ACS OMEGA

http://pubs.acs.org/journal/acsodf
Article

Fatal Food: Silver-Coated Grain Particles Display Larvicidal Activity in *Culex quinquefasciatus*

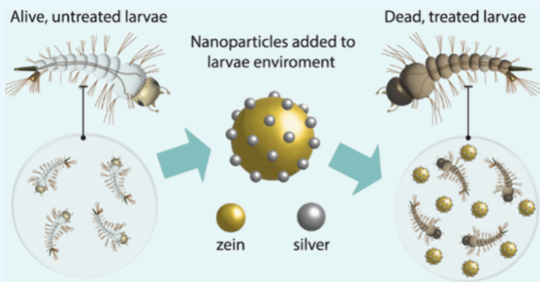
Amie E. Norton,* Robert Ewing, Michael Tilley, Jeff Whitworth,* and Lee W. Cohnstaedt*

Cite This: *ACS Omega* 2023, 8, 33437−33443

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Mosquitoes pose a significant risk to millions of people worldwide since they can transmit pathogens. Current methods to control mosquito populations include the use of synthetic pesticides. Nanotechnology may be a solution to develop new mosquito control. However, one barrier to expanding the impact of nanomaterials is the ability to mass-produce the particles. Here, we report a novel hybrid particle synthesis combining micro- and nanoparticles using the coprecipitation technique with the potential for mass production. These particles may have applications as a mosquito larvacide. The particles reported here were designed using a microparticle zein polymer as the core and a nanoparticle silver as the active ingredient. The hybrid NPs reported here targeted a late-stage mosquito larvae and that resulted in a high larval mortality concentration (1.0 ppm, LC_{90}) and suppression of pupal emergence at 0.1 ppm. This research demonstrates the efficacy of a plant-based core with a metal-based AI coating (AgNPs) against larval mosquitoes.



■ INTRODUCTION

Millions of people worldwide are at risk from mosquitoes, as they can transmit serious pathogens such as malaria, dengue, yellow fever, Zika virus, and West Nile virus.[1] Mosquitoes belonging to the Culicidae family pose a dual threat to humans and animals. In addition to being a nuisance, they can spread diseases and parasites that can affect dogs and horses, including heartworm, West Nile virus, and Eastern equine encephalitis. Therefore, it is imperative to take preventative measures to protect both human and animal populations from these potential health risks.[2] Current methods to control mosquito populations include the use of synthetic pesticides. The overuse or misuse of pesticides, harms nontarget organisms and the environment.[3,4] As pesticide use has increased, so has pesticide resistance in targeted pests. The rising concern over mosquito control has sparked interest in the development of new methods. One of the new methods in pesticide development that has gained significant attention is the application of nanotechnology.[5] Nanotechnology is defined as a material that is between 1 and 100 nm. Nanomaterials have improved bioreactivity and unusual physical properties due to the increased surface area to volume ratio.[6,7] The current methods for synthesizing nanoparticles involve either toxic chemicals in chemical processes or high pressure, energy, and temperature in physical processes.[8,9] Despite the numerous beneficial properties of nanoparticles, their large-scale production may become an issue due to the use of toxic chemicals or costs of products.[10] When considering the expansion of NP production, various factors must be considered, including cost, environmental impact, and health concerns.[11−13] Environmentally friendly methods can reduce the cost of production and facilitate large-scale production of NPs for large-area pest applications.[9]

Here, we report a hybrid NP targeting a specific pest that resulted in a high larval mortality concentration (1.0 ppm, LC_{90}) and suppression of pupal emergence at 0.1 ppm. This research demonstrates the efficacy of a plant-based core with a metal-based AI coating (AgNP) nanoparticle against larval mosquitoes. Silver was selected for its antimicrobial properties. It is important to note that the effects of silver nanoparticles on human health are not fully understood. However, smaller silver nanoparticles from 0 to 10 nm are more cytotoxic.[14] The cytotoxic properties of the particles are affected by surface modification of the nanoparticles.[15] The silver nanoparticles described here are between 15 and 20 nm and are attached to a zein microparticle of 463 nm, thus potentially reducing the cytotoxicity of the particles. Insecticidal silver nanoparticles are not novel, with at least 18 studies reporting mosquito larvicidal efficacy (Table S1). Prior nanoparticle synthesis did not produce "green NPs" with production scalability and environmental safety being a priority and without these two considerations, NPs cannot be produced used for large-area insecticidal applications without potential harm to the environment.

