Copper Oxide Nanoparticles Stimulate Cellular Damage and Histological Architecture Deterioration in Tissues of Culex Pipiens Larvae (Diptera: Culicidae).

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INTRODUCTION

Culex pipiens, is one of the considerable vector-borne diseases widely distributed all over the world. The extensive use of synthetic insecticides caused various human health hazardous, insect resistance and environmental pollution. As an alternative control strategy, nanoparticle applications in insect management are mandatory. Mainly, the aim of this current study is to elucidate the toxic effect of copper oxide nanoparticles (CuONPs) on larval tissues of Culex pipiens regarding the histological and cellular damage aspects. The lethal concentrations LC_{10}, LC_{25}, LC_{50} and LC_{90} were determined from the established regression log concentrate-response lines after 24 hours of treatment. Four replicates were considered for each concentration where twenty-five third instar larvae were involved for each replicate. Results revealed significant increase in mortality rate upon increasing copper oxide nanoparticles (CuONPs) concentrations which recorded 0.040, 0.099, 0.268 and 1.767 mg/ ml for LC_{10}, LC_{25}, LC_{50}, and LC_{90} respectively. Data in this study showed a significant increase in cellular damage enzyme levels namely, lipid peroxidase and nitric oxide, and a significant decrease in levels of total protein while, an increase in albumin proportions were detected in tissue homogenates of treated larvae of the increasing mentioned lethal CuONPs concentrations. Meanwhile, histological studies implied severe deterioration in tissue architecture of the treated larvae which increases markedly as the CuONPs concentration increase as well. In conclusion, CuONPs increase the cellular damage and deteriorate the histological structure of tissues of Culex pipiens larvae which render them promising and smart larvicidal agents.

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ABSTRACT

Culex pipiens, is one of the considerable vector-borne diseases widely distributed all over the world. The extensive use of synthetic insecticides caused various human health hazardous, insect resistance and environmental pollution. As an alternative control strategy, nanoparticle applications in insect management are mandatory. Mainly, the aim of this current study is to elucidate the toxic effect of copper oxide nanoparticles (CuONPs) on larval tissues of Culex pipiens regarding the histological and cellular damage aspects. The lethal concentrations LC_{10}, LC_{25}, LC_{50} and LC_{90} were determined from the established regression log concentrate-response lines after 24 hours of treatment. Four replicates were considered for each concentration where twenty-five third instar larvae were involved for each replicate. Results revealed significant increase in mortality rate upon increasing copper oxide nanoparticles (CuONPs) concentrations which recorded 0.040, 0.099, 0.268 and 1.767 mg/ ml for LC_{10}, LC_{25}, LC_{50}, and LC_{90} respectively. Data in this study showed a significant increase in cellular damage enzyme levels namely, lipid peroxidase and nitric oxide, and a significant decrease in levels of total protein while, an increase in albumin proportions were detected in tissue homogenates of treated larvae of the increasing mentioned lethal CuONPs concentrations. Meanwhile, histological studies implied severe deterioration in tissue architecture of the treated larvae which increases markedly as the CuONPs concentration increase as well. In conclusion, CuONPs increase the cellular damage and deteriorate the histological structure of tissues of Culex pipiens larvae which render them promising and smart larvicidal agents.
The application of eco-friendly insecticides has received the world's attention as alternative effective insecticides (Nathan et al., 2005). Meanwhile, copper oxide nanoparticles (CuONPs) are promising metal oxides which drew great attention in recent times due to their diverging applications in biological, pharmaceutical, chemical, industrial and medical research fields (Ali et al., 2021). Copper oxide nanoparticles consist of copper and oxygen, in which copper is the central metal ion that binds with four oxygen molecules (Kumar and Kumar, 2020). These copper-oxide nanostructures can act as an organic dye-degradation material which is effective in the prevention of water pollution. Also, they are known to be involved in catalytic reactions (Ben-Moshe et al., 2009). In biology, CuONPs play a crucial role in preventing fungal, bacterial and microbial attacks. CuONPs act effectively in inhibiting various bacterial growth such as *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* (Ahamed et al., 2014). Additionally, CuONPs exhibit biocidal properties and they are known to be used in many biomedical aspects (Grigore et al., 2016). In medicine, CuONPs have shown their effectiveness in biomedical concerns, however, the major drawbacks in the medical field are attributed to their toxicity (Ostaszewska et al., 2015). CuONPs are shown to be toxic for mammalian cells as well as for vertebrates and invertebrates depending on many related factors including the increased production of reactive oxygen species (Ruiz et al., 2015). Such nanoparticles induced oxidative stress in pulmonary epithelial cells which promotes toxicity via damaging DNA and mitochondria (Sankar et al., 2014). Promising biomedical applications of CuONPs would focus on the detection of diseases as in the detection of viruses that infect human beings (Ahamed et al., 2014). In a study described by Li et al. (2012), a smart method for the detection of the flu virus was adopted. Currently, CuONPs are used in hospitals as antimicrobial agents due to their antimicrobial ability to control almost all types of bacteria (Lazary et al., 2014). CuONPs also exhibit a widely spread antifungal spectrum. Interestingly, they are safe to cause skin irritation or sensitization. In general, they are safe for humans if they are used externally and in low amounts (Borkow and Gabbay, 2008). Hence, the aim of this current study is to investigate and elucidate the toxic effect of copper oxide nanoparticles on tissues of *Culex pipiens* larvae regarding histological and cellular damage aspects to be regarded as a promising larvicidal agent.

