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Comparative analysis of the insecticidal activity of Biogenic Zinc Oxide Nanoparticles, Iron Oxide Nanoparticles and *piper nigrum* fruits extract against 1st instar larva of House fly, (*Musca domestica*) (Diptera: Muscidae)

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Abstract

The housefly (*Musca domestica*) is a common and important medical insect that can transfer over a hundred pathogens. Thus, there is a crucial need for effective strategies with biocompatibility properties to be considered in eliminating the widespread of this insect. Green-manufactured Zinc oxide nanoparticles ZnONPs and iron oxide nanoparticles (α-Fe_2O_3NPs) have been highlighted as biocompatible and eco-friendly choices to counteract the threat of insecticidal-resistant insects. Thus, the current study evaluates their potential by investigating their activity against 1st-instar larvae of houseflies. ZnONPs and α-Fe_2O_3NPs were synthesised using *Piper nigrum* fruit extract as a reducing and capping agent. The nanoparticles were characterised using an ultraviolet-visible spectrophotometer (UV-vis), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscope (SEM) measurements. The study explored the impacts of various concentrations of ZnONPs and α-Fe_2O_3NPs using a feeding assay of 1st-instar larvae and investigated the mortality and morphological changes in larvae, pupae, and emerging adults. Biogenic nanoparticles had a sub-spherical shape with a size of 20-30 and 30-40 nm for ZnONPs and α-Fe_2O_3NPs, respectively. The nanoparticles have caused obvious morphological abnormalities in the larvae, pupae, and adult emerging. The LC50 values of ZnONPs, α-Fe_2O_3NPs, and *Piper nigrum* fruit extract were 2, 1.2, and 75 mg/g, respectively. ZnONPs and α-Fe_2O_3NPs may accord to reducing the attempts, risk indications, and economic amounts raised from applying chemical insecticides. Thus, insect administration using nanoparticles could unfold deep, advanced sides.

Keywords: *Musca domestica*, 1st-instar larvae, insecticidal, ZnONPs, α-Fe_2O_3NPs, *Piper nigrum*

Introduction

The house fly (*Musca domestica*) is one of the most widespread insects worldwide and impacts the environment and public health. It can be found in all places humans have found, and it adapts easily to different environmental conditions (1). The house fly feeds on a wide range of materials, including human and animal waste and decaying food, and can break down solid food using its secretions and then absorb it in liquid form (2,3). House flies can be bred in decomposing organic materials, like garbage, compost heaps, animal waste, or sewage (4). The warm and humid habitats are considered the main points of their geographical

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distribution (5). Houseflies can transmit diseases such as cholera, typhoid, and diarrhea, and consequently, can become a worldwide health hazard (6). Chemical control approaches, routinely applied against the house fly, cause risk effects on the health, accumulate in the environment, and develop resistance problems in pests (7, 8).

Nanoparticle synthesis implicates different methods, including physical, chemical, and biological methods (green synthesis) (9, 10). However, chemically synthesized nanoparticles showed significant toxic effects on human cells, including the release of cytochrome C, mitochondria of treated cells, nucleus enlargement, lectin degradation, significant genetic damage, and changes in the gene expression due to the release of Reactive oxygen species (ROS) as the main cause of these changes (11,12). On the contrary, biogenic synthesized nanoparticles are lately deemed eco-friendly alternatives to traditional chemicals (13). Many studies used different types of nanoparticles for house fly control, such as AgNPs (14-16), ZnNPs (16-18), and Al_2O_3NPs (18).

Biogenic iron oxide nanoparticles (α-Fe_2O_3NPs) offer potential in many applications due to their environmental friendliness, lack of impact on the ecosystem, and low manufacturing cost (19). α-Fe_2O_3NPs have been widely used in various applications, such as magnetic resonance imaging and removing hydrocarbons and heavy metals from water, as well as used as antimicrobial (20). The use of α-Fe_2O_3NPs to control house flies is a contemporary area of study that promises to introduce new and effective strategies to control the house fly (21). The α-Fe_2O_3NPs offer different mechanisms to control the house fly, such as magnetic targeting using a magnetic field of α-Fe_2O_3NPs. α-Fe2O3NPs act as pesticide carriers and target specific areas, thus enhancing the efficiency of pesticide delivery to the house fly (22, 23). In addition, α-Fe2O3NPs can directly target insects without adjuvants or complex killing aids (24). The use of iron oxide nanoparticles in controlling first-stage housefly larvae has demonstrated the unique potential of these particles (25).

