


Sensitivity of the house fly, (*Musca domestica*) to the zinc oxide nanoparticles produced by *Bacillus foraminis*

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ABSTRACT

The housefly, *Musca domestica* Linnaeus (Diptera: Muscidae), is an important pest not only causing annoying to humans but is also known for its ability to transmit different infectious microorganisms to humans and animals. Recently, zinc oxide nanoparticles (ZnONPs) synthesized by microbial organisms have gained much attention in pest control because of their extensive antimicrobial activity, eco-friendliness, and simplicity. Therefore, the green synthesis of ZnO nanoparticles was applied in the current study as a new and effective biopesticide against the house fly, *Musca domestica* by feeding the first instar larvae on different concentrations of ZnONPs synthesized by *Bacillus foraminis* (0.01, 0.025, 0.05, 0.1, 0.25, and 0.5 mg). Results showed significant mortality values in the larval stage (1.84 ± 0.10 , 3.50 ± 0.29 , 5.75 ± 0.25 , 7 ± 0.41 , 8 ± 0.41 and 8.75 ± 0.25) while pupae recorded mortality values of (1.75 ± 0.14 , 1 ± 0.00 , 1.63 ± 0.13 and 1 ± 0.00) at the higher concentrations (0.05, 0.1, 0.25, 0.5 mg), respectively and some morphological changes appeared in some pupae. Also, the adult emergence had a significant reduction (100 ± 0.00 , 82.5 ± 2.50 , 62.5 ± 4.79 , 37.5 ± 2.50 , 28.35 ± 0.95 , 17 ± 0.08 and 0 ± 0.00), in comparison with the control group C (10 ± 0.00). ZnONPs showed effectiveness against *M. domestica*, suggesting it as a new and promising method in pest control.

Keywords: *Musca domestica*, zinc oxide nanoparticles, *Bacillus foraminis*, control

INTRODUCTION

The housefly *Musca domestica* Linnaeus (Diptera: Muscidae) is a vector insect that is distributed worldwide and highly adaptable to various environments (Abbas *et al.*, 2014; Bahrndorff *et al.*, 2014). Houseflies are mechanical transmitters of diseases gained during their feeding on excrement and decaying organic waste. Gastroenteritis, a serious public health issue, might result from human ingestion of contaminated food that has not been warmed (Nwankwo *et al.*, 2020). The control of houseflies depends mostly on chemical insecticides (Naqqash *et al.*, 2016). The most common way to prevent houseflies is by using insecticides, therefore several chemicals, including organophosphates, chlorinated hydrocarbons, pyrethroid, and carbamates have been used to control houseflies over the past seventy years (Shono *et al.*, 2004). Although there are many alternative approaches available, the management of pests still primarily focuses on the use of pesticides as ingredients based on organic chemicals that are applied to crops, foods, or the environment. Moreover, the use of pesticides has been connected to chemical accumulation and degradation in the atmosphere, causing injuries to mammals. Nitzko *et al.* (2022), reported the disadvantages of chemicals when used in cultivation are their residues that persist in food and. In addition, there is an increased rate of tolerance production by several insect species to many of the commonly used compounds. These factors are a major problem in agriculture and can considerably reduce effective active ingredients (Athanasassiou *et al.*, 2018). The systemic application of chemicals produced advances, but the damage they caused soon became apparent, including the removal of beneficial arthropods and environmental imbalances as well as the pollution of agricultural soil, the environment, water sources, food, and workers. These factors contributed to the resurgence of pests and supported the rapid selection of resistant individuals, leading to irreparable harm to the environment and health, such as cancer, genetic mutations, poisoning, and death (Prabhaker *et al.*, 1998).