**MATERIALS AND METHODS**

**Culex pipiens Larvae:**

Mosquito larvae were obtained by dipping method from stagnant water around the great Cairo region. The collected larvae were identified as *Culex pipiens* (Harbach, 1988) in the Mosquito Research Department, Research Institute of Medical Entomology, Giza, Egypt. *Culex pipiens* colonies were maintained at 29 ± 2°C temperature, 80 ± 10% RH and 12:12 light and dark photoperiod in a laboratory that was totally isolated from any source of insecticides exposure. Thereafter, larvae were transferred into enamel plates where they were fed on yeast granules and rusk powder till the pupation phase. Pupae were then allowed to be collected and placed in plastic bowls filled with water and placed in a wooden-framed cage with a cotton pad impregnated daily with 10% sucrose solution inside the cage for mosquitoes to feed on after emergence (Kauffman et al., 2017). Adult female mosquitoes were offered Pigeons’ blood meals in order to obtain the protein needed for egg production. After egg hatching and breeding of many generations, required specimens were selected for running the bioassay experiments.
Preparation of Copper Oxide Nanoparticles Using Sol-Gel Method:
Copper nitrate powder was dissolved in deionized water. Acetic acid was added dropwise to the copper nitrate solution and the reaction proceeded for one hour at 100°C. Sodium hydroxide solution was added to the reaction mixture and the reaction proceeded for a further one hour at 100°C. A black precipitate was obtained which was filtered using Whatman filter paper and taken to be dried by adjusting the lab oven at 500°C. Copper oxide nanoparticles powder was obtained which was then characterized using transmission electron microscopy (Kayani et al, 2015).

Detection Of Sub-Lethal Concentrations of Copper Oxide Nanoparticles Against *Culex pipiens* Third Instar Larvae:
Gradual concentrations of copper oxide nanoparticles (0.03, 0.07, 0.1, 0.2, 0.5 and 0.7 mg/ml) were prepared using dechlorinated water as diluent. Twenty-five 3rd instar larvae were put into a 500 ml beaker containing the test solution of each concentration. Four replicates were regarded for each concentration. Regarding the control experiments, larvae were placed into dechlorinated water only. Larval mortalities were assessed 24 hours post-treatment. The larva was considered dead if it did not move when prodded using a fine dowel (Ragheb et al., 2020). Lethal concentrations were determined by mortality rates after 24 hours of exposure. Probit analysis (Finney, 1971) was performed for lethal concentrations detection and obtaining the slope values. Data were corrected for control mortality using Abbott’s formula (Abbott, 1925).

Preparation of Larval Tissue Homogenates:
The larvae were incubated with sub-lethal doses of copper oxide nanoparticles following the predetermined concentrations of LC90, LC50, LC25 and LC10 which were obtained from the established regression log concentrate response lines after 24 hours of incubation in step I. Non-mortal larvae were homogenized using UP 200H ultrasonic processor, and one gram of tissue was processed in a 5ml phosphate buffer solution (PH 7.4). The obtained suspension was centrifuged at 4000 rpm for 45 minutes at room temperature. The pellet was discarded while the aliquots of supernatants were involved in determining the activities of cellular damage marker enzymes.

