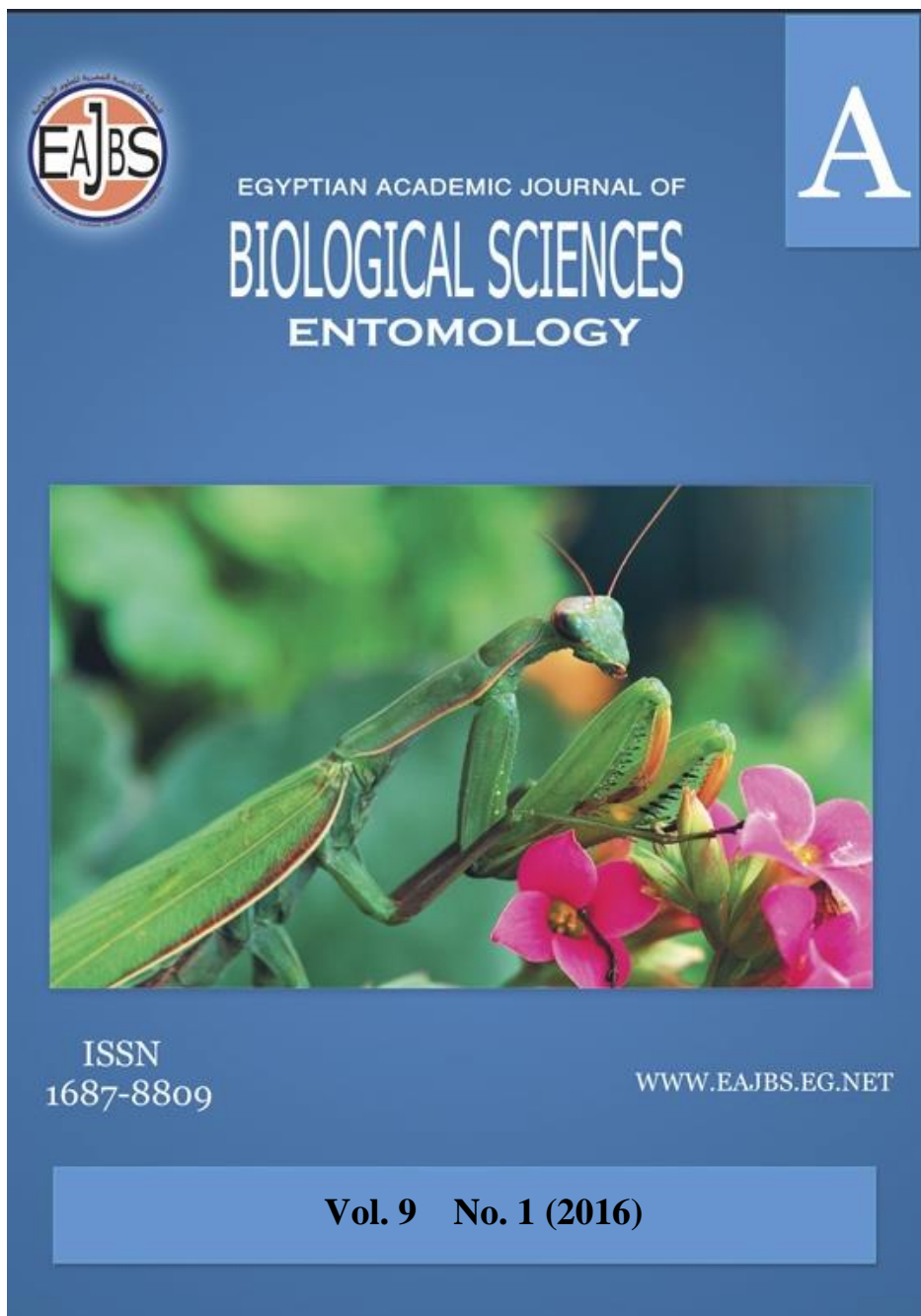


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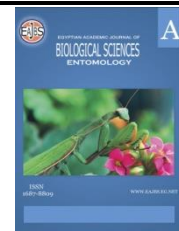


