EFFECT OF COPPER COATED LUPINE EXTRACT NANOPARTICLES ON SOME AQUATIC AND TERRESTRIAL PEST SNAILS

Karima M. Azzam, Eman Abdel-Hady* and Eman K. Khedr
Plant Protection Research Institute, ARC, Dokki, Giza, Egypt

*Corresponding author: mnmn7733@yahoo.com

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ABSTRACT

Effect of lupine extracts nanoparticles (NPs) coated with copper sulphate on the mortality and eggs productivity of aquatic snails, Biomphalaria alexandrina Ehrenberg and Bulinus truncatus Audouin, and terrestrial snail, Eobania vermiculata Müller, were investigated in comparison with copper sulphate nanoparticles and both treatments compare with untreated group in the present and previous investigation.

This experiment was planned to elucidate the molluscicidal properties of lupine extract coated with CuSO4 NPs against adult B. alexandrina B. truncates and E. vermiculata snails after 24 hours exposure, in dark conditions, followed by another 48 hrs. light for recovery.

The results revealed that lupine extracts nanoparticles has more effect than copper sulphate nanoparticles on both aquatic or terrestrial snail mortality. It caused 100% mortality for B. alexandrina and B. truncates, at concentration of 20 ppm, after 24 hours of exposure in dark followed by 48 hours recovery in day light. Copper sulphate nanoparticles at the same concentration caused only 70% and 46.67% mortality for B. alexandrina and B. truncates, respectively, under the same conditions. Mortality rate increased with the increase of concentrations either in lupine NPs or CuO4 NPs. On normal lupine extract the concentration that caused 100% mortality was equal to ten folds of lupine NPs.

Egg productivity of the healthy thirty individuals, of B. alexandrina, B. truncatus and E. vermiculata, were investigated and compared with the survival snails in low concentrations of both Cu NPs and lupine NPs, to study the effect of nanoparticle materials on the snail fecundity.

Both lupine extract nanoparticles and copper sulphate nanoparticles may have a sterilized effect, where B. alexandrina and B. truncatus snails exposed to sub lethal doses from them didn’t laid any egg masses after treatment. E. vermiculata, treated or untreated individuals, didn’t laid any eggs either, because it had a specific season of reproduction which not coinciding with the time of experiment.

Keywords: Lupine coated copper NPs, Aquatic snails, Terrestrial snail

INTRODUCTION

Gastropods are very prevalent animals in the world (Barker, 2002). In Egypt, terrestrial snails are important economic pests, prevalent in many Governorates, infesting and causing severe damage to many economic agricultural crops i.e., orchard trees, vegetables and field crops and ornamental plants (Desoky, 2018). Azzam and Abd El-Hady (2018) recorded seven snail species and three slug species infesting about 47 species of plants in seven Governorates in Egypt, including Cairo and Arish (North Sinai). All terrestrial snails cause damage to their host plants, by feeding on leaves, blooms, flowers, fruits, trunks, limbs and even on barks (Godan, 1983 and Ismail et al 2003).

Azzam and Belal (2006) recorded 20 aquatic molluscs species; including the snails playing as an intermediate hosts of Schistosoma mansoni Sambon, Schistosoma heamatobium Bilharz, Fasciola gigantica Cobbold, F. hepatica L. and Angiostrongylus cantoniensis Chen. Thus, snail control strategies are considered a priority for preventing or minimizing Schistosomiasis transmission (Lardans and Dissous, 1998) as well as reducing damage to economic agricultural crops in Egypt.
These emphasized the necessity of controlling such harmful terrestrial and aquatic snails which predominately achieved by the application of molluscicides which lead to more environmental pollution. However, further innovated techniques are required in this field.

Nanoparticles are naturally occurring in both aquatic and terrestrial environments in form of colloids, “mineral precipitates (Al, Fe, MgO, OH)” and dissolved organic matter (Boverhof et al 2015). The development of nanotechnology produces many nanoparticles (NPs) that are important in medicine, agriculture and industry (Grazyna et al 2014). Fernandes et al (2007), suggested that ENPs may affect organisms’ behavior, survivorship, and community structure, function and biogeochemical processes.