Received: May 9, 2023
Accepted: August 24, 2023
Published: September 11, 2023

■ MATERIALS AND METHODS

The insecticidal particles reported here were consumed by the target insect after they were filtered out of the water by mosquito larva. Therefore, the key structural components of these particles were the edible microparticle core (zein protein polymer) and a nanoparticle insecticidal coating (silver) around the core.

Glycerol, lactic acid, poly(ethylene glycol) acetic acid, zein protein, silver nitrate, and sodium borohydride were purchased from Sigma-Aldrich (Burlington, MA).

Zein Particle Synthesis. Zein particles were synthesized according to protocols outlined by Taylor et al.[16] with slight modifications. In brief, a ratio of 1:1:1 of lactic acid, poly(ethylene glycol) (PEG) (6000 MW), and glycerol (0.66 g) was added to 4.34 mL of glacial acetic acid in a 150 mL flask. The environmental solvent rating for acetic acid was considered using the GSK solvent rating. Acetic acid and glycerol have a life cycle score of 8, and ethylene glycol has a life cycle score of 9.[17] Water has a life cycle score of the highest rating and an environmental solvent score of 10. Pfizer lists acetic acid as usable.[18] Using a hot/stir plate with a magnetic stir bar (Corning, PC-220 pyroceram hot plate stirrer, Fisher Scientific), 1.8 g of zein (powder) was added to the solution, and the temperature was slowly raised to 37 °C. All solids were dissolved in the acidic polymer solution and formed a film residue after evaporation of the solution. DI water was added dropwise to the residue, and the mixture was continually stirred until a total volume of 70 mL was obtained and precipitation of the zein polymer particles was completed. The solution was removed from heat, stirred for 5 min at 21 °C, and then transferred to 50 mL Eppendorf tubes. The tubes were centrifuged (Eppendorf 5810 R centrifuge) for 5 min at 3110*g*, and the supernatant was poured off. The remaining particles were washed and centrifuged for 5 min at 3110*g* three times with 20 mL of DI water.

Zein-Silver Synthesis. AgNPs were synthesized according to protocols previously reported[19] with slight modifications. In brief, a stock solution of silver nitrate was prepared by adding silver nitrate (0.09 g) to 25 mL of DI water while being stirred with a magnetic stir bar on a stir/hot plate. In a separate solution, 0.1 g of zein particles were stirred in 15 mL of DI water on a hot/stir plate with a magnetic stir bar under ambient conditions. After stirring for 30 min, the silver nitrate solution was added to the zein solution and stirred for an additional 30 min. Sodium borohydride (100 ppm, 1 mL) was added dropwise to the solution until the entire solution turned brown. Sodium borohydride was added to speed up the reaction; the reaction would proceed without adding sodium borohydride after 24 h of stirring. The silver-zein particles were then poured into a centrifuge tube and centrifuged for 5 min at 3110*g* after which the supernatant was poured off. Particles remaining at the bottom of the centrifuge tubes were washed for 5 min at 3110*g* three times with 20 mL of DI water.

Particle Characterization Methods. UV−visible spectrum analysis was performed using a Biotek Epoch 2 microplate reader spectrometer to confirm and characterize the size and shape of the silver NP formations. The zein polymer microparticles were UV−visible inactive, meaning they did not have a spectral absorbance in this range.

Transmission electron microscopy (TEM) was used to image the particles. A drop of particle solution was placed on carbon-coated 200 mesh copper grids and viewed at an accelerating voltage of 120 kV on an FEI Tecnai G2 Spirit BioTWIN TEM (FEI Company, Hillsboro, OR). This characterizes the size and shape of the nanoparticles on the surface of the microparticle core.