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Toxicological Effects of Hematite Nanoparticles on the Common House Mosquito, *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

The obtained work aimed to evaluate the efficiency of hematite nanoparticles as larvicidal agents against *Cx. pipiens* larvae. Hematite nanoparticles were synthesized by a simple hypothermal method. The obtained nanoparticles average size was below 50 nm. TEM images determined the size and morphology of nanoparticles. Serial concentrations were applied on mosquito larvae. It was found that LC₅₀ of hematite nanoparticles after 48 hours of treatment was 5.6997 ppm. The obtained results added a new weapon to control mosquitoes. It is suggested to investigate the joint action of nanoparticles with other insecticides to which insects had developed resistance.

INTRODUCTION

Mosquitoes under the genus *Culex* (*Cx.*) are vectors of numerous pathogens that significantly affect public health including arboviruses such as Sindbis Virus Complex, West Nile Virus, and Rift Valley fever virus (RVF) (Diamond 2009 & Becker *et al.* 2010) and the filarial worm, *Wuchereria bancrofti* (Ottesen *et al.* 1997 & Goddard 2008). In Egypt, Lymphatic filariasis (LF) and Rift Valley fever virus (RVF) are transmitted by *Cx. pipiens* (Hoogstraal *et al.* 1979 & Harb *et al.* 1993). Insecticide applications, although highly efficient against the target vector species, are facing a threat due to the development of resistance to insecticides (Liu *et al.* 2006). Other undesirable effects include toxicity to non-target organisms and environmental and human health concerns (Yang *et al.* 2002). Whereas nanotechnology has become omnipresent in the pharmaceutical research and development arena starting as early as the mid-70s (Rosen and Aribat 2005), it has, quite surprisingly, only recently made its way to the field of agrochemical formulations and delivery systems. Nanoparticles constitute a major class of nano-size materials. These particles are zero-dimensional, possessing nanometric dimensions in all the three dimensions.

The diameter of nanoparticles can vary anywhere between one and a few hundred nanometers (Rao *et al.* 2007).

These particles play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, and tissue engineering (Morones *et al.* 2005). Nanoparticles are promising in different fields of agricultural

biotechnology (Rahman *et al.* 2009). Nanoparticles are synthesized artificially by chemical and/ or physical methods. Many of the physical methods involve the evaporation of a solid material to form a supersaturated vapour from which homogenous nucleation of nanoparticles occurs. Emerging chemical methods proved indispensable for synthesizing nanocrystals of various types of materials. Isolated nanocrystals dispersible in solvents (sols) are the most important nano-size materials produced by chemical means in agrochemical biotechnology. These can either be in aqueous media, hydrosols or in organic solvents, organosols (Rao *et al.* 2007).

Recent application of nanoparticles as insecticidal agents resulted in high mortality levels in larvae of *Spodoptera littoralis* (Elek *et al.* 2010), *Sitophilusoryzae* (Depnath *et al.* 2010), *Anopheles subpictus* and *Culex quinquefasciatus* (Santhoshkumar *et al.* 2010). A limited number of studies investigated the toxicological and biological effects of different forms of iron oxide nanoparticles on *Drosophila melanogaster* (Alvarez *et al.* 2014 & Chen *et al.* 2014).

MATERIALS AND METHODS

Mosquito Culture:

The mosquito *Cx. pipiens L.*, was obtained from the Research and Training Centre on Vectors of Diseases, Ain Shams University, Cairo. The sample was reared for several generations in the Entomology Department, Faculty of science, Ain Shams University, Cairo under controlled conditions ($27\pm 2^{\circ}\text{C}$, RH $70\pm 10\%$ and 12-12 light-dark regime). Adult mosquitoes were kept in (24 x 24 x 24 cm) wooden cages and daily provided with cotton pads soaked in 10% sucrose solution for a period of 3-4 days after emergence. After this period the females were allowed to take a blood meal from a bird host. Plastic cup oviposition (15x15cm) containing distilled water was placed in the cage. The resulting egg rafts picked up from the plastic dish and transferred into plastic pans (25 x 30 x 15 cm) containing 3 liters of tap water left for 24 hours. The hatching larvae were provided daily with fish food as a diet (Kasap and Demirhan, 1992).