El-Tarky (2005) noticed that, mortality rate of adult B. alexandrina snails was 52%, when exposed for 4 hrs to 438.8 W/m2 of sunlight after 12 hours of their incubation with 10–5 HpdL... The author added that, the effect of Hpd against snails increased in the alkaline media (pH = 8), while decreased at the acidic ones (pH = 6). It was stated that porphyrins and their derivatives exhibited a potent phytotoxic effect against gram-positive bacteria. Ragheb (2009) stated that, incubation of B. truncatus snails, for 24 hrs at 10–4 M/L gold nanoparticles (GNPs) in dark, followed by 2 hours of exposure to 650 W/m2 irradiance (Solar Simulator), resulted in 100% death.

El-Hommossany and El-Sherbibni (2011) mentioned that, exposed B. alexandrina to 5 × 10–5 Mdm–3 (HpdGNPs) with 4 hours of exposure to sunlight suppressed the survival rate of B. alexandrina snails by 50%. Meanwhile, control snails incubated with 5 × 10–5 Mdm–3 HpdGNPs were not affected and still alive (100%). They also found that exposing B. alexandrina snails to sub lethal concentrations of the photosensitizer Hpd coated GNPs (12 hours incubation, 4 hours exposure to 336.2 W/m2) significantly reduced their reproductive capacity.

Nanomaterial toxicological studies have also shown large bactericidal and mutagenic effects due to the metal ion properties, stabilizing agents and the size and shape of the NMs (Suresh et al 2013). Fish and plants were seen to show an inhibited growth, and even death when coming into contact with specific nanoparticles (Burke et al 2015). However, some conflicting data exist where plants have shown improvement in growth under specific exposure (Lahiani et al 2013).

Adult blue mussels, Mytilius edulis, exposed to 0.7 μg L–1 Ag NPs exhibited shell abnormalities after 72 hours, and 50% of snails, Lymnaea luteola, died after 96 hours of exposure to 48 μg L–1 Ag NPs (Wong et al 2013; Ali et al 2014). The 96-hour LC50 of Ag NPs for gastropods decreased from 2.18 μg L–1 in the absence of sediment to >100 μg L1 when sediment was present (Bernot et al 2013).

Copper compounds have been used as molluscicides. Shaldoum et al (2016), studied the immunological effect of Cu2O nanoparticles on Biomphalaria alexandrina snails.

Nanotechnology is used to modify material at the nano-scale (<100 nm) to create novel properties. Changes in the physicochemical and structural properties of materials caused by the decrease in particle size can lead to new and sometimes unexpected biological effects. Besnaci et al (2016), evaluated the toxicity of Fe2O3 nanoparticles on the embryonic stage of Helix aspersa with different concentrations (1.25mg/ml, 1.5mg/ml, and 2mg/ml). Ali et al (2015), evaluate the possibilities of using silver nanoparticles (Ag NPs) to control the land snail Eobania vermiculata Muller.

Saddik et al. (2017), found that 10.7 (mg/L) of ZnNPs caused 90% mortality of Oreochromis niloticus after 24 hrs, while 10.99 (mg/L) of it caused the same ratio of mortality from Tilapia zillii fish. Organ failure was observed in zebra fish and carp due to acute exposure to metal NPs (Griffit et al 2008). Human organs can also be severely affected by inhaling, ingesting or coming into contact with large amounts of nanomaterial (Tang et al 2015). Nanomaterials have been shown to enter living organisms and “exert toxic effects at the cellular level, including membrane disruption, protein inactivation, DNA damage, and disruption of energy transfer and release of toxic substances (Van Aken, 2015). The effect of chitosan and nanochitosan on the land snails Eobania vermiculata and Monacha obstructa was investigated by Khidr (2018).

To equilibrate between the advantage and disadvantage of metal nanoparticles in the present study, evaluation of the effects of copper nanoparticles on the terrestrial and aquatic snails was made as a mean of control, to reduce the toxicological effect of copper metal nanoparticles on environment and at the same time control the harmful snails.
MATERIALS AND METHODS

Rearing of the terrestrial snail (E. vermiculata) and aquatic snails (B. alexandrina and B. truncates) were carried out by the technique previously described by Azzam and Tawfik (2005).

Lupine-coated cupper nanoparticles

Preparation of the control (CuSO4) was carried out according to Ihegwuagu and Tali, 2009)

Lupine Extract

Preparing extracts was conducted by grinding the grain of lupine until getting a fine powder, then placed it in two flasks each containing 500gm of lupine powder. The powder was soaked in ethanol for 48 hours (two centimeter height above the powder surface). The extracted solution was evaporated by rotary evaporator until complete dryness, then weighted and solved in certain amount of ethanol to prepare the stock solution.