Green-synthesized ZnONPs have important applications as antibacterial, antifungal, antidiabetic, anti-inflammatory, and antioxidant agents compared to chemically synthesized ZnONPs, whose biological applications are limited due to their toxicity (26,27). In addition, ZnONPs have been used to remove heavy elements from water (28), and in dental applications (29). Using ZnONPs to eliminate houseflies is considered a trendy strategy that aims to improve the effectiveness of insect management (30). The mechanism of action of zinc oxide nanoparticles (ZnONPs) is to interact with the cell membranes of insect larvae, which leads to severe effects and damages (31). The use of zinc oxide nanoparticles (ZnONPs) to control houseflies is a novel approach that aims to improve the effectiveness of insect management (32). The advantages of using ZnONPs include the direct effect on the housefly upon contact, making it a fast and effective method for eliminating it. It also reduces the need for conventional pesticides to which insects may develop resistance (33).

Piper nigrum L., commonly known as black pepper, belongs to the Piperaceae family. It is a popular spice that is grown worldwide, primarily for its fruits. Black pepper contains many phytochemicals, including alkaloids, flavonoids, and phenolics. Black pepper also contains metabolically active compounds that are used in various pharmaceutical formulations and as a food preservative (34), volatile oils, oleoresins, minerals, vitamins, carotenoids, and flavonoids (35). These chemical compounds act as reducing agents during the synthesis and capping of nanoparticles (36).

The current study aimed to evaluate and compare the effect of two types of nanoparticles (ZnONPs and α-Fe_2O_3NPs) and black pepper fruit extract against the 1st-instar larvae of house flies for utilizing these nanoparticles as larvicidal tools

using the feeding strategy in breeding locations for eliminating the insects. Further, this study was addressed to define the impacts of least lethal concentrations LC50 of both nanoparticles on the larvae, pupae duration, and emergence of adults.

Materials and Methods

Materials

Dry black pepper fruits (*Piper nigrum* L.), Zinc Acetate ($Zn(CH_3CO_2)_2 \cdot 2H_2O$), Ferric chloride ($FeCl_3$), HCl, NaOH. Milk and Yeast.

Methods

Collection of plant samples

The dry fruits of the black pepper (*P. nigrum* L) (Figure 1) were compiled from local markets in Basra Governorate in October 2023. The fruits were transferred to the laboratories of the Biology Department/ the College of Science, University of Basra, to classify them in the herbarium of the Department by Dr. Sahar Abdul Abbas Malik.

Figure 1. *Piper nigrum* L., plant and dried fruits (37, 38)

Preparation of plant powders

The powder of the plant was made by taking and grinding the dry fruits with an electric grinder. The powders of fruits were kept in clean, closed plastic containers to protect them from moisture and spoilage. The containers were then held in the refrigerator until used in the extraction.

Preparation of ethanolic plant extracts

Plant ethanolic extract was prepared following the protocol described by (39).

Preparation of aqueous *P. nigrum* extract

The dry powder of plant (5 g) was put in a 250 ml conical flask, then 100 ml of distilled water was added, and the mixture was boiled at 70 C° for 30 min. The extract was then filtered using a Whatman No. 1 filter paper, then kept at 4 C° (40).

Gas chromatography/mass spectrometry (GC-MS) analysis

The ethanolic plant extracts were prepared for GC/MS analysis (the Nihran Omer Company, the device is of type (SHIMADZU, Japan) by taking 0.2 g of ethanolic extract and dissolving it in 10 ml of ethyl alcohol as an extraction solution. The sample is injected into the apparatus, heated and gasified, and transported by a carrier gas (usually helium or hydrogen) through a chromatography column. The components are separated based on their differences in fluency and selectivity toward the stationary phase within the column. The separated components are then transported to the mass analyzer, where they are fragmented into ions using various ionization sources such as electron ionization or chemical ionization. These ions are

analyzed for mass and charge in the mass analyzer and are used to determine the chemical structure of the components based on the resulting mass spectra (41).

Synthesis of ZnONPs and α-Fe_2O_3NPs

To prepare ZnONPs, Zinc Acetate ($Zn(CH_3CO_2)_2 \cdot 2H_2O$) from Aldrich, USA was used to prepare a fresh aqueous zinc acetate solution (0.25M) using 100 ml of distilled water (42). The mixture of zinc acetate and P. *nigrum* extract was adjusted for pH at 12 and was left on the magnetic stirrer for two hours at 60-70 °C to ensure the complete reduction of the zinc acetate salt. To prepare Iron oxide nanoparticles (α-Fe_2O_3NPs), ferric chloride ($FeCl_3$) from Hi-Media was used, and 100 ml of distilled water was used to dissolve Ferric chloride (43). The resultant mixture of Ferric chloride and *P. nigrum* extract was left on a magnetic stirrer for 24 hours to complete reduction. The ZnONPs and α-Fe_2O_3NPs were collected and washed thrice with D.W. and once with ethanol. The NPs were dried using an oven at 70°C, collected and ground using a ceramic mortar, and placed in a neat, tightly sealed glass container to prevent exposure to moisture. The container was stored at room temperature in a dark place.