Tested Nanoparticles:

Hematite ($\alpha\text{-Fe}_2\text{O}_3$) nanoparticles were prepared by hypothermal methods according to (Tadic *et al.* 2014). Solution A was formed by mixing 1.1 g NaOH, 3 ml deionized water, 14 ml ethanol, and 11 ml oleic acid in a 250 ml conical flask. Afterwards, mixture was heated on an electromagnetic stirrer at 60°C to produce a homogenous mixture. Solution B was prepared by dissolving 0.8g of ferrous-sulfate heptahydrate $\text{Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$ in 12.5 ml deionized water. Solutions A and B were mixed together in a 250 ml conical flask and heated on an electromagnetic stirrer at 60°C for 6 hrs. The final solution was placed in a 100 ml capacity Teflon liner autoclave and heated at 160°C for 50 hrs. Finally, the solution was cooled to room temperature. Reddish brown powder was collected and washed six times by deionized water and ethanol and dried in a laboratory oven at 75°C for 12 hrs. Stock solution of hematite nanoparticles was prepared by dissolving 0.1 g of nanoparticles in 100 ml 0.5% Triton x-100. Distilled water was used in the preparation of the stock solution.

Characterization by Transmission Electron Microscope:

High resolution transmission electron microscope (HR-TEM, Tecani G20, FEI, Netherlands) was used for the aim of imaging and crystal structure revelation. Eagle charge-coupled device camera with (4k*4k) image resolution was applied to acquire and collect transmitted electron images. TEM Analysis software was used to analyze TEM images.

Bioassay Test:

The larvicidal activity of synthesized nanoparticles were evaluated against the late third instar larvae of *Cx. pipiens* under laboratory conditions (27±2°C, RH 70±10%, and 12-12 light-dark regime). Bioassay tests were performed according to (WHO 2005). Batches of twenty late third instar larvae were transferred by a dropper to four small disposable test cups, each containing 100 ml of water. Small, unhealthy or damaged larvae were removed and replaced. The tested concentrations were 0.025, 0.25, 2.5, 25 ppm. The concentrations were prepared by serial dilution of the stock solution. The appropriate volume of dilution was added to the volume of water in the cups to adjust the desired concentration. Control test was performed for each concentration using the corresponding concentration of Triton x-100. Three replicates were performed for each concentration. After 48 hours exposure, larval mortality was recorded.

Calculations and Statistical Analysis:

- Mortality percentages of larvae were calculated and corrected for natural mortalities by (Abbott, 1925)

$$\text{Corrected mortality} = \frac{\text{observed mortality \%} - \text{control mortality \%} \times 100}{100 - \text{control mortality \%}}$$

- Computed percentage of mortality was plotted versus the corresponding concentrations using Ldp line software to obtain the toxicity regression lines. Percentages of corrected mortalities were statistically analyzed according to (Finney, 1971).

RESULTS

Hematite nanoparticles:

Size and morphology of the nanoparticles was determined by analyzing the recorded TEM images as shown in Figs. (1 & 2). The sample was composed of uniform nanoparticles with narrow size distribution. The estimated average size of the nanoparticles by TEM was < 50 nm. Aggregation of nanoparticles was observed in the sample. Analysis of TEM images were done using a special software in the same unit of TEM.

