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Original Article

Eco friendly approach to pest control using Copper oxide/ sodium alginate/pluronic F-127 for larvicidal activity against *Helicoverpa armigera*

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ABSTRACT

Background: *Helicoverpa armigera* (*H. armigera*), is an important agricultural pest, which is responsible for sizeable crop reduction worldwide. The traditional chemical insecticides are controlled this problem, often leading to environmental contamination, pest-resistant and killing on other non-targeted species. In present work, we fabricated a novel larvicidal system based on copper oxide (CuO)-Sodium alginate (SA)-Pluronic F127 (PF127) nanoparticles, aimed at controlling *Helicoverpa armigera*. **Materials and Method:** The CuO-SA-PF127 NPs were synthesized green process. The synthesized NPs where characterization was employed by structural, optical, antibacterial and larvicidal bioassay. **Results:** The CuO-SA-PF127 NPs were significantly controlling larvicidal activity, with positive mortality, when our increasing concentration also increased larvicidal activity and low toxicity to non-targeted organisms. Which is alternation for conventional insecticides. **Conclusion:** The CuO-SA-PF127 NPs exhibits potential to killing the *Helicoverpa armigera*, offering a fascinated approach for integrated pest managements (IPM) strategies. This is a potential achievement for nanotechnology industrial applications for next-generation agrochemical and environmentally safety responsibility.

Keywords: *Helicoverpa armigera*, Copper oxide, Sodium Alginate, Larvicidal.

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INTRODUCTION

The cotton bollworm, also known as *Helicoverpa armigera*, is considered a very damaging pest throughout the continents because it feeds on many hosts, including legumes, cotton, and cereals. This pest has developed resistance to several conventional insecticides, which is a major drawback to sustainable solutions to agricultural practices as well as food security. This has increased the demand for the need to formulate new types of larvicidal agents that are also environmentally safe and sustainable.[1] Recent studies have also reported the use of novel materials based on nanotechnology in the production of new insecticidal materials. New generation of insecticidal materials are synthetic nanoparticles and among them, CuO nanoparticles have exhibited potent biocidal activity against various pathogens including mosquitoes. However, the utilization of medicinal effective nanoparticles like CuO alone may be limited due to problems with stability, dispersibility and non-specific neurotoxic for other living organisms.

Difficulties like these have been addressed by using sodium alginate, a biopolymer as a nanoparticle carrier. Sodium alginate is a natural polymer obtained from brown algae which is biocompatible, biodegradable, and nontoxic. Incorporation of pluronic, block copolymer will also aid in increasing nanoparticle stability. The goal is to evaluate this nanocomposite as a promising and environmentally friendly larvicidal agent.[2] Using CuO nanoparticles coupled with biocompatible, biodegradable carriers, we are attempting to break through the restrictions of current insecticides and be a part of building more sustainable vector control. This is where nanotechnology could become a promising means of creating new materials that can do more biology.

Among nanomaterials, CuO nanoparticles have been widely studied due to their high antimicrobial activity against a wide range of pathogens and insect pests. However, CuO nanoparticle application in larvicidal formulations faces several constraints, such as the need for a stable and biocompatible carrier system to improve efficiency and reduce possible toxicity.[3] Preparations of biopolymers, such as sodium alginate extracted from brown marine algae, present an environmentally friendly host for the retention of nanoparticles. Sodium alginate is known for its biocompatible, biodegradable, and gel-forming material for improved stability with controlled agent release. This further

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enhances formulation performance by acting as a surfactant, stabilizing nanoparticles, and dispersing active agents in an aqueous system.[4]

We evaluate the efficacy of this composite material against *H. armigera* larvae, with a focus on its potential as a sustainable and eco-friendly alternative to conventional larvicides.

MATERIALS AND METHODS

Materials

The chemical and reagents including sodium alginate, Pluronic F127, and copper oxide all purchased from Sigma-Aldrich in the United States.

Preparation of Plant Extract

The plant materials (pepper) washed thoroughly with distilled water to remove dust and mites, the pepper was air dried at room temperature for 30 min. The extraction process involved placing 10 g of dry and chopped leaves 200 mL of double distilled water and cooking at 80°C for 1 hr and observing that its color changed into yellow-brown. The extract was cooled to room temperature and filtered through Whatman No. 1 filter paper.

Synthesis of CuO-SA-PF127 HNMs

For CuO-SA-PF127, bioactive compounds were extracted from 10 g of pepper and boiled in 150 mL of distilled water at 80°C for one hour. In a separate solution, dissolve 0.1 M CuO, 0.5 g of sodium alginate and 0.5 g of Pluronic F-127 in 100 mL of leaf extract. The solution was stirred at 26°C for 20 min and placed in an autoclave bottle with a polypropylene cap. CuO-SA-PF127 HNMs was prepared by exposing the solution to 800 W microwave energy for 10 min. The nanoparticle solution was washed with deionized water and ethanol to remove inactive particles. The residue was then dried at 120°C. The prepared CuO-SA-PF127 HNMs was heated at 200°C for 5 hr to make it more stable.