**Detecting the Levels of Total Protein and Albumin in Tissues of *Culex pipiens* Larvae Treated with Copper Oxide Nanoparticles.**
In this experiment, total protein and albumin levels were detected spectrophotometrically as indicators for the assessment of cellular functions in the larval tissue homogenates previously prepared in step I. Total protein levels were detected following the instruction manual of DiaSys diagnostic systems, Gmbh, Germany. While, albumin levels in the tissue homogenates were examined following the instruction manual of Diamond diagnostics, Egypt.

**Detecting the Levels of Cellular Damage Enzymes in Tissues of *Culex pipiens* Larvae Treated with Copper Oxide Nanoparticles.**
In this experiment, lipid peroxidation and nitric oxide levels were detected spectrophotometrically as indicators for the assessment of cellular damage in the larval tissue homogenates previously prepared in step I. Lipid peroxidation levels were detected following the colorimetric method of BIODIAGNOSTICS kit Egypt, instruction manual. Nitric oxide in the tissue homogenates was examined following the colorimetric method of the BIODIAGNOSTICS kit Egypt, instruction manual as well.

**Histological Studies:**
*Culex pipiens* third instar larvae treated with LC10, LC50 and LC90 of copper oxide nanoparticles along with larvae of the control group were fixed using BOUIN’s fixative (picric acid and formalin) than embedded, sectioned (5-8 µm), and stained with Delafield's
hematoxylin and eosin according to (Raguvaran et al., 2021).

**Statistical Analysis:**
Statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 25 (SPSS Inc., Chicago, IL, USA.). Data were recorded as means ± standard deviations. Differences in parameters in more than two groups were evaluated by one-way analysis of variance (ANOVA). Differences between groups were considered significant at p < 0.01 (Shafagh et al., 2015).

**RESULTS**
The larvicidal efficacy of copper-oxide nanoparticles (CuONPs) against mosquito larvae *Culex pipiens* was assessed by estimating the levels of LC$_{10}$, LC$_{25}$, LC$_{50}$ and LC$_{90}$ and the slope of the log-concentration probit response lines after 24 hours of treatment were tabulated in tables (1 and 2). Results showed that CuONPs were very effective against mosquito larvae. The levels of LC$_{10}$, LC$_{25}$, LC$_{50}$ and LC$_{90}$ were 0.040, 0.099, 0.268 and 1.767 mg/ml respectively. The ratio of LC$_{90}$/LC$_{50}$ indicates the steepness of the log-concentration Probit lines in a reversal way to the slope value. Data indicated that the slope of the efficacy regression line of copper oxide nanoparticles was (1.659±0.096), and LC$_{90}$/LC$_{50}$ ratio was 6.59.

**Table 1:** Response of *Culex pipiens* larvae to gradual concentrations of copper oxide nanoparticles.

<table>
<thead>
<tr>
<th>CuONPs concentration (mg/ml)</th>
<th>No. of larvae tested</th>
<th>Died</th>
<th>Alive</th>
<th>Mortality%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>100</td>
<td>11</td>
<td>89</td>
<td>11</td>
</tr>
<tr>
<td>0.07</td>
<td>100</td>
<td>15</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>0.1</td>
<td>100</td>
<td>24</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>0.2</td>
<td>100</td>
<td>30</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>0.5</td>
<td>100</td>
<td>60</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>0.7</td>
<td>100</td>
<td>90</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>0.0</td>
<td>100</td>
<td>0.0</td>
<td>100</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Table 2:** Efficacy of different lethal concentrations of copper oxide nanoparticles against 3rd instar larvae of *Culex pipiens*.