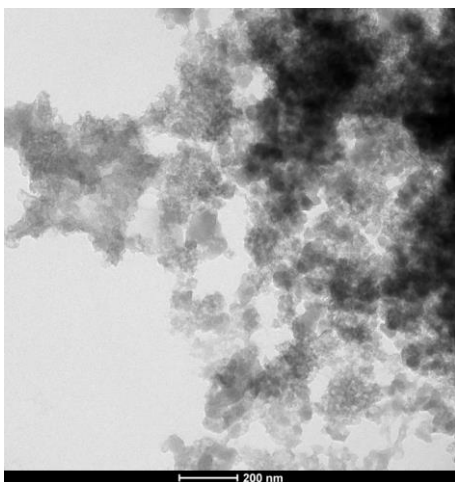


Fig. 1: TEM showing hematite nanoparticles (200 nm)

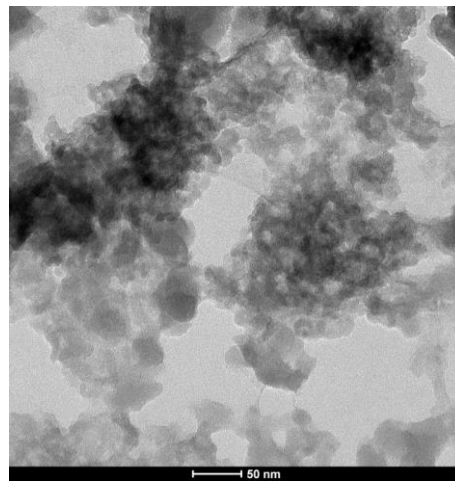


Fig. 2: TEM showing hematite nanoparticles (50 nm)

Toxicity of Hematite Nanoparticles against 3rd instar larvae of *Cx. pipiens* L.:

The toxicity of four tested concentrations (0.025, 0.25, 2.5 and 25 ppm) of HNPs against 3rd instar larvae of *Cx. pipiens* was evaluated by bioassay test. The observed mortality percentage after 48 hours was 6.67, 23.34, 35, and 68.34 ppm, respectively. Medial lethal concentration (LC₅₀) was calculated using Ldp line software and was of 5.6997 ppm. Dead larvae were observed and counted every 12 hours. Survival rate of mosquito larvae started to decrease after 36 hrs. Observed mortality was corrected to exclude control mortality. (Table 1) (Figs. 3 & 4)

Table 1: Toxicity data of hematite nanoparticles against 3rd instar larvae of laboratory *Cx. pipiens* strain

Concentration (ppm)	Observed Mortality %
0.025	6.67±1.67
0.25	23.34±1.67
2.5	35±2.88
25	68.34±3.34
Slope	0.6234±0.0689
Chi-Square (χ^2)	2.7123 (tabulated 6)
Correlation Coefficient (r)	0.9882 (tabulated 0.95)
LC ₅₀ (Its limits at 95%)	5.6997 (3.3173-11.1746)
LC ₉₀ (Its limits at 95%)	648.1186 (199.51-3830.6984)

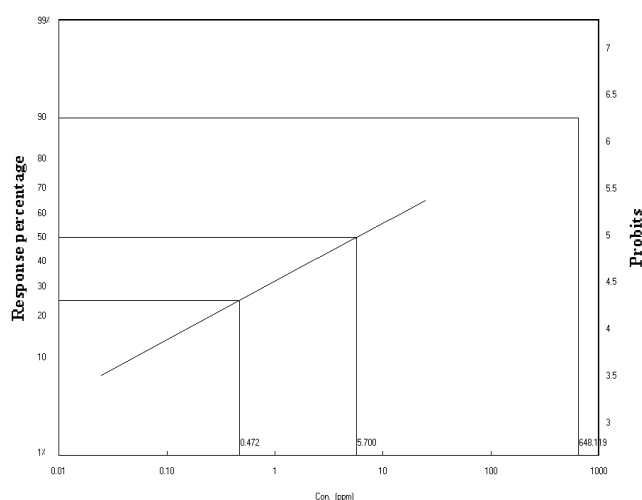


Fig 3. Toxicity regression line of hematite nanoparticles bioassay against 3rd instar larvae of *Cx. pipiens*