Characterization process

CuO-SA-PF127 HNMs is characterized using an X-ray diffractometer (model: X'PERT PRO PANalytical). For sample CuO-Alginate-Pluronic F127 HNMs, diffraction patterns were obtained in the range of 25-80°C using the monochromatic wavelength of 1.54. The spectrum was obtained at ultra-high vacuum pressure, Al K excitation, 250 W. Morphology and chemical composition were determined on Carl Zeiss Ultra 55 FESEM using EDAX (Model: Inca). JY Fluorolog-3-11 spectrometer is used for Photoluminescence (PL) spectrum analysis at room temperature. FT-IR spectra collected using Perkin-Elmer spectrometer in the range of 400×4000 cm^{-1} were used to identify various functional groups. (HORIBA, Kyoto, Japan) for the analysis of HNM. Dissolve CuO-SA-PF127 HNMs using 1 mL of 5% DMSO solution as stock solution.

Antimicrobial assay

Bacteria such as *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), *Vibrio cholerae* (*V. cholerae*), *Proteus vulgaris* (*P. vulgaris*) and *Candida albicans* (*C. albicans*) were cultured on Nutrient agar and PDA agar plates and 6 mm wells were created on the surface of the agar plates. The wells were then filled with CuO-SA-PF127 HNMs at different concentrations (1, 1.5 and 2 mg/mL). The antibiotic amoxicillin was used as a positive control. After 24 hr of incubation, the zone of inhibition around the well was assessed and the data tabulated.

Bioassay

The deceased were witnesses. Each experiment included 20 infants per hypothesis and was repeated five times using a total of 100 infants (n=100). Lethal concentrations were determined by observation of the results. Percent treatment group mortality was adjusted as necessary to account for control group mortality.

Histological studies

Bollworm larvae were obtained from laboratory larvae and third instar larvae were selected for histological analysis. Larvae were fixed on ice and dispersed in phosphate-buffered saline. Carefully remove the midgut, fat body, and other tissues of interest using fine dissecting tools under a stereomicroscope (Leica MZ75). This fixative was chosen to preserve cellular detail. After fixation, tissues were washed with 70% ethanol to remove picric acid. The tissue was then dehydrated through an ethanol series (70%, 80%, 90%, 95%, and 100%) for 30 min each. After dehydration, tissues were processed in xylene and embedded in paraffin (melting point 56-58°C). Paraffin blocks were cooled and stored at 4°C until sectioning.

Statistical Analysis

For studies conducted in triplicate, the results were presented as mean±standard deviations. To detect any significant changes, one-way ANOVA and Tukey's *post hoc* test were employed to compare the mean values from the treatment and control groups.

RESULTS

XRD Analysis

The XRD pattern of green produced CuO-SA-PF127 HNMs. As shown in Figure 1, the hkl planes of 70.72°, (110), (002), (111), (-202), (020), (202), (-113), (-311), (220), (311), and (-222) reveal the monoclinic structure in CuO NPs (JCPDS 80-1916), respectively. The crystallite size of the resulting powder can be determined using the Debye–Scherrer equation; size D=(0.9×)/(×cos×). The average size of CuO-SA-PF127 HNMs was found to be 55 nm.

Morphology and chemical composition analysis

Figure (2a-b) shows the lower and higher magnification FE-SEM image of the green synthesized CuO-SA-PF127 HNMs. The CuO-SA-PF127 HNMs formed polymorphic nanostructure and average particle size was observed at 50-100 nm. The EDAX spectrum employed the chemical composition, and CuO-SA-PF127 HNMs present elements were carbon, copper, and oxygen molecules, as shown in Figure (2c) and no other impurity peaks not observed in the EDAX spectrum. The atomic percentages were found to be: 20.99% for C, 62.13% for O, and 16.87% for Cu in the CuO-SA-PF127 HNMs.

DLS spectrum

Figure 3 shows the Dynamic Light Scattering (DLS) of CuO-SA-PF127 HNMs in an aqueous medium. The hydrodynamic size was observed at 70 nm for CuO-SA-PF127 HNMs.