Fourier-transform infrared spectroscopy (FTIR) measures the functional groups of the particles. A Thermo Nicolet Nexus 670 FTIR spectrophotometer equipped with a smart collector analyzed both the zein polymer core and silver-zein particles. The analysis was carried out in the wavenumber range of 400−4000 cm^{-1}, with the detector at a 4 cm^{-1} resolution and 32 scans per sample. OMNIC 6.1a software (Thermo-Nicolet Corporation, Madison, WI, USA) was used to determine the peak positions and intensities.

Dynamic light scattering (DLS) was conducted on a Malvern Zetasizer (Malvern, United Kingdom) to determine the particle size in water.

Larval Treatments. The *Culex quinquefasciatus* (*Cx. quinquefasciatus*) colony was established in 2016 in Orange County, CA, by UC Davis and has been maintained at the USDA Insectary since 2017. Colony rearing conditions: density, 0.8−1 larvae cm^2; temperature, 26 ± 0.5 °C; relative humidity, 70−80%; photoperiod, 13:11 L/D; diet, 1:1 ratio of tropical fish flakes and ground cat food and, on rearing day five, 0.1 g of beef liver powder.[23] Twenty larvae (third and fourth instars) were exposed to specified concentrations of AgNPs in three replicates unless otherwise noted. Larvae were placed in disposable weigh-boats with 10 mL of water and contained within mini mosquito breeders (BioQuip products, Rancho Dominguez, CA) at ambient temperature. Three treatments were added via disposable pipettes per treatment: 100, 1.0, and 0.1 ppm, using 10 mL of silver-zein NPs. Controls of water, zein polymer particles, a polymer mixture, and sodium borohydride were recorded. Mortality determination occurred by slightly probing unmoving larvae within each treatment.

Mode of Action. Optical images of mosquitoes (treated and nontreated) were recorded on a Keyence VHX-6000 (Keyence, Itasca, IL) digital microscope. Imaging of the mosquito midgut (treated and nontreated) was completed using scanning electron microscopy (SEM). Samples were fixed in 4% formaldehyde and 1% glutaraldehyde overnight, washed in buffer, and dehydrated through a graded ethanol series followed by critical point drying (Samdri 790 B, Tousimis, Rockville, MD, USA). Dried samples were placed on double-sided conductive carbon sticky tape atop an aluminum stub and then sputtered with palladium by using a Denton Vacuum Desk II sputter coater (Denton Vacuum, Moorestown, NJ, USA). Samples were analyzed under a Hitachi S-3500N SEM instrument (Hitachi Science Systems Ltd., Tokyo, Japan) at an accelerating voltage of 15 kV. Energy-dispersive X-ray spectroscopy (EDS) confirmed the presence of the silver element using an Oxford EDS detector (Oxford Instruments Microanalysis Group, High Wycombe, England).

■ RESULTS AND DISCUSSION

Characterization of the Particles. Hybrid microparticles were created by dissolving a mixture of zein and a polymer (lactic acid:glycerol:PEG (1:1:1)) (zein particles) in acetic acid followed by the gradual addition of water to alter the solvent polarity and generate microparticles with a zein core. Zein MPs and insecticidal zein polymer cores with AgNPs were characterized by UV−visible spectroscopy, TEM, FTIR, and DLS.

https://doi.org/10.1021/acsomega.3c03210
ACS Omega 2023, 8, 33437−33443

The zein particles were UV−visible inactive, which was demonstrated by Patel et al.[20] The AgNPs on the zein polymer microparticles showed a maximum of 400 nm UV−visible spectroscopy (Figure S1). The silver nanoparticle UV−visible spectrum band maximum wavelength ranges from 391 to 491 nm. The AgNP band at 400 nm corresponds to the plasmon absorbance of silver nanoparticles, which is similar to previous reports.[21,22] Haiss et al. developed a methodology that utilizes UV−visible spectroscopy to quantify the size and density of gold nanoparticles.[23] Paramelle et al. built on this methodology and determined the size of silver nanoparticles by using the UV−visible peak maximum compared to TEM measurements of silver nanoparticles.[22] The study by Paramelle et al. measured the size of silver nanoparticles capped with citrate. The study found a slight variation in size, with a range of only ±5%. The techniques employed in this research are contingent on the size of the silver nanoparticles and the impact it has on their UV−visible peak maximum.[24,25] Based on the Paramelle et al. table of wavelength maximum vs size of silver nanoparticles measured by the TEM, silver nanoparticles with a maximum wavelength band at 400 nm have a size of approximately 20 nm.