Characterization of nanoparticles:

A UV-vis spectrophotometer (UV-1900i-Shimadzu, Japan) was used to measure the absorption spectra of the nanoparticles in the visible and near-infrared 200-800 nm at a scanning speed of 100 nm/s. Fourier transform infrared spectroscopy (BRUKER, Germany) in the region of 400-4000 C-1 of the electromagnetic radiation spectrum was used to obtain the absorption or emission spectrum of the nanoparticles. The XRD device (Philips, PW1730, Netherlands) was used to obtain the X-ray diffraction values of the nanoparticles. Field emission scanning electron microscopy (FESEM-EDX) (MIRA III, Tescan, Czech Republic) was used to acquire images of the nanoparticles for size, shape and distribution determination at 5.00 kV, 8.9 mm imaging window, and 80.00 kx resolution for ZnONPs and at 15.00 kV, 4.97 mm imaging window with a resolution of 350.00 kx for α-Fe_2O_3NPs.

The house fly collection and pure colonies Preparation:

Adults of the house fly (*M. domestica*) were collected from different farms in the Khor Al-Zubair area/Basra Governorate (longitude of E 47.7687° and latitude of N 30.2331°) using a large insect net during October 2023. The flies were transferred to the laboratories of the Biology Department/ the College of Science, University of Basra, to classify them. The house flies were classified in the Research Center and Natural History Museum of Professor Dr Kazim Saleh Al-Hadlak/ Department of Biology/ College of Science/ University of Basra. Following, twenty flies were reared in the breeding cages with dimensions of (50 x 25 x 25 cm) to get pure strain to raise them for conducting further experiments.

Housefly breeding:

Adults of house flies in breeding cages were fed using milk dissolved in sterilized distilled water (6 g/ 10 ml) as a nutritional medium by putting it in a glass petri dish provided with cotton pads to lay eggs (44). In addition, containers of larval media consisting of milk, yeast, and sterilized animal manure were placed in breeding cages and were constantly moistened with sterilized tap water to ensure maintaining the appropriate humidity of 75% and a temperature of 28±2 °C as considered standard conditions for raising the insect (45).

Bioassay:

Different concentrations of chosen NPs (0.2, 0.4, 0.8, 1.6, 3.2, 6.25, 12.5, 25, 50, and 100 mg/g) were used for bioassay analysis using feeding procedures for 1st-instar larvae (8). The weights of NPs (0.2, 0.4, 0.8, 1.6, 3.2, 6.25, 12.5, 25, 50, and 100 mg) were separately added to 1 g of previously wetted larvae diet with distilled water in plastic containers

and mixed well to get the above concentrations. Three replicates of the 1st instar larvae for each concentration (ten larvae in each) were applied. The control group was given 1g of larvae diet wetted with distilled water. The test and control containers of larvae were maintained at standard conditions. Statements of tested and the control groups of 1st-instar larvae, pupal development, and adults' emergence were recorded at 24, 48, and 72 hr to investigate mortality or morphological changes. The dead larvae were collected and moved from the containers for counting; larval mortality was documented until the pupation stage, and pupae mortality was recorded until 6 days after pupation without adults emerging.

Statistical Analysis:

The Graph Pad Prism program version 5.0 (GraphPad Software Inc, USA) for the statistical analysis was applied. The data was presented based on the mean ± SD of at least three independent replicates, and the differences were analysed to determine any statistically significant differences between the mean values of the treatments with time and concentrations using Two-way ANOVA followed by Bonferroni posttests, $P \leq 0.0001$. Further, the lethal concentration of nanoparticles and extract needed to eradicate 50% (LC50) of the house fly larvae 72 hr post-treatment was investigated.

Results:

GC-MS analysis

The chemical analysis (GC-MS) of black pepper fruit extract exhibits the existence of 25 different active compounds (Figure 2 and Table 1). Piperine compound came in the highest percentage estimated at 53.66% which was higher than the sum of the percentages of the other compounds combined. The caryophyllene compound came in the second sequence with a percentage of 10.18%. The third percentage was for the compound (E)-5-(Benzo[d][1,3] dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one, which reached 5.34%. The rest of the compounds were in varying percentages less than that.

Efficacy of ZnONPs, α-Fe_2O_3NPs, and black pepper extract on 1st-instar larvae of the house fly:

Data in Table 2 showed varied effectiveness of ZnONPs, α-Fe_2O_3NPs, and *P. nigrum* extract in the mortality rate against 1st-instar larvae of house flies using feeding bioassay during 24, 48, and 72 hr. The rate of larval mortality via feeding bioassay indicated its importance for treated groups. The treated 1st-instar larvae with ZnONPs displayed clear activity, that increased with increasing the concentration of ZnONPs. The concentration (25-100) mg/g, recorded percentage of mortality started from the first hours of treatment and reached (100%) 24 hr post-treatment (Figure 3), and that the LC50 value is 2 mg/g. The mortality rates varied between the times of 24 hours to 72 hours. The mortality rates of 1st-instar larvae treated with α-Fe2O3NPs revealed maximum activity at high concentrations of (100-12.5) mg/g, recorded mortality rates of (100%) in the first hours of treatment till 24 hr post-treatment, (Figure 4), and LC50 value is 1.2 mg/g. The ethanolic extract of black piper fruits against 1st instar larva of *M. domestica* exhibited activity in the mortality rate started from 48 hours of treatment at concentrations of 25 and 50 mg/gm, which reached 23.3% for both concentrations after 72 hr of treatment, and the LC50 value is 75 mg/g (Table 3) and (Figure 5).