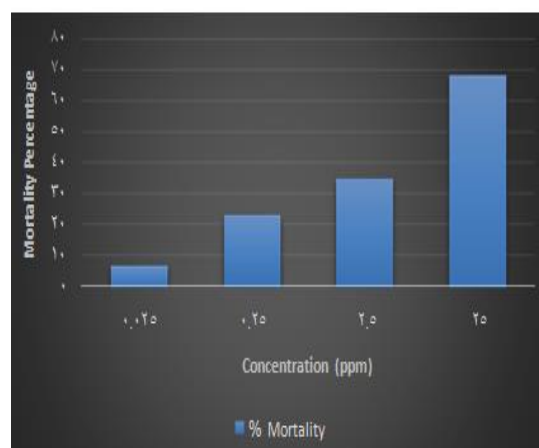


Fig. 4: Histogram showing observed mortality % of 3rd instar larvae of *Cx. pipiens* after treatment with serial concentrations of hematite nanoparticles

DISCUSSIONS

Hypothermal methods for preparation of hematite nanoparticles was illustrated briefly by (Zhu *et al.* 2012 & Wang *et al.* 2013 & Tadic *et al.* 2014). The obtained TEM images showed that hypothermal methods is a key method for preparation of hematite particles in nanoscale. The authors hypothesized that using a nonionic surfactant to disperse nanoparticles may result in aggregation of nanomaterial.

Application of nanoparticles as insecticidal agents is a recent approach in insecticide design. (Zhou *et al.* 2012) assessed the toxicity of gold and iron oxide nanoparticles against the discoid cockroach, *Blaberus discoidalis*. Nanoparticles were injected into the central nervous system of the cockroach. Insects treated with iron oxide nanoparticles showed hyperactivity, which was an indication of the neurotoxicity of the particles in insects.

The overall survival of treated insects dropped dramatically after 40 days of the

injection. (Vecchio *et al.* 2013) proposed a quantitative ranking of nanoparticles toxicity *in vivo*. The authors applied different nanoparticles on *Drosophila melanogaster* to evaluate their toxic effects in terms of lifespan reduction of the organisms, as compared to the untreated organisms. Magnetite (Fe₃O₄) nanoparticles, which is another form of iron oxide, was found to be highly toxic displaying 30% of viability reduction at low concentration. (Vega-Alvarez *et al.* 2014) found that iron oxide nanoparticles synthesized by co-precipitation coated with 3-Aminopropyltriethoxysilane and iron oxide nanoparticles synthesized by thermal decomposition showed the highest mortality level in *Drosophila* embryos among eight different types of nanoparticles tested. The obtained data showed that iron oxide nanoparticles may act as a larvicidal agent against mosquito larvae. Serial dilution prepared from a stock solution (0.1%) was applied against the late 3rd instar larvae. Significant mortality was observed after 36 hours of application. Several investigations are needed to understand the mode of action of nanoparticles on insects.

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ARABIC SUMMERY

التأثيرات السمية للجسيمات النانومترية من الهيماتيت على بعوضة كيولكس بيبينز

رضا فضيل على بكر^{1&3} - نهى عوني جنيدى¹ - محمد سعيد عطية² - إبراهيم ربيع السباعى¹

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تهدف الدراسة إلى تقييم كفاءة الجسيمات النانومترية من الهيماتيت ضد يرقات بعوضة كيولكسيبيينز. وقد تم تخليق الجسيمات النانومترية للهيماتيت بطريقة فوق حرارية بسيطة. متوسط حجم الجسيمات النانومترية التي تم الحصول عليها أقل من 50 نانومتر. صور الميكروسكوب الإلكتروني النافذ حددت حجم والشكل الظاهري للجسيمات النانومترية. وتم تطبيق تركيزات متتالية على يرقات البعوض. فكان التركيز القاتل لنصف العشرة بعد 48 ساعة هو 5.6997 جزء في المليون. واطهرت النتائج التي تم الحصول عليها اضافة سلاحا "جديدا" لمكافحة البعوض. ومن المقترح التحقق من العمل المشترك للجسيمات النانومترية مع المبيدات الأخرى التي طورت لها الحشرات مقاومة.