<table>
<thead>
<tr>
<th>LC values (mg/ml)</th>
<th>LC$_{10}$(lower-upper)</th>
<th>LC$_{25}$(lower-upper)</th>
<th>LC$_{50}$(lower-upper)</th>
<th>LC$_{90}$(lower-upper)</th>
<th>LC$<em>{90}$/LC$</em>{50}$</th>
<th>Slope ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.026-0.062)</td>
<td>0.040</td>
<td>0.099</td>
<td>0.268</td>
<td>1.767</td>
<td>6.59</td>
<td>1.659±0.096</td>
</tr>
</tbody>
</table>

Results of this study showed a significant decrease in total protein levels and enzymes of cellular damage as well in tissues of *Culex pipiens* larvae as shown in Table (3) and Figures (1&2).

**Table 3:** Total protein, Albumin, Lipid Peroxidase (LP) and Nitric oxide levels among different lethal concentrations of CuONPs against *Culex pipiens* mosquitos’ larvae.

<table>
<thead>
<tr>
<th>Copper oxide nanoparticles concentrations (mg/ml)</th>
<th>Total protein (g/dL) ± SE</th>
<th>Albumin (g/dL) ± SE</th>
<th>Lipid peroxidation level (nmol/g. tissue) ± SE</th>
<th>Nitric oxide (umol/L) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC$_{10}$(0.040)</td>
<td>0.0816 ±0.00004**</td>
<td>0.046±0.00044**</td>
<td>24.82±0.004**</td>
<td>16.33±0.004**</td>
</tr>
<tr>
<td>LC 25 (0.099)</td>
<td>0.06±0.0040**</td>
<td>0.051±0.00044**</td>
<td>31.41±0.004**</td>
<td>16.83±0.004**</td>
</tr>
<tr>
<td>LC50 (0.268)</td>
<td>0.04±0.00044**</td>
<td>0.058±0.00044**</td>
<td>33.33±0.004**</td>
<td>17.82±0.004**</td>
</tr>
<tr>
<td>LC90 (1.767)</td>
<td>0.02±0.0040**</td>
<td>0.066±0.00044**</td>
<td>75.44±0.004**</td>
<td>23.26±0.004**</td>
</tr>
<tr>
<td>0.00</td>
<td>0.142±0.00044**</td>
<td>0.033±0.00044**</td>
<td>11.32±0.004**</td>
<td>14.85±0.004**</td>
</tr>
</tbody>
</table>

Values are represented as mean± SE. ** is a highly significant= P<0.001.
Copper Oxide Nanoparticles Stimulate Cellular Damage

Fig. 1: Levels of total protein and albumin in tissue homogenates of *Culex pipiens* larvae among different CuONPS lethal concentrations.

Fig. 2: Levels of lipid peroxidase and nitric oxide in tissue homogenates of *Culex pipiens* larvae among different lethal CuONPs concentrations.

Visualization of histological damages in this study is illustrated in figure 3 (A-D) which showed histological deterioration in the midgut tissues of *Culex pipiens* larvae treated with CuONPs in a way that indicated an increase of such damages as the concentration of CuONPs increased as well.

Fig. 3: Light micrographs show the larval mid-gut cells of *Culex pipiens* third instar larvae stained with eosin and hematoxylene (10X). The brush border (bb) in the mid-gut epithelial cells is tight and intact in control sample (A) while, upon treatment with CuONPs, protrusions of the brush that encircles the mid-gut epithelial cells (p), with the brush border tending to become more thinner in a gradual manner that indicated that protrusion of epithelial cells of mid gut tissues, with the brush boundary are completely disordered and thinning out with tissue compartments tend to lose their integrity as CuONPs concentration increase (figures B, C and D for CuONPs sub-lethal concentrations LC_{10}, LC_{50} and LC_{90} respectively).
DISCUSSION