As a result, there is a growing challenge to explore alternative control materials that are safe and highly efficient; therefore, nanomaterials have great attention in recent years because of their antimicrobial and pesticide activities (Rai and Ingle, 2012). On the other hand, chemical techniques have different hazards for the atmosphere and the person performing them; therefore, a cost-effective and environmentally friendly nanoparticles synthesis technique is required (Agarwal *et al.*, 2017). Biosynthesis of nanoparticles uses microorganisms and plants with biomedical methods to synthesize nanoparticles. This method is environmentally sustainable, effective, biocompatibility, healthy and green (Abdul *et al.*, 2014). Furthermore, this method of nanoparticle technology is more useful because it helps to synthesize nanoparticles at low temperatures, pressure, and pH (Seferos *et al.*, 2007; Raut *et al.*, 2009). Some plant-synthesized nanoparticles have been studied for their efficiency in managing economic arthropod pests, such as mosquitoes (Benelli 2016a, b). Zinc is the second most common metal after iron and is the only metal used in the six enzyme groups (transferases, lyases, hydrolases, isomerases, ligases, and oxidoreductases) (Nakahara *et al.*, 2001). Artificial pesticides could be replaced by green-produced nanoparticles (Siddique *et al.*, 2022). Research on the characterization synthesis and properties of ZnONPs in recent years has received a lot of attention because of its simplicity, eco-friendliness, extensive antimicrobial activity, and applications (Singh *et al.*, 2011; Kalpana *et al.*, 2018). Previous studies have confirmed that at a very low concentration of gram-negative and gram-positive bacteria ZnONPs have a very strong antibacterial effect, indicating a stronger antibacterial effect than ZnONPs that are chemically synthesized (Hazra *et al.*, 2013; Vimala *et al.*, 2014). Therefore, the current study aimed to investigate the effect of ZnONPs synthesized by microorganisms such as bacteria, specifically *Bacillus foraminis*, against the housefly *Musca domestica* as an eco-friendly, efficient, safe, and green method of pest control.

MATERIALS AND METHODS

Collection of house fly:

Samples of house fly *Musca domestica* Linnaeus (Diptera: Muscidae), (10 individuals) were collected randomly from different sheep and poultry farms in Makkah Region using an insect sweep net, during the period from October to December 2019.

Zinc oxide nanoparticle samples:

Samples were provided from a previous study analysed in the Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre, Cairo, Egypt. *Bacillus* species were isolated from soil samples of a cattle farm in Makkah Region, Saudi Arabia and identified genetically as "*Bacillus foraminis*"; they were used for biosynthesizing zinc oxide nanoparticles (El-Ghwas, 2022).

Preparation of housefly colony:

An endoscopic anatomy microscope was used for the examination of housefly samples, and both sexes were sorted and identified through taxonomic procedures (Service, 1980; Hall and Smith, 1993; Al-Saffar, 2003). Following, houseflies (10 males and females) were reared in the Insect laboratory at the Faculty of Science at the University of Jeddah. Houseflies were cultured for three generations to obtain laboratory strain and then used to evaluate their sensitivity to different concentrations of ZnONPs.

Housefly breeding:

Adult stages were reared in cubic wooden cages of dimension (35 × 35 × 35 cm). The cages were covered with iron mesh, and the front part was equipped with a muslin sleeve to manage the insects inside and to deliver food. Adults were fed with 10% sugar solution and provided with a three-inch cellulose cotton pad one inch thick, immersed in 10% milk solution, and placed in Petri dishes as a pillar for depositing eggs. Flies were kept in the laboratory at 27 ± 2°C and 60 ± 5 % RH with a light period of 12L:12D-hour, and the diet was revived every two days according to Bahareth *et al.* (2018). The first instar larvae (8–12 hours post-hatching) were removed and placed in labelled plastic jars containing 5 g of larval diet, with 10 larvae in each. The larvae diet was prepared by a mixture of 5 g wheat bran, 0.25 g yeast, 0.25 g powdered milk, and 10 ml distilled water (5: 0.25: 0.25: 10), according to Ruiu *et al.*, (2006), with some modification. The diet was mixed with soft paste, distributed in plastic jars, and 10 larvae were placed in each jar. Later, plastic jars containing pupae were placed in separate cages for adult emergence.

Bioassay:

Throughout the six treated groups (G1, G2, G3, G4, G5, and G6), different concentrations of ZnO nanoparticles (0.010, 0.025, 0.05, 0.1, 0.25, and 0.5 mg) were diluted with 10 ml of distilled water and added to 5 g of the larvae diet into plastic jars, respectively. Each group had three replicates with 10 larvae in each. The control group (C) was provided with a 5 g of larvae diet moistened with 10 ml of distilled water. Plastic jars of each treated group were placed in a 500-ml glass beaker allowing larval development to pupation and kept at 27 ± 2°C and 60 ± 5% RH with

a light period L12: D12. Observations of treated larvae (first, second and third instar) and pupal transformation were recorded daily at different intervals of 1, 6, 12, and 24 hours for any mortality or morphological changes. Any dead larvae were removed from the jars and counted; mortality data was recorded until pupation. Adult emergence was observed daily and counted. Adults were observed 24 hours post-emergence and mortality of individuals was recorded.