Images by TEM showed the porous zein polymer surface (Figure S2a). After the addition of silver nitrate, the AgNPs appeared as dark spherical objects on the surface (Figure S2b), similar to those previously reported.[26] No dark circles were observed on the untreated zein polymer particles. The AgNPs on the surface of the zein measured about 15−20 nm, which agreed with our UV−visible spectrum band maximum estimates.

The FTIR-ATR spectrum was recorded on a zein polymer and silver-zein particles (Figure S3 and Table 1). The FTIR bands for zein particles are in agreement with previous reports.[27,28] After the addition of silver, new bands were observed at 1380, 1320, 1020, and 931 cm^{-1}. The observed bands at 1380 and 1320 cm^{-1} may be attributed to an Ag−N bond, which also aligns with Zhang et al.'s observation.[27,29] However, the silver-zein nanoparticles reported here showed new peaks at 1020 and 931 cm^{-1}, which were not previously observed by Dashdorj et al.[27] or Velayutham et al.[28] Zhang et al. conducted FTIR-ATR spectroscopy on silver-zein particles that were synthesized under acidic conditions with a pH of 3.3, as well as those prepared at a pH of 6.5. The spectral analysis revealed that the particles synthesized under more acidic conditions exhibited bands at 1020 and 931 cm^{-1}, but the bands were absent in the particles prepared under neutral pH conditions. The synthetic route used in this paper was under acidic conditions, which agrees with Zhang et al. observing these bands at more acidic pH.

Table 1. Solid-State FTIR-ATR Describing the Functional Groups and the Values of Zein Polymer MPs and Silver-Zein NPs Corresponding to the Following Groups

zein particle (cm^{-1})	silver-zein NPs (cm^{-1})	functional group
3320	3290	O−H
2920	2930	C−H
1650	1650	C=O
1520	1490	aromatic C=C
	1380	C−C
	1320	C−N−silver
	1020	
	931	

The size of the particles was determined by using DLS, as shown in Figure S4. The zein polymer particle core had a diameter of 463 nm (Figure S4), whereas the silver-zein particles had a diameter of approximately 100 nm (Figure S4). The zein polymer is larger than what has been previously reported.[20,30,31] One reason for this is the zein particle's ability to aggregate. Argos et al.[32] reported self-assembly by zein proteins by examining the particles in circular dichroism. Another possible reason for slightly larger particles is that the synthesis of the particles differed slightly from the previously published procedures, and the polyethylene polymer molecular weight differed from those previously used. However, when the silver nanoparticles are added to the zein polymer, the larger microparticle aggregates are broken, making smaller nanoparticles.

Larvicidal Mode of Action. The insecticidal particles were placed in solutions at 100, 1.0, and 0.1 ppm. Larvae exposed to particles at a 100 ppm concentration resulted in 100% mortality in minutes and 90 ± 4% mortality at 1.0 ppm 40 h postexposure (Figure 1). It has been reported that $AgNO_3$ has an LC_{50} of 45 ppm.[33] A previous report of synthesized silver nanoparticles using isoamyl acetate as the reductant shows the LC_{90} mortality rate of 32 ppm.[28]

Treatments were compared to the following control sodium borohydride, which exhibited 8% mortality after 90 h of exposure. This further demonstrates the need to remove less environmentally friendly reactants if the products are used for wide-area applications. The mixture of polymers (lactic acid, glycerol, and PEG (the "greener solvents")) and zein was not lethal. In the first trial, one adult emerged from the 20 larvae that survived at a concentration of 1.0 ppm, and three adults emerged from the 20 larvae at 0.1 ppm. In the second and third trials, no larvae died, but no adults emerged after exposure to silver-zein particles. This suggests that AgNPs may also have sublethal effects on inhibiting pupal/adult development.