Chemicals

Copper Sulphate (CuSO4.5H2O), 98%, product of Adwic Chemika Co., Glucose (C6 H12 O6, with average molecular weight =180.2) product of El-Nasr Pharmaceutical Chemicals Co., Starch soluble 99% powdered solid ((Cs H10 O5)n, with average molecular weight =81.37) product of Chemajet Pharmaceutical Co.

Synthesis of starch-copper nanoparticle-encapsulated

Starch—copper nanoparticle-encapsulated formulated according to (Ihegwuagu et al 2016), with the modification that encapsulation was completed in situ during synthesis of the copper nanoparticles by direct physical gelation (Ihegwuagu et al 2016 and Tali, 2009). The synthesis was carried out via chemical reduction of Copper sulphate by glucose as follows: to a mixture of 5% CuSO4.5H2O and 0.2 M glucose solution (1 : 3 volume ratio) in a loosely covered flask containing 1% starch dispersion (1 g in 100 ml distilled water), then added 10 ml of (lupine extract). This formulation was stirred and heated at 80-90°C in a fume cupboard for 3 hours. Heating was stopped and stirring continued till cooling to room temperature then centrifuged at 11 000 rpm for 20 minutes.

A similar procedure was performed for the preparation of the control (CuSO4.5H2O) without adding lupine extract.

Characterization of Lupine and CuO2 nanoparticles

Particle size and size distributions were determined by a Malvern Zetasizer 3000 HAS Nano s. England (Malvern Instruments) Figs. (1 & 2).

Molluscidal effect of Lupine coated cupper nanoparticles

The present experiment was carried out by preparing six replicates of gradual concentrations from each stock solution, for each concentration, then applied on the terrestrial species (Eobania vermiculata) and the two aquatic species (Biomphalaria alexandrina and Bulinus truncatus). Five snails, 8-10mm diameter from B. alexandrina, 9 -11ml heights from B. truncatus and 20-22ml widths from E. vermiculata, were used in each replicate. The snails were exposed to the tested concentrations (1.25, 2.5, 5, 10 and 20 PPM of lupine NPs coated with Cu SO4) for 48 hours under dark conditions, then removed from the experimental conditions, then removed from the experimental concentration, washed thoroughly and kept in dechlorinated tap water for the next 24 & 48 hours for recovery (25±1°C). Similar groups were treated with Cu SO4 NPs and unexposed snails (control) were assayed simultaneously as treated groups.

Snails that did not respond to a gentle prodding with forceps were considered to be dead (Musee et al 2010). The toxicological endpoints measured were; the mortality rates after 24, 48 and 72-hrs. of exposure. Dead snails were recorded as the average of the six replicates.

The effectiveness of the different concentrations was expressed in terms of LC 25, LC 50 and LC 90. Slope of regression lines were represented and analyzed by Chi-square value. Statistical analysis with LC50, LC90, and slope values were based on LDP line program.

Effect of Lupine coated cupper nanoparticles on snail’s productivity

Survival snails in low concentrations from both Cu NPs and Lupine NPs were maintained in laboratory until death to investigate its capability to laying egg masses, for aquatic snails, and eggs in terrestrial snails, in comparison with untreated snails. The snails were daily fed on fresh lettuce.
The numbers of eggs/snail were recorded weekly for four weeks. Each bioassay was conducted at approximately 22–26°C, and a 24h: 48h dark: light: photoperiod.

Statistical Analysis

Survival rates of treated snails were analyzed by ANOVA and T test values of contingency tables using SSp statistical program. Concentrations were considered to be toxic if a given test endpoint, namely survival and reproduction, were statistically different from those of the control tested organisms (P < 0.05), and at least 20% lower than the mean tested organism response in the negative control sample (Thursby et al 1997).

RESULTS AND DISCUSSION

Toxicity test

This experiment was planned to elucidate the molluscicidal properties of lupine extract coated with CuSO4 NPs against adult B. alexandrina, B. truncatus and E. vermiculata snails after 24 hours of exposure, in dark conditions, followed by another 48 hrs., light for recovery.