FT-IR analysis

Figure 4 shows the FT-IR spectrum of CuO-SA-PF127 HNMs. The metal-oxide stretching band Cu-O stretching peaks were observed at 616 and 529 cm^{-1} for CuO-SA-PF127 HNMs. The broad O-H stretching water molecules absorbed on the CuO-SA-PF127 HNMs at 3433 cm^{-1}. The biopolymer sodium alginate peak is at 2923 and 2852 cm^{-1}, owing to the asymmetric and symmetric C-H peaks and the carboxylate anions (C=O) and O-H bending bond at 1638 cm^{-1}. The C-H stretching was observed at 1417 cm^{-1} for plutonic F127 molecules.

Photoluminescence (PL) analysis

The PL spectrum of the synthesized green CuO-SA-PF127 HNMs is shown in Figure 5 with an excitation wavelength of 325 nm. The PL emission wavelengths of CuO-SA-PF127 HNMs are 365, 395, 412, 439, 461, and 481 nm. The UV emission observed between 365 and 395 nm is due to the combination of electrons in the holes in the valence band. Color emission - Pluronic F127 HNMs due to oxygen vacancies and Cu interstitial defects.

Antimicrobial activity

Antimicrobial performance of CuO-SA-PF127 HNMs against bacteria (bacterial and fungal) *S. aureus, S. pneumoniae, B. subtilis, E. coli,V. cholerae, P. vulgaris* and *C. albicans* were evaluated using the diffusion method (Figure 6A). The inhibition zone results for this assay are shown in Figure 6B. Microbial results show that CuO-SA-PF127 HNMs has significant antibacterial properties and can inhibit the growth of laboratory bacteria such as *Staphylococcus aureus, Streptococcus pneumoniae*, *Bacillus subtilis* and *Escherichia coli. Escherichia coli, Vibrio cholera*e, *Proteus vulgaris* and *Candida albicans*. A higher inhibition zone around the well was observed with increasing doses (1, 1.5 and 2 mg/mL) of *S. aureus, S. pneumoniae, B. subtilis, E. coli, V. cholerae, P. vulgaris* and *C. albicans* were found on the growth plate. The difference in inhibition around the well depends on the characteristics of the bacteria (bacteria and fungi) and the concentration of *S. aureus, S. pneumoniae, B. subtilis, E. coli, V. cholerae, P. vulgaris* and *C. albicans*

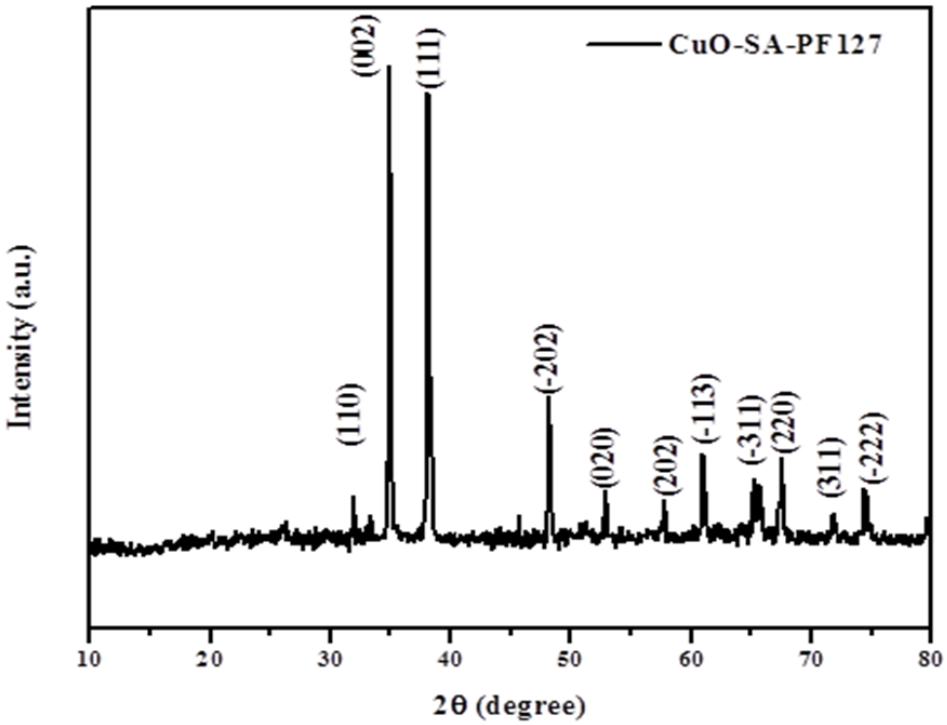

Figure 1: X-ray diffraction patterns of CuO-SA-PF127 HNMs.

Bioassay

The larvicidal activity of the CuO-Sodium alginate composite was evaluated against *Helicoverpa armigera* larvae across various concentrations. The mortality rates were observed over a period of 24, 48, and 72 hr post-exposure, with significant dose-dependent effects noted.