Table S1 provides a comprehensive compilation of publications reporting insecticidal properties of synthesized silver nanoparticles from numerous plant sources. Mortality from prior silver nanoparticle papers reported a wide range of mortalities from 3 to 150 ppm, which may be due to the difference in size, shape, and chemical reduction source of the nanoparticles. The lowest concentration of nanoparticles causing mortality was 3 ppm after 90 h. For example, Amala et al. used synthesized AgNPs from an Azolla plant (*Azolla pinnata R. Br*). Insecticidal silver-zein particles reported here have a 90% mortality level at 1 ppm after 40 h using AgNPs.[34]

To gain a better understanding of the insecticidal mode of action, a combination of optical microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) was employed to capture visual representations. Figure 2 depicts the optical microscope images of two mosquito larvae, one having ingested silver-zein particles (Figure 2b) and the other belonging to the control group (Figure 2a). Additionally, the control larva displayed normal movement, and the midgut was still visible, in contrast to the larva exposed to silver-zein particles. The NPs caused the larva to melanize, and midgut bacteria were no longer visible. Sap-Iam et al.[35] recorded pictures of mosquito larvae exposed to AgNPs with an optical microscope and reported dark spots in the larva's abdomen, which agrees with what was observed here.

https://doi.org/10.1021/acsomega.3c03210
ACS Omega 2023, 8, 33437−33443

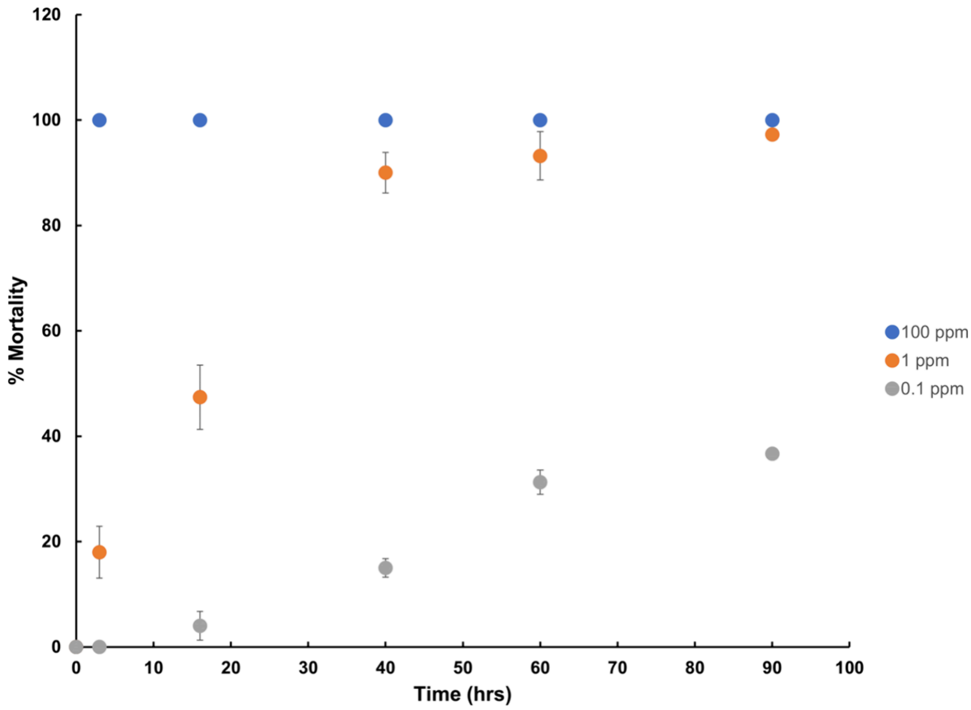


Figure 1. Graph of larval mortality vs time (h): blue, 100 ppm silver-zein particles; orange, 1.0 ppm silver-zein particles; gray, 0.1 ppm silver-zein particles. Five trials of 20 third and fourth instar larvae were exposed to different concentrations of silver-zein insecticidal particles in solution. In addition, treatments of sodium borohydride (0.1M), glycerol (0.1 M), zein (100 ppm), water, and a solution of the combined ingredients of lactic acid (0.1 M), glycerol (0.1 M), and PEG (0.1 M) were recorded. Only sodium borohydride caused a mortality of 8 ± 5%. These control treatments were selected because they were used in the synthesis of the particles.