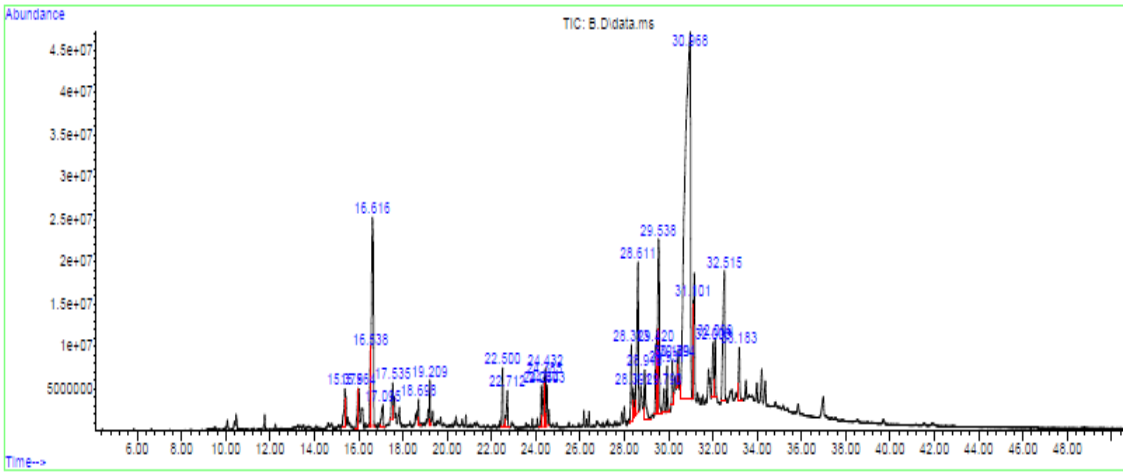

Figure 2: Chemical compounds in the ethanolic extract of black pepper fruits

Table 1: Chemical compounds in the ethanolic extract of black pepper fruits

Peak	Library/ID	R.T.	Area %
1	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-	15.375	1.3594
2	Copaene	15.964	0.8951
3	Caryophyllene	16.538	10.1879
4	Humulene	17.095	0.9734
5	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	17.535	1.2046
6	Caryophyllene oxide	18.698	0.5064
7	Isospathulenol	19.209	0.7476
8	2,4-Decadienamide, N-isobutyl-, (E, E)-	22.5	1.276
9	n-Hexadecanoic acid	22.712	0.9816
10	(E)-3-Phenyl-1-(piperidin-1-yl)prop-2-en-1-one	24.244	0.8893
11	10E,12Z-Octadecadienoic acid	24.385	1.7523
12	Oleic Acid	24.432	1.0683
13	(2E,4E)-1-(Piperidin-1-yl)deca-2,4-dien-1-one	24.503	0.6899
14	1,2,3,4-Tetrahydro-9-acridanone	28.313	1.8789
15	Dibenz[b,f]][1,4]oxazepine	28.391	0.6578
16	(E)-5-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one	28.611	5.3493
17	(E)-1-(Piperidin-1-yl)hexadec-2-en-1-one	28.91	1.8082
18	Piperine	29.42	53.6835
19	5,5'-((2R,3S)-2,3-Dimethylbutane-1,4-diyl)bis(benzo[d][1,3]dioxole)	29.931	0.9259
20	(5R,8R,8aS)-8-Ethyl-5-(pent-4-yn-1-yl)octahydroindolizine	30.159	0.9932
21	2-(4-Hydroxy-3-methoxyphenyl)-3,5,7-chromanetriol	30.394	0.4795
22	(2E,6E)-7-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)hepta-2,6-dien-1-one	31.101	1.8484
23	(E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(pyrrolidin-1-yl)non-8-en-1-one (Piperoleine B)	32.005	6.9188
24	3-(1,3-Benzodioxol-5-ylmethyl)-4-[(3,4-dimethoxyphenyl)methyl]oxolan-2-one	32.099	1.5251
25	Pipernonaline	33.183	1.3996
			100%

Table 2: The effectiveness of ZnONPs, α-Fe_2O_3NPs, and pepper extract on the 1st-instar larvae of the house fly (24, 48, and 72 hours)