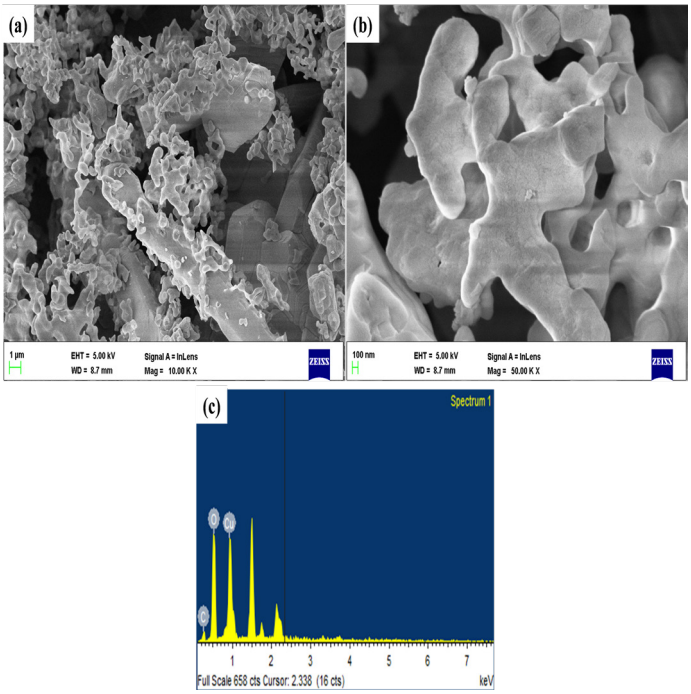

Figure 2: (a-b) Lower and higher magnification FE-SEM image and (c) EDAX spectrum of CuO-SA-PF127 HNMs.

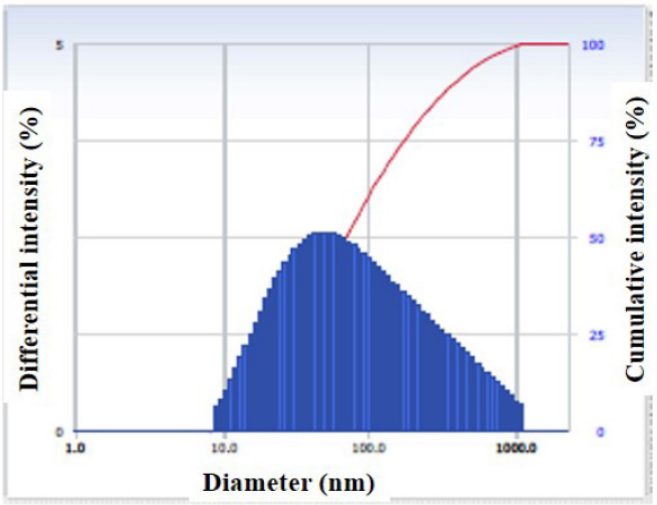

Figure 3: DLS spectrum of CuO-SA-PF127 HNMs.

Concentration-Dependent Mortality

The results demonstrated a clear correlation between the concentration of CuO-SA-PF127 and larval mortality. At the highest concentration tested (X mg/L), a mortality rate of 70% was observed after 24 hr, which increased to 85% by 72 hr. In contrast, at the lowest concentration (1 mg/L), the mortality rate was significantly lower, with 50% mortality after 24 hr and 70% by 72 hr (Figure 7).

Time-Dependent Mortality

Time-Dependent Mortality Increasing mortality rates with time of exposure were also observed at all concentrations tested. For example, at the middle concentration (1 mg/mL), after 24 hr no mortality was observed; however, at the same concentration following the next 24 hr period, mortality was recorded. The LC_{50} concentration was calculated to be 970 µg/mL. At 1 mg/L, mortalities were recorded at 85% within 24 h, increasing to 95% after 48 h and reaching 99% by the end of 72 hr. Deaths recorded over this period, a trend consistent for all concentrations, signify that the larvicidal effect of the CuO-Sodium alginate composite increases with lengthened time of exposure.

Helicoverpa armigera Larval Mmidgut Histology

Histological examination of midgut tissue indicated marked structural changes in response. Control midgut epithelium showed well-organized columnar epithelial cells, goblet cells, and microvilli, with centrally located nuclei, and intact peritrophic membrane proper cellular activity and gut integrity. Larvae treated with the candidate formulation exhibited marked histopathological alterations. A complete disarray of the midgut epithelium including vacuolization, cytoplasmic disintegration, and nuclear pyknosis was observed. Columnar cells were deformed, irregular in shape and size, and many were necrotic. Some erosion either in part or as a whole of the layer of microvilli caused impaired absorption of nutrients (Figure 8).

