findings of **Jian-Hui *et al.* (2011)** , **Waleed *et al.* (2011)** and **Mostafa *et al.* (2018)**. Results obtained by **Mir *et al.* (2020)** agree with the current study results in peaks which were investigated by XRD. The present work confirmed the pure phase of ZnO, and this result coincides with those of **Muthukumaran and Gopalakrishnan (2012)** and **Palacios-Padros *et al.* (2013)**. The morphology of the ZnO-NPs detected by TEM, XDR or FTIR agrees with some other studies with respect to the sharpness and intensity of the peaks which reflect the high crystallinity and crystal size of ZnO **(Zhang *et al.*, 2004; Raja *et al.*, 2014)**, and also the result detected by FTIR agrees with that of **Mir *et al.* (2020)**.

The larvicidal activity of the crude and synthesized *Ovabunda macrospiculata* zinc oxide nanoparticles (*Om*-ZnO-NPs) was evaluated against the larvae of housefly, *Musca domestica.* The newly moulted last instar larvae were treated with five

concentrations with different ranges (20 - 100 ppm) for the crude extract and (8 - 40 ppm) for the *Om*-ZnO-NPs extract. Based on the obtained results, synthesized *Om*-ZnO-NPs extract showed 2-3 folds higher larvicidal activity than crude extract. However, different marine organisms were reported previously as a rich-source of various compounds or derivatives with insecticidal or in particular larvicidal properties. In the same context, **Kalimuthu *et al.* (2014)** investigated the effect of seaweed *Gracilaria firma* with copepod, *Megacyclops formosanus*, against the dengue vector, *Aedes aegypti*, larvae giving similar lethal concentrations as those reported here. **Hasaballah and El-Naggar (2017)** investigated the marine sponges, *Negombata magnifica* and *Callyspongia siphonella* for their larvicidal activity against the mosquito, *C. pipiens* with LC_{50} values of 47.6 ppm for *N. magnifica* and 610.3 ppm for *C. Siphonella* for the crude extract.

For the *Om*-ZnO-NPs extract, results are in agreement with the findings of **Murugan *et al.* (2015)** who tested the toxicity of *Caulerpa scalpelliformis* AgNPs against *C. quinquefasciatus* where larval toxicity recorded LC_{50} values with ranges 3.08 - 7.33 ppm. Similarly, **Madhiyazhagan *et al.* (2015)** reported that *Sargassum muticum* AgNPs were highly toxic against larvae of *Ae. aegypti*, *Anopheles pharoensis* and *C. quinquefasciatus*; **Kalpana *et al.* (2020)** reported that *Lagenaria siceraria* and its mediated ZnO-NPs induced larvicidal activity of *An. stephensi* with LC_{50} (56.46 ppm); and **Gutiérrez-Ramírez *et al.* (2021)** stated that ZnO-NPs caused increased mortality (88 %) of the *Bactericera cockerelli* (Hemiptera: Triozidae) at 250 ppm. The promising larvicidal activity reported here for the *Om*-ZnO-NPs extract may be attributed to the small size of synthesized nanoparticles that allow easy penetration into the insect cuticle and cells where they interfere with moulting and other physiological processes **(Benelli, 2016)**.

Little information is available regarding the impact of different soft coral extracts against the houseflies, or in particular, its effects on the fecundity and the egg-hatchability of these disease vectors. In this study,the fecundity of the housefly, *M. domestica* females, treated with *Om*-ZnO-NPs extract, were 3-4 folds lower than those treated with the crude extract. In the same context, **Roni *et al.* (2015)** reported that concentrations ranging from 100 - 500 ppm of *Hypnea musciformis* fabricated AgNP strongly reduced the female fecundity of *Ae. Aegypti*. Furthermore, **Madhiyazhagan *et al.* (2015)** found that *S. Muticum* synthesized AgNP reduced the oviposition rates to more than 70% in *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus* when treated with concentration of 10 ppm. As a confirmation of results obtained in the present study, data illustrated in the transmission electron microscope photographs revealed different levels of degeneration and disruption in oocytes, nurse cells, nucleus, ovarian sheaths and theses signs were increasingly detected in females treated with higher concentrations specifically those treated with *Om*-ZnO-NPs extract as previously reported in the results section. However, those signs of damaged ovaries resulted from treatment with different extracts may be the main factor responsible for the very low fecundity and consequently egg-hatchability percentages in females tested.

CONCLUSION

In conclusion, eco-friendly synthesis of ZnO-NPs extract was carried out using the soft coral, *Ovabunda macrospiculata*. Synthesized ZnO-NPs were characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infra-red (FTIR) spectroscopy. Results of characterization revealed that ZnO-NPs were semi-spherical in shape and crystalline in nature with particles diameter that ranged from 20.4 to 21.3 nm. Synthesized ZnO-NPs induced promising larvicidal and ovideterrent activities against the housefly, *Musca domestica*, the mechanical vector of many disease pathogens of public health importance. Generally, obtained results suggest that coral-mediated synthesis of ZnO-NPs is more feasible to eco-friendly synthesis of ZnO-NPs with improved properties.

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