Statistical Analysis:

Statistical analysis for data was expressed as the mean \pm SE, which was evaluated by using SPSS version 16. The statistical significance levels in non-parametric data were done by the Kruskal-Wallis test, followed by the Mann-Whitney U test. Values were considered significantly different when the $P \leq 0.05$, and the LC_{50} were calculated for the larval stage.

RESULTS

Effect of zinc oxide nanoparticles on the larval stage:

The results of the present study, which are represented in **Table (1)** and shown in **Fig. (1)**, showed that feeding the first instar larvae on the treated diet with different concentrations of ZnONPs (0.01, 0.025, 0.05, 0.1, 0.25 and 0.5 mg) was effective against larval stage recording a mortality percent ranging from $18.35 \pm 0.95\%$ to $87.5 \pm 2.50\%$ throughout the different concentrations 0.01–0.5 mg. Furthermore, the mortality showed a significant ($P \leq 0.05$) gradual increase in value (1.84 ± 0.10 , 3.50 ± 0.29 , 5.75 ± 0.25 , 7 ± 0.41 , 8 ± 0.41 and 8.75 ± 0.25) throughout the different concentrations in comparison with the control group C, which recorded (0 ± 0.00). The results also showed that the LC_{50} of ZnONPs against the larval stage of *M. domestica* was at a concentration of 0.218 mg (**Fig. 2**).

Effect of zinc oxide nanoparticles on the pupation:

Results in **Table (1)** and **Fig. (3)** showed that there was a significant decrease in pupal development. The reduction continued throughout the different concentrations and recorded significant ($P \leq 0.05$) values: (8.50 ± 0.29 , 6.50 ± 0.29 , 4.25 ± 0.25 , 3.33 ± 0.24 , 2.67 ± 0.19 and 1 ± 0.00) proportionally to the increasing concentrations 0.010, 0.025, 0.05, 0.1, 0.25 and 0.5 mg, respectively.

Table 1. Effect of Zn Oxide Nanoparticles on house fly' mortality of larvae (No.), pupation (No.), and Mortality of larvae (%)

Groups		Treatment (mg)	Mortality in Larvae (No)	Pupation (No)	Mortality in Larvae (%)
			Mean \pm SE	Mean \pm SE	
Treated groups	G1	0.010	$1.84^* \pm 0.10$	$8.50^* \pm 0.29$	$18.35^* \pm 0.95$
	G2	0.025	$3.50^* \pm 0.29$	$6.50^* \pm 0.29$	$35^* \pm 2.89$
	G3	0.05	$5.75^* \pm 0.25$	$4.25^* \pm 0.25$	$57.5^* \pm 2.50$
	G4	0.1	$7^* \pm 0.41$	$3.33^* \pm 0.24$	$70^* \pm 4.08$
	G5	0.25	$8^* \pm 0.41$	$2.67^* \pm 0.19$	$80^* \pm 4.08$
	G6	0.5	$8.75^* \pm 0.25$	$1^* \pm 0.00$	$87.5^* \pm 2.50$
Control	C1	10	0 ± 0.00	10 ± 0.00	0 ± 0.0

Values are expressed as means \pm S.E.; n= 40 for each group. A statistically significant test for comparison was done by Kruskal-Wallis, followed by post hoc Mann-Whitney U test. *P < 0.05 statistically significant compared to the control group.

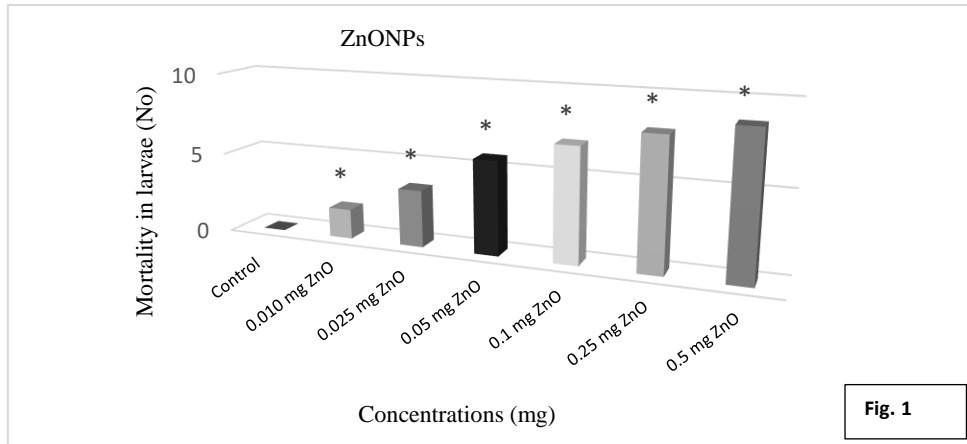


Fig. 1. Effect of ZnONPs on the mortality of house fly larvae (No)