Furthermore, it was also seen that in many parts the peritrophic membrane was significantly disrupted or even absent, compromising the protective nature of the gut against pathogens and toxins. This finding was further supported by the presence of cellular debris in the gut lumen, which indicated the extent of tissue damage.[5] These histological changes thus indicate that the treatment perhaps does exert a direct cytotoxic effect on midgut cells belonging to *H. armigera*, and this may result into decreased digestive and absorptive functions contributing to larval mortality.

DISCUSSION

This study reveals that CuO nanoparticles encapsulated in sodium alginate pluronic had a significant reduction of larval population of Helicoverpa amigera. The effectiveness could be most likely attributed to special properties of CuO nanoparticles, like their small size and high surface area, which enhance their interaction with the larval cells disrupt critical physiological function.[6] Encapsulation in sodium alginate pluronic was considered most advantageous in improving the delivery, stability of CuO

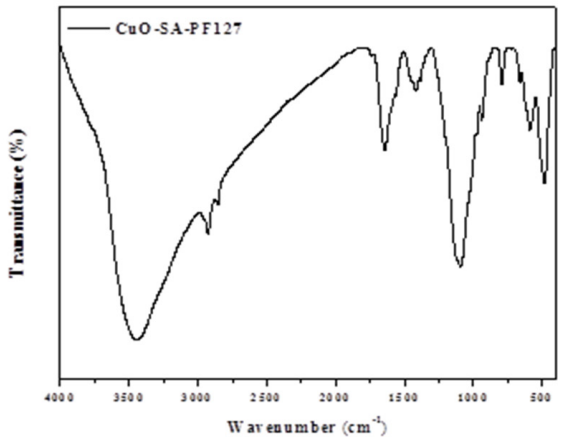

Figure 4: FT-IR spectrum CuO-SA-PF127 HNMs.

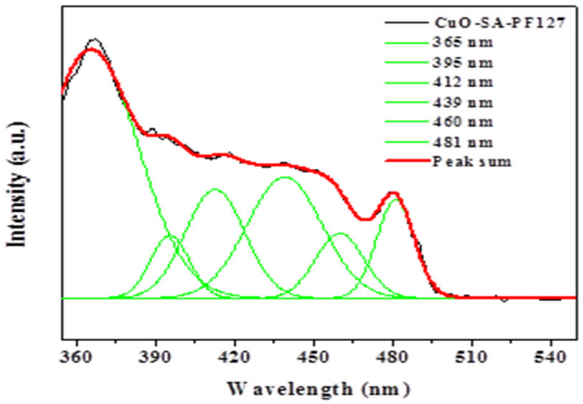

Figure 5: PL analysis of CuO-SA-PF127 HNMs.

nanoparticles by providing sustained release and protection from environmental degradation.[7] There is now less need for frequent reapplications because of the sustained larvicidal activity brought on by the controlled release from the matrix. The study shows that the number of Helicoverpa amigera larvae was considerably lower in CuO nanoparticles embedded with sodium alginate pluronic. The efficiency may result from particular characteristics of CuO nanoparticles, such as their tiny size and large surface area, which improve their ability to interact with larval cells and interfere with vital physiological processes.[6] By enhancing prolonged release and shielding CuO nanoparticles from the impacts of environmental deterioration, sodium alginate pluronic encapsulation offers significant advantages.[8,9]

These compounds have to be reapplied less frequently because of the matrix-controlled release, which prolongs the larvicidal efficacy. This work proves that the larvicidal activity of CuO nanoparticles on *H. armigera* can be attributed to oxidative stress. Just as it was furnished to generate ROS by other metal nanoparticles, CuO, as well is most often recorded in the literature.[10-12] The actions of ROS on causing disruption of cellular integrity ultimately brought about apoptosis together with several other cellular dysfunction mechanisms in insects are noticed. Importantly, this study offers a sustainable alternative to traditional chemical pesticides.[13] The use of nanoparticles, particularly when encapsulated in biodegradable polymers like sodium alginate, reduces the environmental footprint and lowers the risk of resistance development in pest populations.[14] This is crucial in the current context of increasing pesticide resistance in *H. armigera* populations.[15] In contrast to synthetic chemical pesticides, which can persist in the environment and harm