Treatment / Con (mg/g)	ZnONPs			α-Fe_2O_3NPs			*P. nigrum* extract		
	Exposure time (hr)			Exposure time (hr)			Exposure time (hr)		
	24	48	72	24	48	72	24	48	72
0.2	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
0.4	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
0.8	*3.33±3.33	16.66±3.33	26.6±3.33	16.66±3.33	23.33±3.33	33.33± 3.33	0 ± 0.00	0 ± 0.00	0 ± 0.00
1.6	16.66±3.33	36.33± 6.66	40 ±0.00	23.33±3.33	43.33±3.33	66.66 ±6.66	0 ± 0.00	0 ± 0.00	0 ± 0.00
3.2	20 ± 10.00	50.00± 0.00	60±0.00	36.33±6.66	50.00±0.00	86.66±6.66	0 ± 0.00	0 ± 0.00	0 ± 0.00
6.25	56.66±5.55	100±0.00	100±0.00	60±0.00	100±0.00	100±0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
12.5	93.33±11.5	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
25	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	0 ± 0.00	6.66±3.33	23.33±3.33
50	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	0 ± 0.00	6.66±3.33	23.33±3.33
100	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	23.33± 3.33	43.33±3.33	66.66±6.66
Control	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

Control = no mortality

*** Numbers represent the mean of the killing percentage of three replicates ± SD**

Table 3: LC50 of ZnONPs, α-Fe_2O_3NPs, and pepper extract on the 1st-instar larvae of the house fly (72 hours)

Treatment/ Con (mg/g)	LC50 24hr	LC50 48hr	LC50 72hr	*P value*
ZnONPs	8.1	3.7	2	*
α-Fe_2O_3NPs	4.5	1.85	1.2	*
P. nigrum extract	214.3	115.4	75	*
P value	*	*	*	

LC50 = lethal concentration of 50% of the larvae

*** = significant (P≥0.05)**

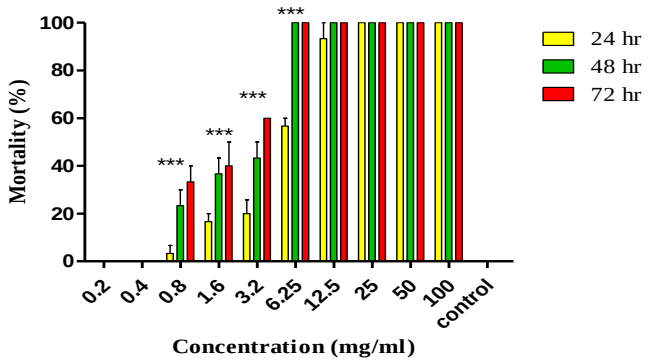

Figure 3: Treatment of 1st-instar larvae with ZnONPs for 24, 48, and 72 hours

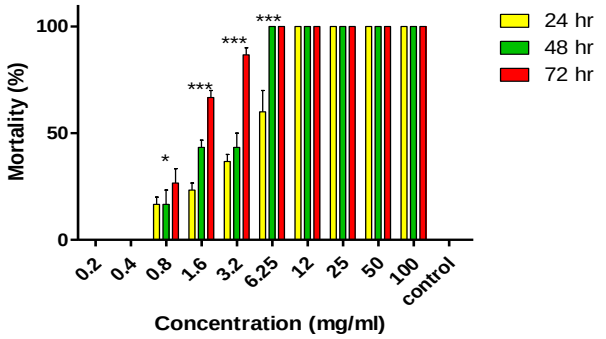

Figure 4: Treatment of 1st-instar larvae with α-Fe_2O_3NPs for 24, 48, and 72 hours

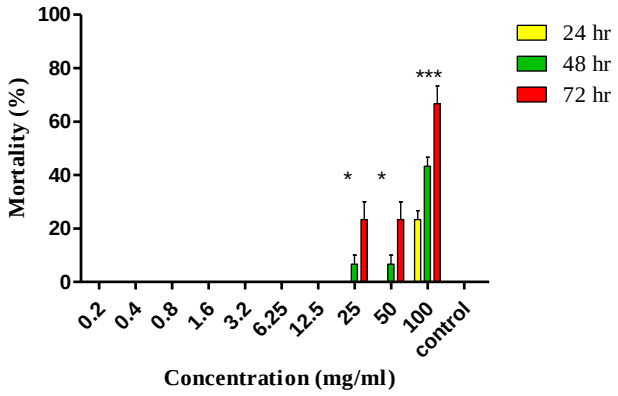

Figure 5: Treatment of 1st-instar larvae with pepper fruit extract for 24, 48, and 72 hours

Efficacy of ZnONPs, α-Fe_2O_3NPs, and black pepper extract on 1st-instar larvae of the house fly:

According to the data in Table 2, when comparing the percentage of killing rates among different treatments (ZnONPs, α-Fe_2O_3NPs and pepper fruit extract), we find that there is an increase in the killing rates of 1st-instar larvae treated with α-Fe_2O_3NPs 16.66% compared to 3.3% for ZnONPs at the concentration of 0.8 mg/g (* $P < 0.05$) and 36.33% for α-Fe_2O_3NPs compared to 20% for ZnONPs at the concentration of 3.2 mg/g (** $P < 0.01$), with no significant differences for other concentration.