Data presented in Table (1) show mortality of B. alexandrina and B. truncatus snails treated with tested lupine NPs carrying on CuSO4 NPs concentrations (1.25, 2.5, 5, 10 and 20 ppm) in comparative with those exposed to the same concentrations from CuSO4 NPs. The unexposed group (control) shows no mortality, therefore excluded from the tables.
Table 1. Comparison between molluscicidal effect of lupine NPs carrying Cu$_2$O$_4$ NPs and Cu$_2$O$_4$ NPs against adult Biomphalaria alexandrina and Bulinus truncatus snails

| Conc. | Lupine NPs | CuO$_2$NPs |
|-------|------------|-------------|
| ppm   | Mean of mortality % of B. | Mean of mortality % of B. | Mean of mortality % of B. | Mean of mortality % of B. |
|       | alexandrina after | truncatus after | alexandrina after | truncatus after |
| 1.25  | 0±0 | 10.67±10.66i | 10.67±10.66i | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| 2.5   | 16.73±10h | 16.67±8.16h | 23.33±8.16g | 16.67±8.16h | 26.67±16.67g | 30±10.95g | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| 5     | 20±12.6h | 20±12.96 h | 26.67±10.32g | 26.67±10.33g | 53.33±10.33c | 73.33±10.33c | 0±0 | 16.67±8.16 | 30±6.94 | 0±0 | 0±0 | 0±0 |
| 10    | 46.66±16.33f | 56.66±15.05e | 96.67±8.16a | 40±0f | 66.67±10.33d | 93.33±10.33b | 30±8.94 | 50±14.14f | 60±14.14d | 0±0 | 0±0 | 0±0 |
| 20    | 53.33±10.33e | 63.33±8.17d | 100±0a | 46.67±10.33f | 100±0a | 100±0a | 60.32±6.32c | 70±23.66c | 70±8.94c | 40±12.65 | 43.33±8.16 | 46.67±10.33 |

Means with the same letter have no significant differences.
It was noted that both lupine NPs and CuSO4 NPs were agglomerated on the mantle and shell opening of B. alexandrina and B. truncatus snails (Figs. 3 & 6). Musee et al (2010) reported agglomerated C-TiO2 nanoparticles attached to the egg mass of Physa acuta Draparnaud.

As seen in this table, the concentration 20 ppm caused 100% mortality at 72 hrs., in both adults of B. alexandrina and B. truncates, with very highly significant differences between them and other concentrations, and the concentration at different times in the case of lupine extracts.

In a previous investigation, on normal lupine extract, Azzam et al (2014) recorded 100% mortality of B. alexandrina after 72 hrs. at concentration of 200 ppm which equal to ten folds of Lupine NPs that gave the same result. Also, the same authors recorded 1250 ppm for 100% mortality of B. truncates after 48hrs. Which was more than fifty folds of Lupine NPs that gave the same result in this study.

Also, very highly significant differences were found between results of lupine NPs and CuO4NPs at different concentration. Insignificant differences were recorded between other data. No mortality occurred in B. truncatus exposed to CuO4NPs concentrations of 10, 5, 2.5 and 1.25 ppm, while for B. alexandrina, no mortality occurred at concentrations of 2.5 and 1.25 ppm only. Shaldoum et al (2016), found that 2.29 ppm of Cu2O NPs killed 90% from adult B. alexandrina. Fahmy et al (2014) found that 2.7 (mg/L) of Zn NPs killed 90% from B. alexandrina snails. El-Hommosany and El-Sheribini (2011), found that 5 x 10–5 Mdm–3 (HpdGNPs) with 4 hours of exposure to sunlight suppressed the survival rate of B. alexandrina snails by 50%. Wong et al (2013) reported that, 50% of the snail Lymnaea luteola, died after 96 hours of exposure to 48 μg L-1 Ag NPs.

Table (1) also showed that mortality rate increased with the increase of concentrations either in lupine NPs or CuO4NPs.

Statistically, high correlation was found between concentration and mortality (r = 0.85) in the case of Lupine NPs on B. alexandrina and (r = 0.99) on B. truncatus with Chi² = 47.93.

Mortality of the terrestrial snail E. vermiculata exposed to lupine NPs. and CuO4NPs. at different concentrations (2. 1.0. 0.5 and 0.25 %) were recorded in Table (2). As seen in this table, the highest rate of mortality (93.33%) was recorded at the concentration of 2% of lupine NPs. after 72 hrs., while the lowest rate (20%) was recorded at concentration of 0.25%. No mortality occurred at the concentration of 0.125 of lupine NPs.. and all the other concentrations of CuO4NPs. Statistically, highly significant differences (P>0.001) were recorded between data reported for concentration 2% and 1% at 24 hrs., and those reported at 48 and 72 hrs. Also highly significant differences (P>0.001) were recorded between different concentrations, but insignificant differences existed between the concentrations 1% and 0.5% at 48 and 72hrs. Ali et al (2015) found that, the exposure of the snails E. vermiculata to Ag NPs in a laboratory experiment reduced the activity and the viability of the land snail (20% of Ag NPs treated snails died).