In recent studies, many researchers have described the larvicidal potency of copper oxide nanoparticles towards different vector diseases including *Aedes aegypti* (Selvan et al., 2018), *Culex quinquefasciatus* mosquito (Chakrabarti and Patra, 2020) and *Anopheles stephensi* (Vivekanandhan et al., 2021). In fact, many features could influence cellular toxicity of CuONPs and consequently influence their capability of being effective pesticides. These features include their size where, small particles are more toxic than larger ones, their surface charge as nanoparticles toxicity is synergized by positive charge which enhances interactions between CuONPs and cells, their dissolution which depends on the temperature and pH of the solution and CuONPs concentration in the medium (Chang et al., 2012). In this study, the sol-gel technique was used as a simple and fast method for preparing CuONPs (Jayaprakash et al., 2014) and to ensure proper CuONPs size synthesis with dimensions ranging between 10 and 40 nm (Karthik et al., 2011). The molecular toxicity mechanisms of CuONPs on animal cells might be elucidated via promotion of mitochondrial damage, DNA damage and oxidative DNA damage which eventually lead to cell death (Zhang et al., 2014). In addition, Isani et al. (2013) had reported that CuONPs actually cause cell membrane damage. Thus, CuONPs cause cytotoxicity even at low concentrations and are capable of inducing cell death (Fahmy et al., 2009). Substantially, CuONPs toxicity do not depend only on nano-size and structural shape of the particles but also on their concentration (Grigore et al., 2016) hence, gradual increase of mortality rate by increasing CuONPs concentrations was observed in results of this study, a finding which was also described by Ragheb et al., (2020 and 2022). These CuONPs increasing concentrations produce dose-dependent cell membrane damage (Anreddy et al., 2010) thus, increasing mortality rate. Results in this study showed significant decrease of total protein levels in tissue homogenates of *Culex pipiens* larvae as CuONPs concentrations increase. In biological media, CuONPs either interact with biomolecules found in the cell like proteins, phospholipids, nucleic acids, glucolipids and even cellular metabolites or proteins might be adsorbed on the CuONPs surface (Saptarshi et al., 2013). This interaction or adsorption that could take place might be one cause of protein levels declination which was observed in this study. Moreover, CuONPs surfaces could alter the structure and hence the function of the adsorbed protein thus affecting their target bio-reactivity (Verma and Stellacci, 2010). Actually, CuONPs induced protein conformational and structural changes that affect the downstream protein-protein interactions, DNA transcription and even cellular signaling as well, which eventually lead to loss of enzyme activity and hence, loss of bio vital functions and death of the organism (Karajanagi et al., 2004) which may be one other cause of mosquitos’ mortality. Elevated levels of albumin in tissue homogenates of *Culex pipiens* larvae were significantly observed in this study. It has been showed that albumin is an important cellular marker which is involved in many bioactive functions and thus its level might give an indication of cellular viability and functionality. Albumin vital functions include; binding and transport of various endogenous or exogenous compounds, regulation of osmotic pressure and it plays a significant role in extracellular antioxidant defenses. It exhibits antioxidant properties as it is the capability of binding to copper tightly and iron weakly. Also, it could scavenge free radicals and provide thiol group in pathological conditions. Thus, increasing albumin levels might be an outstanding biomarker of increasing oxidative protein damage (Sitar et al., 2010).
2013). Thus, it is most likely that albumin levels increase in cases of cellular stress which might be due to dehydration or oxidative stress generated as a consequence of CuONPs actions they exert on Culex pipiens cells (Theodore et al., 2005). Lipid peroxidation and oxidative stress have been reported as the most accepted mechanism for CuONPs toxicity (Alarifi et al., 2013). Oxidative stress with metal nanoparticles like CuONPs may be related to the surface properties or to the elaborated metal ions or both (Wang et al., 2016). In the present study, the ability of CuONPs to induce oxidative stress was assessed by measuring the levels of lipid peroxidase enzyme and nitric oxide. In this research, oxidative stress and its consequent cellular damage was indicated by the elevated lipid peroxidase. In accordance to these results, Ragheb et al., (2020 and 2022) described an increased lipid peroxidation levels upon treatment of Culex pipiens and Musca domestica third instar larvae with green silver nanoparticles and zinc oxide nanoparticles respectively. Consistent to the notion of dealing with biological entities, copper nanoparticles caused significant elevation of lipid peroxidase levels in spleens of treated rats (Zhou et al., 2019) and in livers of rats exposed to CuONPs. Anreddy (2018) and Tang et al. (2019) had reported dose dependent increase in lipid peroxidase levels. Also, Elkhatteeb et al. (2020) had demonstrated increased lipid peroxidation levels in kidney of CuONPs treated rats and recently, Sakr et al., 2021 described the same results upon treating spleens of albino rats with CuONPs. The increased levels of nitric oxide and lipid peroxidase levels always go along with each other. Meanwhile, lipid peroxidase, nitric oxide levels and oxidative stress have been suggested to play a crucial role in the mechanisms of nanoparticles toxicity including copper oxide (Abdelazeim et al., 2020). Data in this study also showed an increased levels of lipid peroxidation along with increased nitric oxide levels which consequently lead to oxidative stress and cellular damage. These results are in accordance with Ragheb et al., (2020 and 2022) and with Abdelazeim et al., (2020).