In addition, there are differences recorded between nanoparticles and black pepper fruit extract *** $P < 0.001$ (Figure 6). For 48 hr post-treatment, Figure (7), the results revealed significant differences between the mortality rates of 1st-instar larvae treated with ZnONPs and iron oxide nanoparticles compared to black pepper fruit extract, while the differences were recorded for 6.25 and the higher concentrations. Further, there are significant differences between the mortality rates of 1st-instar larvae treated with ZnONPs and α-Fe_2O_3NPs using the concentrations of 3.2 and 1.6, while the differences between nanoparticles compared to black pepper fruit extract started with the higher concentrations *** $P < 0.001$ (Figure 8). Further, significant differences were also recorded when comparing the treatments and control groups at all times.

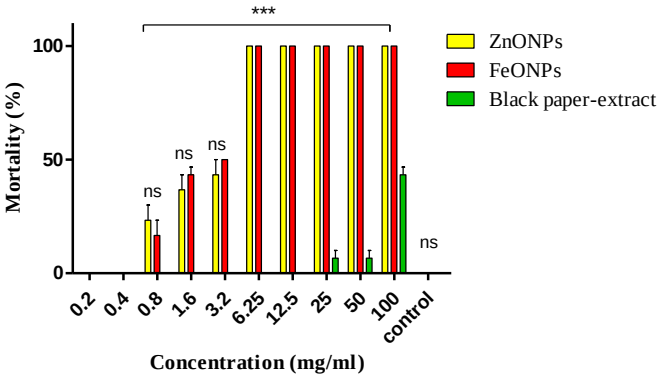

Figure 6: Treatment of 1st-instar larvae with ZnONPs, α-Fe_2O_3NPs, and black pepper extract (24 hours)

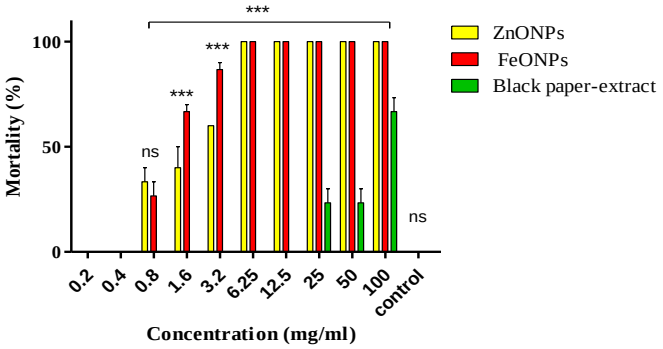

Figure 7: Treatment of 1st-instar larvae with ZnONPs, α-Fe_2O_3NPs, and black pepper extract (48 hours)

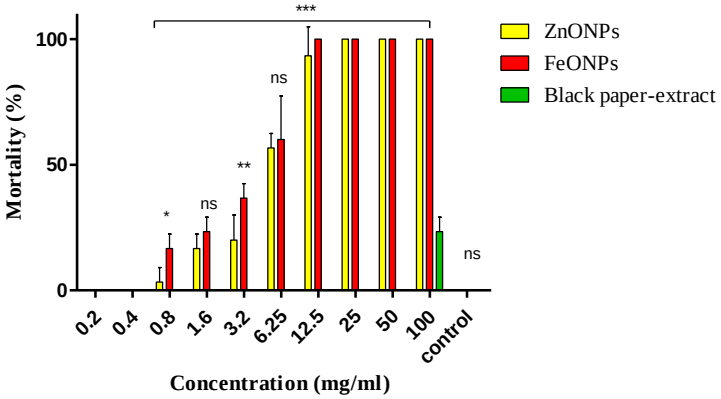

Figure 8: Treatment of 1st-instar larvae with ZnONPs, α-Fe_2O_3NPs, and black pepper extract (72 hours)

The percentage of larval mortality and pupation percent:

Data in Table 4 reveal a significant reduction in pupation rate that declined to 6.66, 46.66, and 56.66% and declined to 6.66, 23.33, and 33.33% at concentrations 0.8, 0.4, and 0.2 mg/gm of ZnONPs and α-Fe_2O_3NPs, respectively compared to 97% for *P. nigrum* extract and control group. Furthermore, significant decreases in pupation rate (76.66, 73.33, 36.66%) were registered for *p. nigrum* at concentrations (25, 50, and 100 mg/g), respectively, compared to the control group. The data also indicated that the duration of treated pupae with ZnONPs and α-Fe_2O_3NPs was 6 days, which was longer than the pupae's duration of the control (5 days).

The percentage of adult emergencies:

The data in Table 4 revealed that the adult emergence declined from 97% for the control to 82.3% and 78.5% for larvae treated with ZnONPs and declined to 89.3% and 57.1% for larvae treated with α-Fe_2O_3NPs using concentrations (0.2 and 0.4 mg/g), respectively.