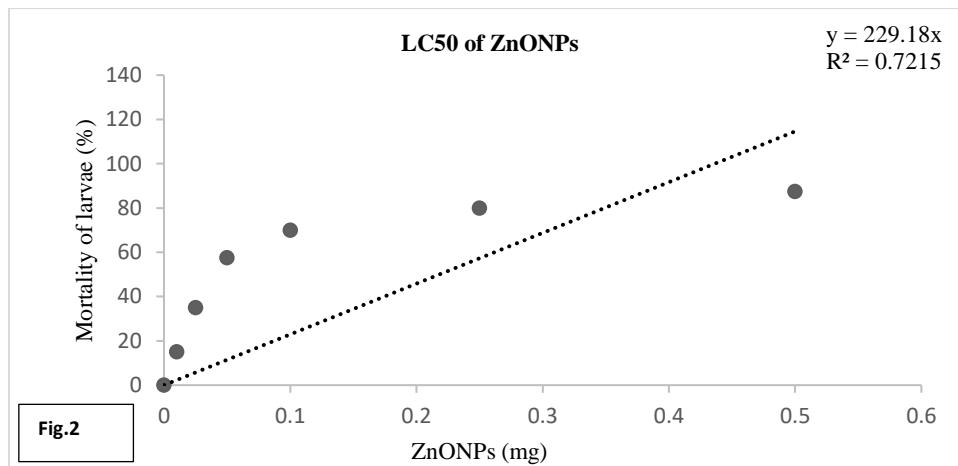


Fig. 2. Lethal concentration (LC50) of ZnONPs against larval stage of *Musca domestica*

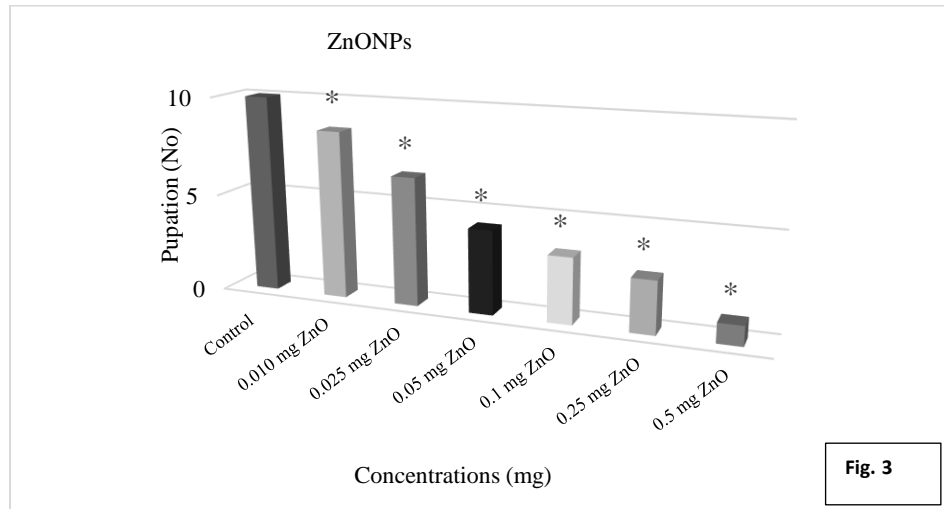


Fig. 3. Effect of ZnONPs on the pupation (No)

Effect of zinc oxide nanoparticles on the mortality of pupae:

Treating the first instar larvae with ZnONPs showed effectiveness in the pupal stage which appeared in the significant ($P \leq 0.05$) mortality values at the higher concentrations of 0.05, 0.1, 0.25, 0.5 mg recording 1.75 ± 0.14 , 1 ± 0.00 , 1.63 ± 0.13 and 1 ± 0.00 , respectively, in comparison to the control group C, which did not affect the pupal mortality (0 ± 0.00). However, the lower concentrations of 0.010 and 0.025 mg recorded non-significant ($P \geq 0.05$) values (0 ± 0.00 and 0 ± 0.00 , respectively), (Table 2 and Fig. 4). Furthermore, some pupae underwent morphological changes and appeared darker in color and smaller in comparison with pupae in the control group C, which appeared bigger and lighter in color. Moreover, some individuals failed to emerge into the adult stage and died. These morphological changes were significant ($P \leq 0.05$) and recorded (1 ± 0.00), which was fixed throughout the different concentrations from 0.010–0.5 mg, compared to the control group C which did not show any morphological changes (0 ± 0.00) (Fig. 5).