Recent in-vivo studies have implied that treatment with CuONPs induces enhanced ROS generation, oxidative stress, various pathological manifestations, inflammation, cellular mal functionality, apoptosis, and consequently histopathological alterations in vital tissue organs (Tulinska et al., 2022). In the present study, histological studies of Culex pipiens third instar larvae treated with CuONPS implied severe tissue damages in the larval mid gut region as indicated by brush protrusions that encircle the mid-gut epithelial cells, with the brush border tending to become more thinner compared to that of the intact and tight brush border in the mid-gut epithelial cells of the control sample. This finding was also described by Raguvaran et al., (2021) which described silver nanoparticles potency in inducing epithelial cells and brush border cell damages as compared to the control untreated larval groups.

Conclusion:
In the end, this study pointed out to the crucial role of copper oxide nanoparticles to be used as a larvicidal agent against third instar larvae of Culex pipiens. Such nanoparticles are capable of altering the protein function mechanism via altering their configurational structure, causing tissue dehydration, inducing oxidative stress mechanism with consequent cellular damage which lead to deterioration in the histological architecture of the organisms and eventually lead to their death. Hence, copper oxide nanoparticles could be considered as larvicidal agents in the control programs of their prevalence.

REFERENCES


Copper Oxide Nanoparticles Stimulate Cellular Damage

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Copper Oxide Nanoparticles Stimulate Cellular Damage


تهدف هذه الدراسة إلى تقييم قدرة الجسيمات النانوية لأكسيد النحاس للحد من انتشار يرقات البعوض (كيولكس بيبايز) التي كانت و لا لا تزال أحد أبرز أمراض الأنهار وأخطرها تأثيرها على الصحة العامة. وقد عنيت هذه الدراسة ببحث قدرة جزيئات أكسيد النحاس النانوية على مكافحة يرقات البعوض وفقًا للظروف المعملية في مصر ومصر بذلك تقليل الآثار السلبية الناجمة عن استخدام المبيدات الكيميائية والاستخدام الأمثل والفعال لتطبيقات علم النانو في هذا المجال. وقد شملت هذه الدراسة تجربة العمر اليرقى الثالث للبعوض لجسيمات أكسيد النحاس النانوية بتكريرات مختلفة ثم تم عمل بعض الاختبارات البحثية على الخلايا و الانسجة و مقارنتها بالمجتمع الضابط للتوصيل إلى نتيجة تأثير هذه المواد على اليرقات محل الدراسة. وقد أظهرت نتيجة البحث أن التركيزات التي تؤدي إلى نسبة موت اليرقات أعلى 10، 25، 50، 90% بعد التعرض لجزيئات أكسيد النحاس النانوية هي 0.4، 0.99، 0.0، 0.268، 0.0، 1.767 من جرام/مل لتر. وقد أوضحت النتائج فاعلية جزيئات أكسيد النحاس النانوية على استعداد خلل واضح في معدلات نسب البروتين الكلى والألبومين في أنسجة اليرقات المعملة بجزيئات أكسيد النحاس النانوية مما يترتب عليه ارتفاع ملحوظ في آنثيمات النحل الخفیة (أكسيد النترك) و الليبيد بروكسيدز) لليرقات المعملة مقارنة بيرقات المجموعة الضابطة مما أدى إلى موت اليرقات المعملة بخفلة ملحوظة مقارنة بيرقات المجموعة الضابطة. كما أظهرت النتائج تقليل واضح للداء النسيجي لدى اليرقات بعض الكيولكس بيبايز نتيجة معالجتها بجزيئات أكسيد النحاس النانوية مقارنة بيرقات المجموعة الضابطة. و بناءً على تلك النتائج فقد توصلت الدراسة إلى أن جزيئات أكسيد النحاس النانوية من الممكن أن تعد من الوسائل الفعالة والواضحة في مكافحة يرقات البعوض كيولكس بيبايز في مصر.