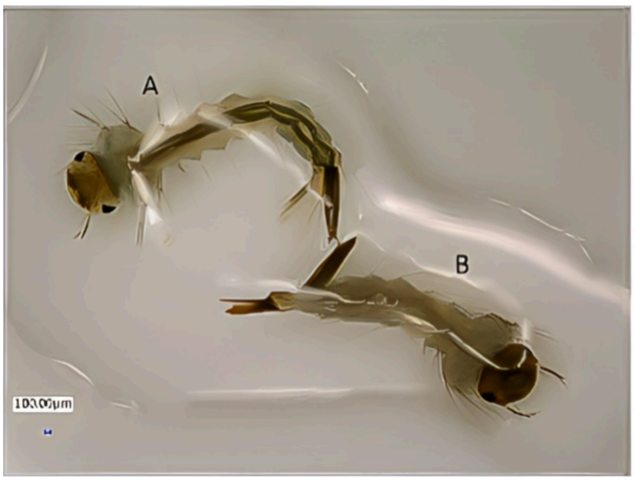


Figure 2. Optical image of mosquito larva (a) nontreated and (b) exposed to 100 ppm silver-zein particles for 1 h. Larvae were randomly selected from a group of 20. The larvae were removed by a pipet and transferred to a microscope slide.

SEM images of the mosquito larva abdomen were recorded for a mosquito larva not exposed to silver-zein particles and a larva exposed to 100 ppm (Figure 3). The nonexposed mosquito showed bacteria inside the midgut (Figure 3a). However, the mosquito exposed to 100 ppm silver-zein particles showed an empty gut. It is possible that the absence of bacteria in the treated mosquito is a result of the silver's antimicrobial properties, and this would delay or halt adult emergence from the pupae, as was observed at 0.1 ppm.

TEM images were recorded on a transverse tissue of a mosquito's gut exposed to 1 ppm silver-zein particles (Figure S5). A silver-zein particle aggregate is seen in the TEM image in the transverse tissue (Figure S5). The size and shape of the particles play a vital role in the reaction of the particles to the substrate.[36] As the size of the nanoparticle decreases, its surface area to volume ratio increases. This results in a greater degree of contact with cells. Nanoparticles with a size of 10−20 nm provide better contact between the cell and the particle,[26,37] which is the size of the silver nanoparticles.

Energy-dispersive spectroscopy (EDAX) measuring the different chemical components in the mosquito gut was conducted on nontreated and treated larvae. The larvae treated with the hybrid particles showed the EDAX peak for silver, while the nonexposed one did not (Table 2). The untreated larval EDAX showed phosphorus and sulfur peaks, while the exposed larvae did not. The absence of the phosphorus and sulfur peaks in the silver nanoparticle larva suggests that the silver could have possibly attacked the sulfhydryl groups of the respiratory system and the phosphorus in the ATP.[38] There was a silver peak of 2.3%, which is to be expected, suggesting that the hybrid particles were in the gut of the mosquito larvae. Metal-based NPs, having antimicrobial effects, can play an important role in pest control, targeting the microbiota of the insect gut, which is essential to the survival of some mosquito species;[39,40] as an example, larvae of *Cx. quinquefasciatus* requires gut bacteria for development and survival.[41,42] The main advantage of the core and coating technology is the ability to change the active ingredient in the coating, which is responsible for larvicidal activity. This will reduce the insecticide resistance through product rotation.