Table 2. Effect of ZnONPs on the house fly 'mortality of pupae (No.), morphological changes in pupae (No.), and the adult emergence (No).

Groups		Treatment (mg)	Mortality in Pupae (No)	Morphological changes (No)	Adult emergence (No)
			Mean \pm SE	Mean \pm SE	Mean \pm SE
Treated groups	G1	0.010	$0^* \pm 0.00$	1 ± 0.00	$8.25^* \pm 0.25$
	G2	0.025	$0^* \pm 0.00$	1 ± 0.00	$6.25^* \pm 0.48$
	G3	0.05	$1.75^* \pm 0.14$	1 ± 0.00	$3.75^* \pm 0.25$
	G4	0.1	$1^* \pm 0.00$	1 ± 0.00	$2.84^* \pm 0.10$
	G5	0.25	$1.63^* \pm 0.13$	1 ± 0.00	$1.64^* \pm 0.12$
	G6	0.5	$1^* \pm 0.00$	1 ± 0.00	$0^* \pm 0.00$
Control	C1	10	0 ± 0.00	0 ± 0.00	10 ± 0.00

Values are expressed as means \pm S.E.; n= 40 for each group. A statistically significant test for comparison was done by Kruskal-Wallis, followed by post hoc Mann-Whitney U test. *P < 0.05 statistically significant compared to the control group.

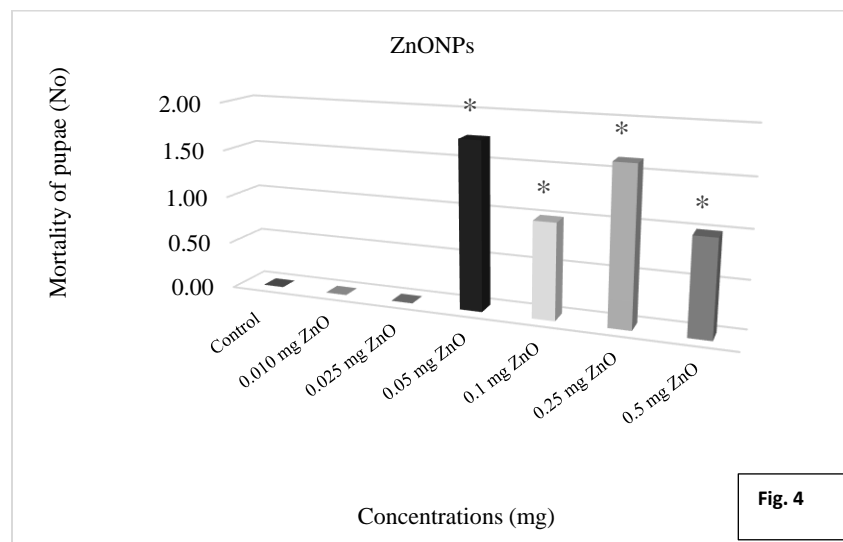


Fig. 4. Effect of ZnONPs on the mortality of pupae (No)