Morphological malformation:

Figure 9 showed that evident distortions of larvae of *M. domestica* were produced following treatment of the 1st-instar larvae with seven concentrations (1.6, 3.2, 6.25, 12.5, 25, 50, 100 mg/g) of ZnONPs and α-Fe_2O_3NPs, images 1, 2, 3, 4, 5, 6, 7, respectively. Morphological larvae malformations (Figure 9) incorporate change in the brown color, body swelling, cuticle frailty and no uniform body shapes compared to control larvae (image 8). The abnormalities are clearer in larvae treated with α-Fe_2O_3NPs than in ZnONPs. Further, morphological malformations of the pupae indicated in images (1, 2, and 3, for larvae treated with (0.2, 0.4, and 0.8 mg/g), respectively (Figure 10) included a black color, smooth surface, somewhat small diameter, lopsidedly shape and shrinkage in the middle compared to the control (image 4).

Table 4: Development of the 1st-instar larvae of the house fly after treatment with ZnONPs, α-Fe_2O_3NPs, and pepper fruit extract after 72 hours

Treatment / Con (mg/g)	ZnONPs			α-Fe_2O_3NPs			*P. nigrum* extract		
	Pupation	duration	New emergent	Pupation	duration	New emergent	Pupation	duration	New emergent
0.2	56.66*	6	82.3	33.33	6	89.3	97	6	100
0.4	46.66	6	78.5	23.33	6	57.1	97	6	100
0.8	6.66	/	0	6.66	/	0	97	6	100
1.6	0	/	0	0	/	0	97	6	100
3.2	0	/	0	0	/	0	97	6	100
6.25	0	/	0	0	/	0	95	6	100
12.5	0	/	0	0	/	0	95	6	98
25	0	/	0	0	/	0	76.66	6	97
50	0	/	0	0	/	0	73.33	6	77.27
100	0	/	0	0	/	0	36.66	6	90
Control	97	5	100	97	5	100	97	5	100

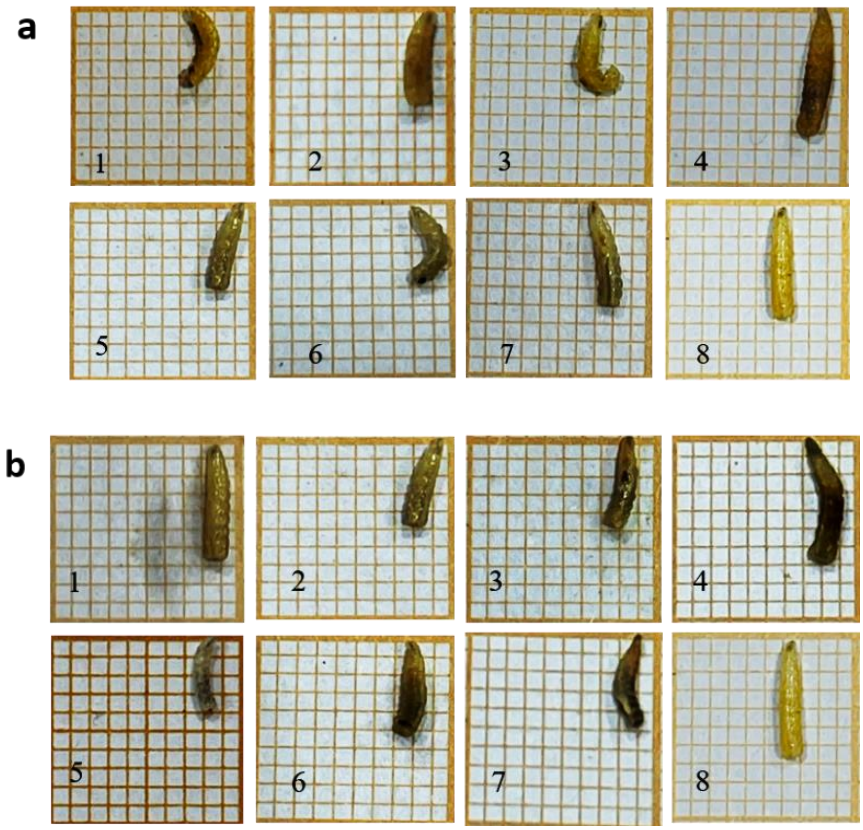

Figure 9: 1st-instar larvae of house flies treated with (a) ZnONPs and (b) α-Fe_2O_3NPs