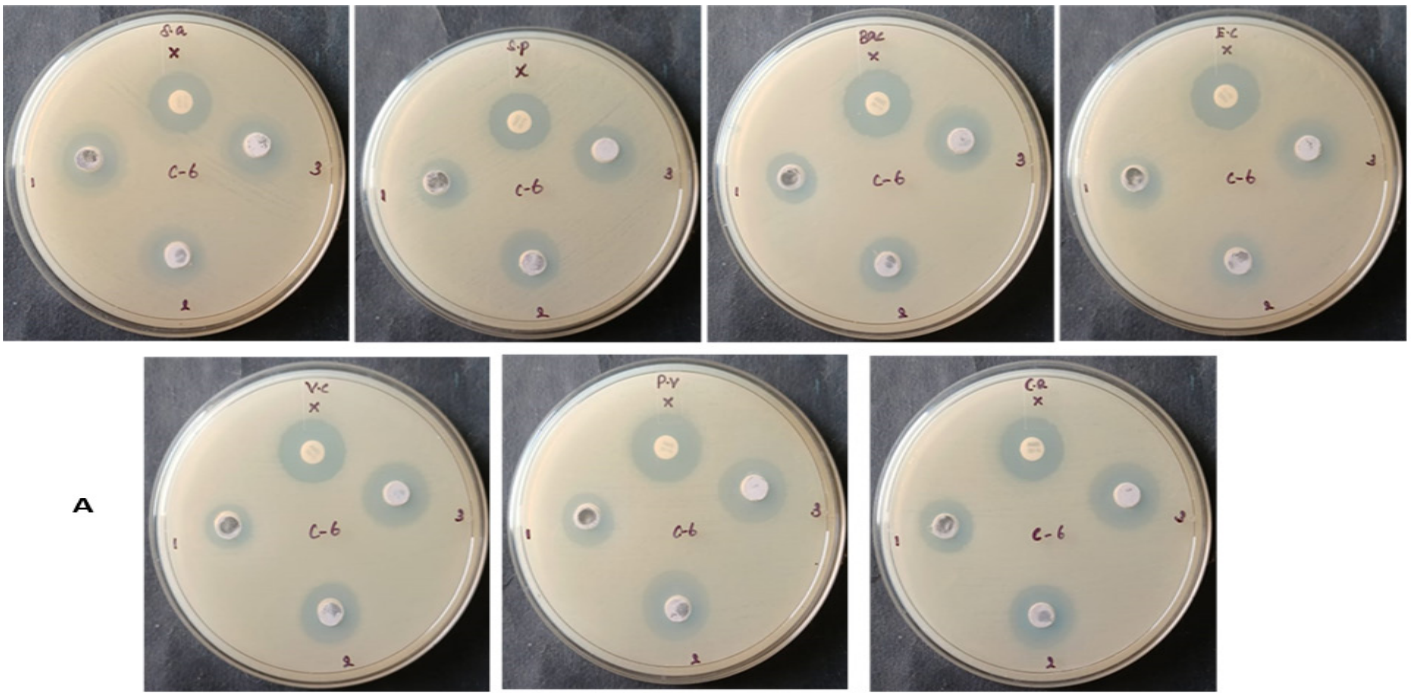

Figure 6A: Antimicrobial performance of CuO-SA-PF127 HNMs against different micro-organisms.