Khidr, (2018), also, reported significant increasing of mortality rate of E. vermiculata with the increase of concentrations of chitosan NPs.

Table (2) also showed significant correlations between concentrations and rate of mortality in the three snail species either in lupine NPs or Cu NPs.

| Concentration % | Lupine NPs | CuO4 NPs |
|-----------------|------------|----------|
|                 | Mean of mortality % E. verniculata after | Mean of mortality % E. verniculata after |
|                 | 24hrs. | 48 hrs. | 72 hrs. | 24hrs. | 48 hrs. | 72 hrs. |
| 0.125           | 0±0    | 0±0      | 0±0      | 0±0   | 0±0    | 0±0 |
| 0.25            | 0±0    | 20±6.32e | 20±6.32e | 0±0   | 0±0    | 0±0 |
| 0.5             | 20±5.16e | 56.67±23.38c | 56.67±23.38c | 0±0 | 0±0 | 0±0 |
| 1               | 36.67±15.06d | 60±7.3c | 60±7.3c | 0±0 | 0±0 | 0±0 |
| 2               | 80±10.33b | 93.33±6.67a | 93.33±6.67a | 0±0 | 0±0 | 0±0 |

Means with the same letter have no significant differences
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Fig. 3. Agglomeration of lupine NPs on the mantle and opening of B. truncatus snails

Fig. 4. Agglomeration of CuSO₄ NPs on the mantle and opening of B. truncatus snails

Fig. 5. Agglomeration of lupine NPs on the mantle and opening of B. alexandrina snails

Fig. 6. Agglomeration of CuSO₄ NPs on the mantle and opening of B. alexandrina snails

Effect of Lupine coated copper nanoparticles on snail productivity

To study the effect of nanoparticle materials on the snail fecundity, egg productivity of the healthy thirty individuals of B. alexandrina, B. truncatus and E. vermiculata, were investigated and compared with the survival snails in low concentrations from both Cu NPs and lupine NPs. Results were summarized in Table (3).

As seen in this table, the terrestrial snail E. vermiculata hadn't laid any eggs either for treated or untreated individuals because it had a specific season of reproduction which was not coinciding with the time of the current experiment. Healthy or untreated individuals of both B. alexandrina and B. truncatus laid 3±0.71 and 3.6±0.89 egg masses/snail, respectively. Each contained 11.47±2.2 and 10.44±2.01 eggs/mass, respectively. While the treated individuals from both species, either with lupine NPs or Cu NPs, didn’t lay any eggs throughout the recovery period. Thus nanoparticles may affect the fecundity of the snails.

El-Hommossany and El-Sherhibni (2011) found that, 5 × 10−5 Mdm−3 (HpdGNPs) with 4 hrs. of exposure to sunlight, treated snails laid low number of eggs throughout the recovery period (4 weeks), in comparison with that of control ones. Musee et al (2010) indicated that an increase in c-alumina and a-alumina NPs concentrations caused a significant decrease (P<0.01) in the percentage of embryo hatched for Physa acuta snail as well as number of egg masses and eggs. But none of the different concentrations of TiO₂, commercial TiO₂, c-alumina and a-alumina prevented egg laying. Besnaci et al (2016), revealed a deformation of egg membrane and accumulation of this molecule at the back of the egg. They also noted a low rate of hatching in the 12th day, and the mortality rate is found to be high at the highest concentration of Fe₂O₃.

The same table showed significant differences between values of LC25, LC50 and LC90 of B. alexandrina treated with lupine NPs, and those treated with CuO₄NPs. Whereas, the LC90 value recorded in the case of CuO₄NPs was equal to more than four folds than that recorded for lupine NPs. These indicated that lupine NPs was more effective than CuO₄NPs. Thus it could be conclude that lupine NPs enhanced the effect of CuO₄NPs.
Table 3. Egg production and hatchability of *B. alexandrina*, *B. truncatus* and *E. vermiculata* treated with lupine extracts NPs and CuO NPs. in comparison with healthy untreated snails