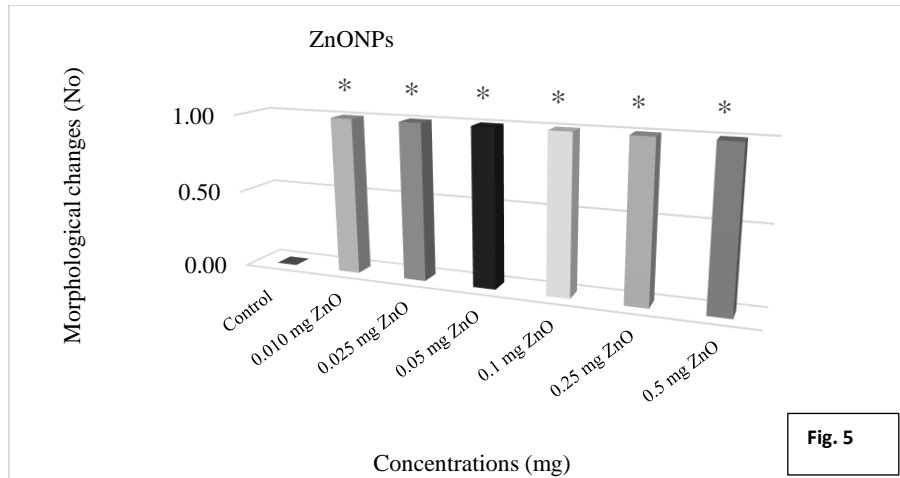


Fig. 5. Effect of ZnONPs on the morphological changes in pupae (No)

Effect of zinc oxide nanoparticles on the adult stage:

Results recorded in **Table (2)** showed that treating the first instar larvae with ZnONPs caused a significant ($P \leq 0.05$) reduction in the number of adult emergences recorded (10 ± 0.00). (8.25 ± 0.25 , 6.25 ± 0.48 , 3.75 ± 0.25 , 2.84 ± 0.10 , 1.64 ± 0.12 and 0 ± 0.00) proportionally to the increasing concentrations: 0.01, 0.025, 0.05, 0.1, 0.25 and 0.5 mg, respectively compared to the control group C. The highest concentration of 0.5 mg was the most effective against adult emergence, with a value of 0 ± 0.00 (**Table 2 and Fig. 6**).

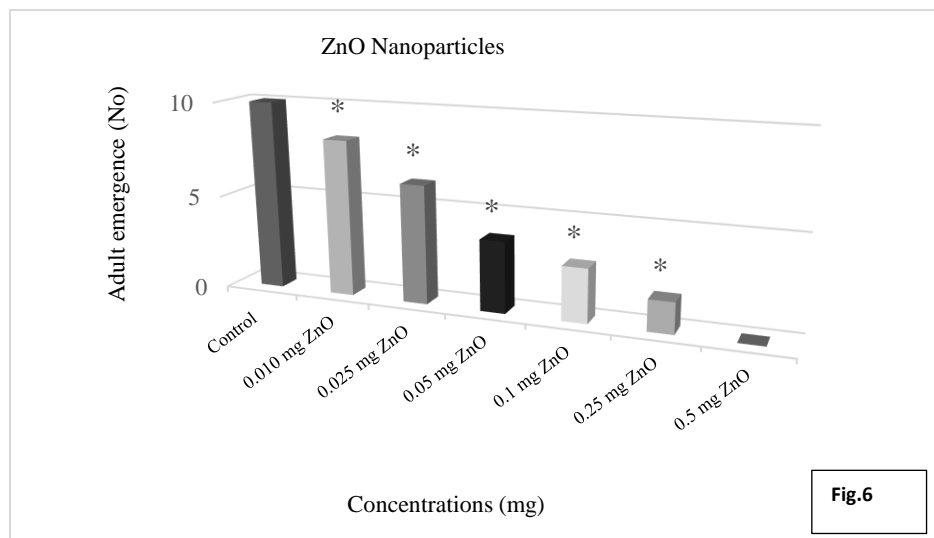


Fig. 6. Effect of ZnONPs on the Adult emergence (No)

DISCUSSION

In the present study, zinc oxide nanoparticles (ZnONPs) synthesized by the *Bacillus foraminis* bacteria were used to investigate their effectiveness against the housefly *M. domestica* (Diptera: Muscidae). Results revealed that the first larvae of *M. Domestica* feeding with different concentrations (0.01, 0.025, 0.05, 0.1, 0.25 and 0.5 mg) of ZnONPs showed significant larval mortality values (18.35, 35, 57.5, 70, 80 and 87.5%) that increased proportionally with increasing concentrations. A previous study recorded the effectiveness of biosynthesis AgNPs against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Kalaimurugan et al., 2019).

The obtained results showed that the ZnONPs caused significant mortality to the pupae after treating the first instar larvae diet with the higher concentrations (0.05, 0.1, 0.25, and 0.5 mg) recording (44.85, 30.42, 56.87, and 100%) mortality, while the lower concentrations did not affect the pupae. The developing pupae were sensitive to ZnONPs and showed decreasing values throughout the different concentrations, and pupae appeared smaller and darker in color than normal ones in the control group. This agrees with a previous study by Abd El-Hamid *et al.* (2018), where they treated the third larval instar (by feeding) with different concentrations of AgNPs, which caused some morphological changes and/or abnormalities in the larvae, pupae, and adult stages. Morphological changes in pupae included: elongation, dwarfism, and swelling, while for the adult stage some individuals showed incomplete emergence and others appeared with wrinkled wings.