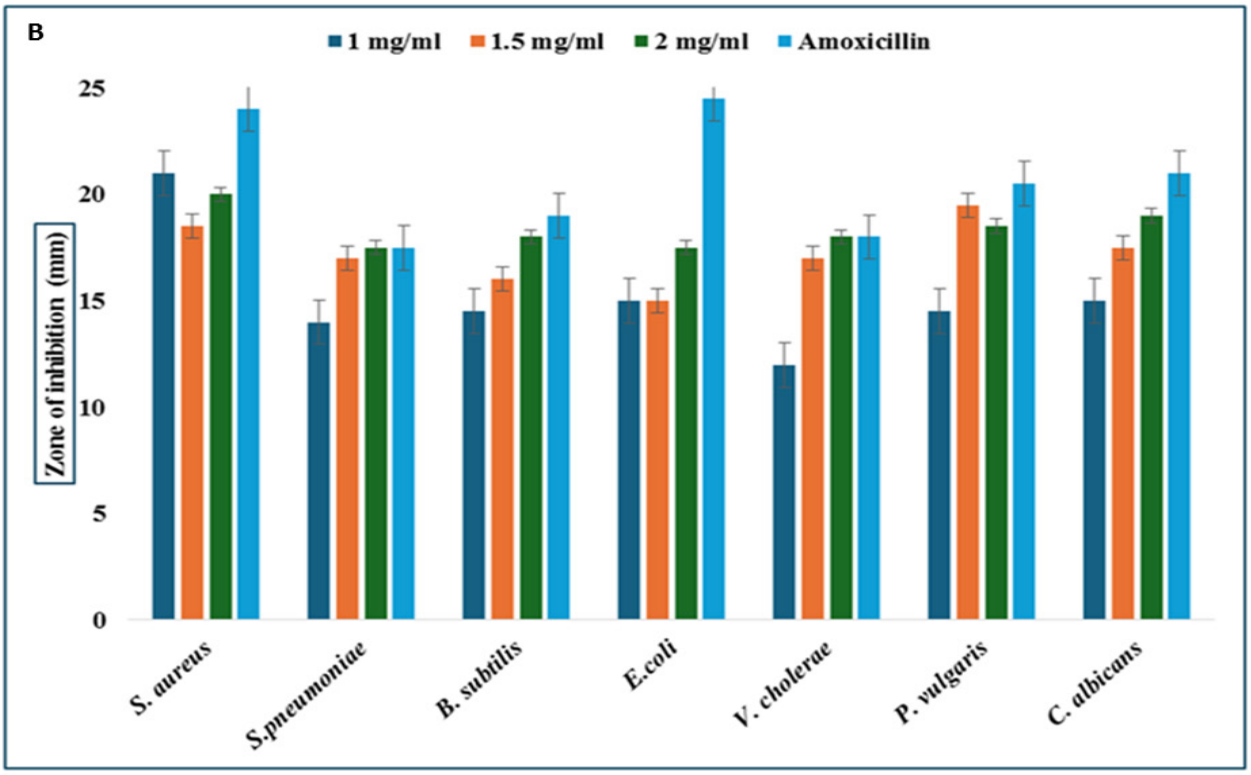

Figure 6B: The treatment of synthesized CuO-SA-PF127 HNMs exhibited an increased zone of inhibition against the tested pathogens. The data were given as a mean±SD of triplicate assays. The values are statistically analyzed by one-way ANOVA and Tukey's post hoc assay.

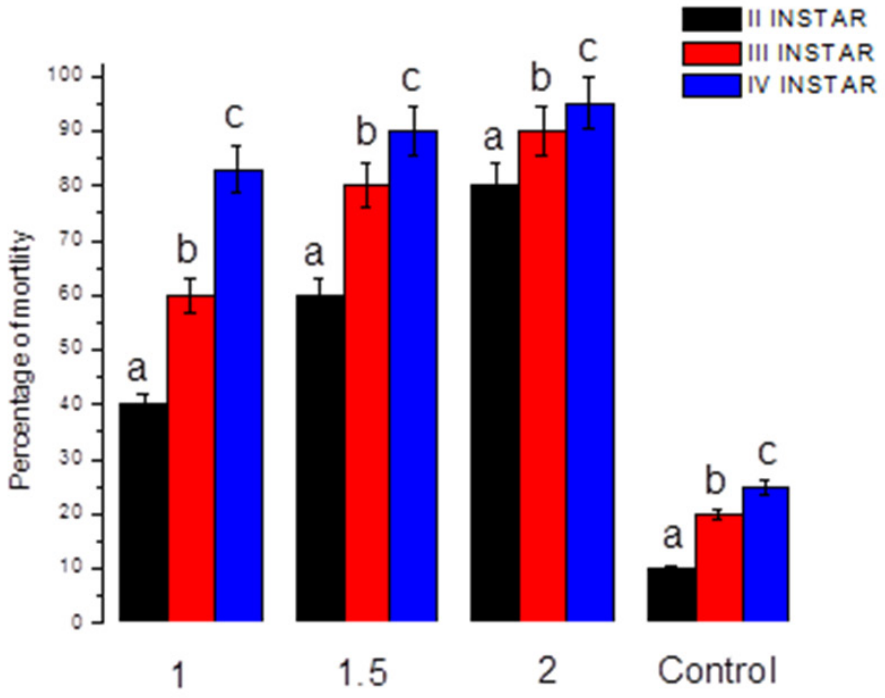

Figure 7: Percentage of mortality rate of *H. armigera* against CuO-SA-PF127 composite with different time duration. The data were given as a mean±SD. The values are statistically analyzed by one-way ANOVA and Tukey's post hoc assay.