| Snail species     | Un treated group(control)     | Treated with Cu NPs     | Treated with lupine NPs |
|-------------------|--------------------------------|-------------------------|-------------------------|
|                   | No. of egg masses/ Snail       | No. of eggs/ mass       | No of hatching | Hatchability % | No. of egg masses/ Snail | No. of eggs/ mass | No of hatching | Hatchability % | No. of egg masses/ Snail | No. of eggs/ mass | No of hatching | Hatchability % |
| *B. alexandrina*  | 3 ±0.71                        | 11.47±2.2               | 10.87±2      | 94.77         | 0                | 0                       | 0                       | 0                       | 0                       | 0                       | 0                       |
| *B. truncatus*    | 3.6±0.89                      | 10.83±2.01              | 10.44±2.01  | 96.41         | 0                | 0                       | 0                       | 0                       | 0                       | 0                       | 0                       |
| *E. vermiculata*  | 0                             | 0                       | 0            | 0             | 0                | 0                       | 0                       | 0                       | 0                       | 0                       | 0                       |
**Table 4.** Values of LC 90, LC 50 and LC 25 against *B. alexandrina*, *B. truncatus* and *E. vermiculata* treated with Lupine NPs. and CuOxNPs

| Nail species | Lupine NPs | CuOxNPs |
|--------------|------------|---------|
|              | LC90 | LC50 | LC25 | Slope | r  | Chi² | LC90 | LC50 | LC25 | Slope | r  | Chi² |
| *B. alexandrina* | 11.97 | 4.56 | 2.75 | 0.97 | 47.79 | 48.26 | 3.64 | 1.74 | 0.96 | 2.59 |
| *B. truncatus* | 8.38 | 3.36 | 2.08 | 0.99 | 2.59 | Nd* | Nd* | Nd* | Nd* | Nd* |
| *E. vermiculata* | 2.08 | 0.55 | 0.27 | 0.96 | 13.15 | Nd* | Nd* | Nd* | Nd* | Nd* |
| Tabulated     | 0.95 | 6    | 0.99 | 7.38 | 0.95 |

Nd* the Ldp line program didn’t estimate these values because there were no enough mortalities that needed to make the regression lines.

**CONCLUSION**

The present study represents the first toxicological tests of lupine coated with copper NPs on the terrestrial and aquatic snails. Using lupine extract to reduce the amount of metal, consequently the side effect of metal nanoparticles on environment simultaneously gaining its toxicological effect on the harmful snail pests.

Both lupine extracts NPs and copper sulphate NPs may have a sterilized effect. When *B. alexandrina* and *B. truncatus* snails exposed to sub lethal doses they didn’t laid any egg masses after treatments.

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تأثير الأجزاء النانومترية المغلفة بالترمس المستخلص على بعض القواقع المائية والأرضية

كريمة محمود عزم - إيمان عبد الهادي - إيمان كامل خضر
مجهد بحوث وقاية النباتات - مركز البحث الزراعي - دقي - جيزة - مصر
*Corresponding author: mnmn7733@yahoo.com
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المحور

تم دراسة تأثير الأجزاء النانومترية المغلفة بكيربات النحاس لمستخلص نبات الترمس المصري Biomphalaria alexandrina Ehrenberg على العائلة. نسبة الموت وضع البيض لكل من Bulinus truncatus Audouin وكذلك القوقع Eobania vermicitulata Müller والمارخية مع الأجزاء النانومترية لكبريتات النحاس بعد مدة تعرض 52 ساعة تحت ظروف ظلام تتبعها فترة إنعاش 24 ساعة تحت ظروف إضاءة عادية.

أوضح النتائج أن الأجزاء النانومترية لمستخلص الترمس يداخلت العديد من القواقع غير محملة ككونترول. صممت هذه التجربة لتوضيح الخصائص الإبادية للأنماط النانومترية لكبريتات النحاس لم ينتظروا أن تكونت القواقع كيمياء للوقوع السابق ذكرها، بعد مدة تعرض 48 ساعة تحت ظروف إضاءة عادية.

أوضح النتائج أن الأجزاء النانومترية لمستخلص الترمس أن قواقع بلهارسيا النحاس كان لها تأثير أكبر من الأجزاء النانومترية لكبريتات النحاس فقط، حيث سبب تركيز 20 جزء في المليون نسبة موت 92.02% تحت الظروف السابقة تشكل في القوقع بالهراسيا النحاس، بينما القوقوق الأرضي لم ينتج نتائج معنوية ومحاولة جري التقاطعات. الجزئيات الدالية: جزيئات الترمس، النانومترية، القواقع المائية، القواقع الأرضية.