In the current study, the value of adult emergence was affected after treatment with different concentrations of ZnONPs. As well, Siddique *et al.* (2022), reported the efficiency of ZnO nanoparticles from *Eucalyptus globulus* L. at different doses (100, 200, 300, 400, 500, and 600 ppm) against *Rhyzopertha dominica* after an exposure period of (3, 7, 11 and 15 days). They mentioned that ZnONPs caused 80.5% of adult mortality at 600 ppm after 15 days while the leaf extract of *E. globulus* caused 62.5% at 1800 ppm. Furthermore, a growth inhibition rate of 75.7% and 87.0% were recorded after 30 days in *R. dominica* against leaf extract and ZnONPs of *E. globulus*, respectively. The present results also agree with some recent studies that reported the effectiveness of different synthesized nanoparticles against houseflies such as the aqueous extract of synthesized AgNPs, AgNO₃, and aqueous extract of *Manilkara zapota* which have been used to evaluate their efficacy on adult houseflies. Kamaraj *et al.* (2012), reported that the synthesized AgNPs were the most effective, with adult mortality values of 72; 89, and 100% after 1, 2, and 3 hours after adults were exposed to a concentration of 10 mg/ml with an LD₅₀ = 3.64 mg/ml. Hence, they suggested that the synthesized AgNPs were effective and could be an ideal environmentally friendly method to control adult houseflies. Similar results were obtained by Gul *et al.* (2016), when they investigated the toxicity of AgNPs synthesized by melon against the adult stage of the housefly, the AgNPs resulted in highly significant mortality of adults 71.6, 9.66, and 100% after 1, 2 and 3 hours of exposure at a concentration of 10 mg/ml and mortalities of 61.67, 70.00, 89.29 and 100% after 1, 2, 3 and 4 hours of exposure at a concentration of 8 mg/ml.

Previous studies have reported the toxic effect of synthesized nanoparticles on the nervous system of the insect, *Cassia fistula*-AgNPs affected the acetylcholinesterase and α and β -carboxylesterase of the fourth instar larva of *A. albopictus* and *C. pipiens* and caused a decrease in their enzyme activity (Solairaj and Rameshthangam, 2016; Fouad *et al.*, 2018). Some studies discussed the effect of different synthesized nanoparticles on insects; nanoparticles are absorbed into the lipids of the cuticle, resulting in physical injury (Barik *et al.*, 2008). Other studies reported that the effect of ZnONPs on the insect became apparent in some morphological and histological changes and accumulations (Banumathi *et al.*, 2017; Abinaya *et al.*, 2018; Ishwarya *et al.*, 2018). Moreover, silver nanoparticles have been reported as highly effective in inducing oxidative stress and detoxification of genes in comparison to the free silver ion effects (Nair *et al.*, 2013). The toxic effect of nanoparticles might be a result of the breakdown of the epithelial lining in the midgut vesicles and damage of the membrane at the apical surface of the epithelial cells (Foldbjerg *et al.*, 2015; Sultana *et al.*, 2018).

CONCLUSION

Pesticides are known to have side effects on humans and the ecosystem, and to overcome these problems it is, therefore, important to use less toxic, more effective, and safe methods. One easy and simple method is the microbial synthesis process for different nanoparticles, which does not involve any hazardous chemicals. The results of the present study showed that the highest concentration of ZnONPs by *Bacillus foraminis* was effective against the larvae, and the latent effect on pupae and adult stages of *Musca domestica* (Diptera: Muscidae), suggesting it as a new promising method in pest control.

List of Abbreviations

ZnONPs: zinc oxide nanoparticles, Mo-LE: *Moringa oleifera* leaf extracts, Mo-AgNPs: Silver nanoparticles synthesized by *Moringa oleifera*, Mo-ZnONPs: Zinc oxide nanoparticles synthesized by *Moringa oleifera*, AgNo3:

silver nitrate, *M. domestica* L.: *Musca domestica* Linnaeus, *A. albopictus*: *Aedes albopictus*, *C. pipiens*: *Culex pipiens*, SE: standard error, LC50: lethal concentration 50, No: number.

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Conflict of Interest: The authors declare no conflict of interest

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