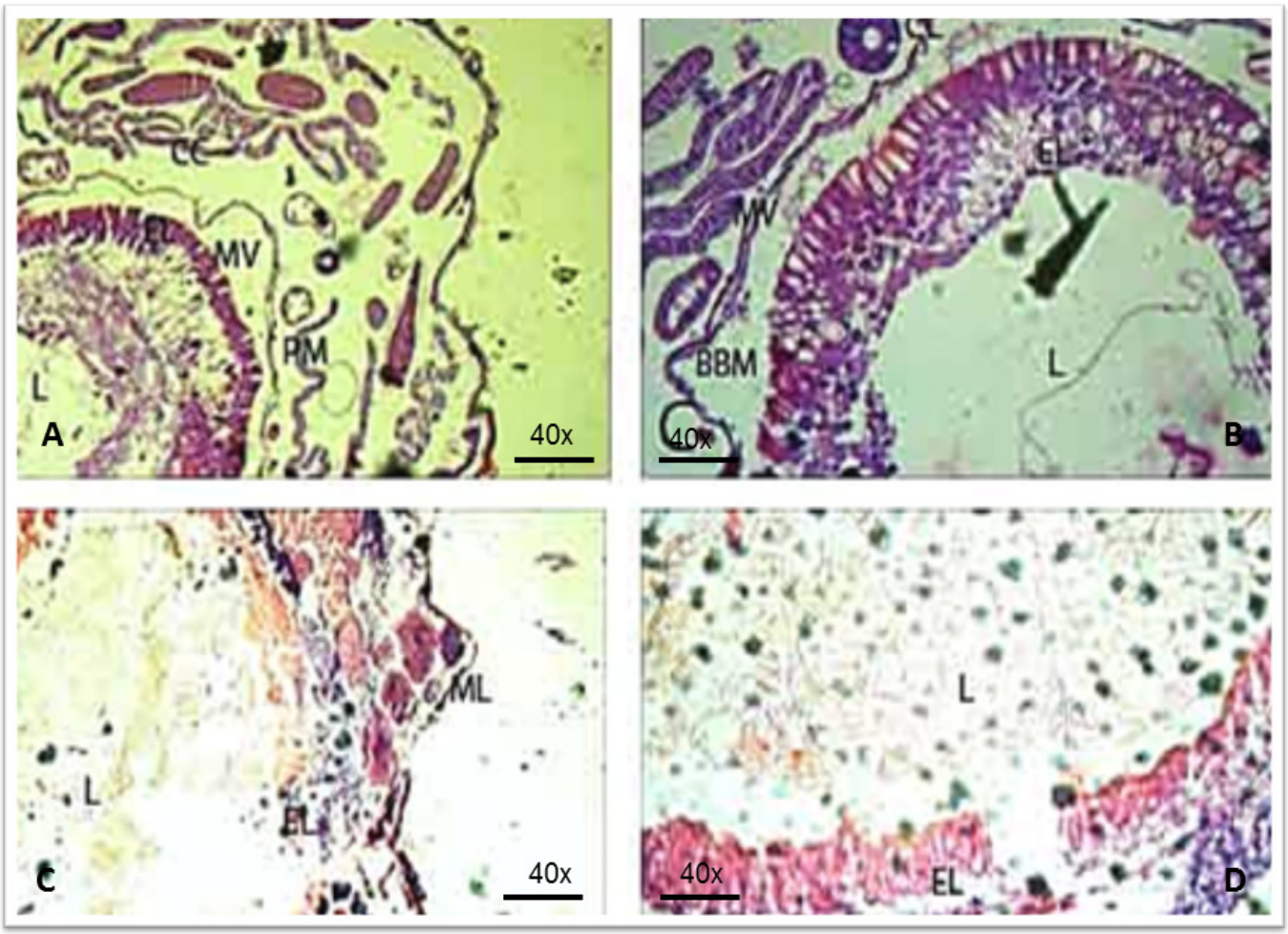

Figure 8: Histological effect of *H. armigera* against CuO-SA-PF127 composite with different time duration. (A) Control, (B) Enlarged Control, (C) Treated and (D) Enlarged Treated

non-target organisms, CuO nanoparticles degrade more quickly and have less long-term environmental impact.[16,17]

One limitation of this study is the potential for nanoparticle accumulation in the environment. While CuO is less toxic compared to conventional pesticides, its long-term environmental impact, especially in non-target organisms, needs further investigation.[18] Future studies should explore the ecotoxicological effects of nanoparticle residues in soil and water ecosystems. Additionally, the scalability of this technology for field applications remains a challenge. The synthesis of CuO nanoparticles and their encapsulation in sodium alginate pluronic need to be optimized for cost-effective large-scale production. The application methods for efficient nanoparticle dispersal in agricultural fields also require further research.

CONCLUSION

CuO nanoparticles encapsulated in sodium alginate pluronic exhibit promising larvicidal efficacy against *H. armigera* and represent a viable, sustainable alternative to conventional pesticides. The development of nanoparticle-based biopesticides could play a crucial role in integrated pest management strategies aimed at reducing the use of harmful chemical pesticides and promoting environmental sustainability.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ABBREVIATIONS

CuO: Copper oxide; ***H. armigera*:** *Helicoverpa armigera*; ***S. aureus*** *Staphylococcus aureus*; ***S. pneumoniae*:** *Streptococcus pneumoniae*; ***B. subtilis*:** *Bacillus subtilis*; ***E. coli*:** *Escherichia coli;* ***V. cholerae***: *Vibrio cholerae*; ***P. vulgaris*:** *Proteus vulgaris*; ***C. albicans*:** *Candida albicans*; **ROS:** Reactive oxygen species.

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