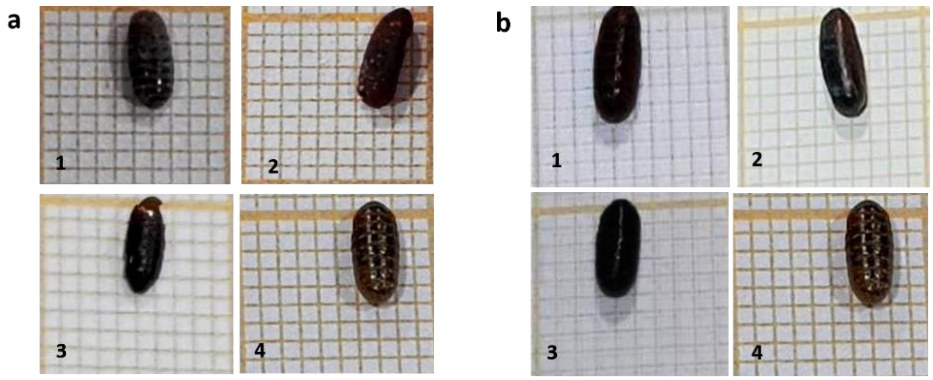

Figure 10: Pupae resulting from 1st-instar larvae treated with (a) ZnONPs and (b) α-Fe_2O_3NPs

Discussion

Black pepper fruit extract was used for synthesizing two types of nanoparticles, Zinc oxide nanoparticles and iron oxide nanoparticles. Black pepper contains various chemical compounds that play a role in the green synthesis of nanoparticles. Piperine is an important alkaloid compound presenting at varying proportions in many plants, but it is in high levels in black pepper, with a percentage that may reach more than 9% (46). Many studies obtained data that agree with the current data. Piperine was found in black pepper in percentages ranging from 3.92 to 3.98% and 6.61% (47). In another study, piperine reached a value of 8.13% (48). Further, the results of the GC-MS of black pepper (*P. nigrum* L.) fruits by (49) (2022) indicate that the main components are alkaloids, including piperidine and caryophyllene, at a rate of 9.97% and 6.67%, respectively. Piperine could be responsible for these nanoparticle syntheses.

Zinc oxide nanoparticles, iron oxide nanoparticles, and black pepper fruit extract were used to evaluate their effectiveness in killing the 1st-instar larvae of the house fly. Larvae were treated using the feeding method. The number of killed larvae was counted in each replicate and for each concentration suggested in the experiment after 24 hours, 48 hours, and 72 hours. Pupation of larvae and emergence of insects from concentrations that did not kill were also monitored. The present data are in line with the data of (33), where ZnONPs with LC50 (1 mg/g) display effects on *M. domestic* larvae, which involved promotion of cellular defect and reduction in oxidative stress enzymes. Another study demonstrated that ZnONPs with an LC50 value of 49.6 mg/g induced 41.9% of larval mortality in *M. domestic;* thus, our study obtained better results as the LC50 of ZnONPs was only 2 mg/g of larval diet (16). On other side, (50) reported that the percentage of 1st-instar larval motility by ZnONPs was 8.75 ± 0.25% using 0.5 mg/g.

Further, the mortality of larvae increased with increasing the period time to 72 hr. Study of (14) found that silver NPs synthesised from crude rice starch paste extract cause mortality that increases with increasing the period of incubation. The present outcomes from ZnONPs agree with the findings of (17), in their assessment of the insecticidal efficiency of zinc oxide nanoparticles against the house fly. Further, the results are comparable with findings of (51), using Fe_3O_4NPs against larvae of *M. domestica*. Moreover, the data revealed that the adult emergence declined with increasing concentrations of nanoparticles. Elevated declines in house fly emergence were also documented by (52) after using cadmium sulfide nanoparticles and against 3rd instar larvae of *M. domestica*. Another study reported a decrease in adult emergence percentage to 100% post-feeding house fly larvae media contaminated with silver nanoparticles at concentrations of 200 ppm (15). The decrease in the emergence of house fly adults was consistent with earlier data for using ZnONPs to control *Spodopetra. Littoralis* (53).

Regarding morphological abnormalities, a study of (54) established morphological abnormalities in larvae of *M. domestica* after feeding it with 45 mg/g of AgNPs. Various morphological anomalies induced by α-Fe_2O_3NPs on larvae and pupae of *M. domestic* house flies were highlighted by (25), which included malformed adults with scrunch-up wings, as well as the inability of pupa to leave puparium, further stream damage in the cuticle tissues and midgut. Also, the toxicity of nanoparticles could be due to the partisan lysis of epithelial cells of the digestive system and the disturbance of the cellular membrane of epithelial cells (55). In addition, ZnONPs, due to their small size, can easily penetrate the cell wall of larvae (56).

Conclusion

In conclusion, α-Fe_2O_3NPs showed a more elevated toxicity influence against *M. domestica* larvae than

ZnONPs and *P. nigrum* extract. Furthermore, the treatment of larvae with nanoparticles induced taller prolongation in the duration of larvae and pupae and a greater decrease in the emergence of adults compared to *P. nigrum*. On the other hand, reductions of the pupation stage were higher in larval groups treated with nanoparticles than in larval groups treated with the same concentration of the *P. nigrum* extract. It is crucial to investigate new green synthesized products to present an adequate strategy against houseflies, and further examinations regarding the application of different nanoparticles in the management of houseflies are recommended.

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