https://doi.org/10.1021/acsomega.3c03210
ACS Omega 2023, 8, 33437−33443

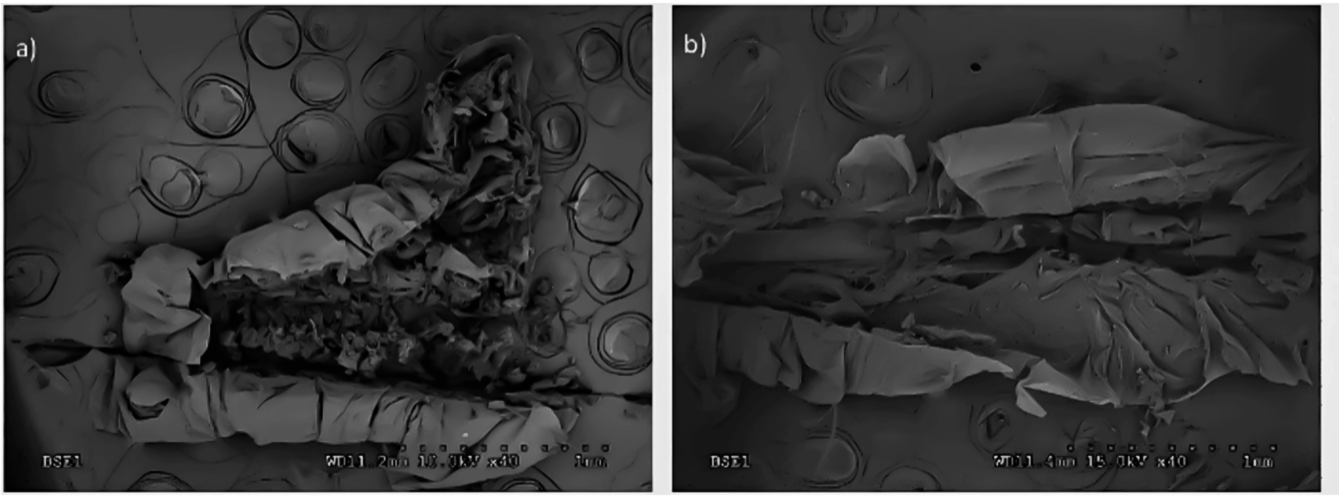


Figure 3. SEM images of (a) nontreated mosquito larva and (b) mosquito larva treated with 100 ppm silver NPs.

Table 2. EDAX of Control Larva and Larva Exposed to Silver-Zein Particles[a]

larva (untreated)			larva (treated)		
element	weight %	atomic %	element	weight %	atomic %
C	58.35	78.54	C	59.32	70.63
O	16.83	17.01	O	28.66	25.62
Na	0.83	0.58	Ca	9.63	3.44
S	2.96	1.49	silver	2.39	0.32
Ca	1.99	0.8			
P	19.04	1.58			

[a]Recorded using energy-dispersive X-ray spectroscopy (EDS) confirming the presence of the silver element using an Oxford EDS detector.

CONCLUSIONS

Zein polymer MPs and silver-zein particles were successfully synthesized, characterized, and utilized as a larvicide for third and fourth instar *Cx. quinquefasciatus*. To demonstrate that hybrid particles were responsible for the mortality, a mosquito gut was characterized and imaged by SEM, TEM, and EDAX. Mosquito larval mortality (90%) was observed at 1.0 ppm after 40 h, which is lower than any previously published value. 0.1 ppm reduced to 5% adult mosquito emergence. The green synthesis used to synthesize the particles also aides in reducing the cost of mass production and later in the environmental impact if the particles are used in wide-area treatments. Nanotechnology and hybrid particles using the core and coating technology can be utilized effectively in an IPM (integrated pest management) program by maintaining the core microparticle and changing the active ingredient coating to reduce the evolution of insecticide resistance.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03210.

(Table S1) Literature comparison of mortality rates, (Figure S1) UV−visible silver-zein NPs, (Figure S2) TEM images of zein particles and silver-zein NPs, (Figure S3) FTIR-ATR of zein particles and silver-zein NPs, (Figure S4) DLS of zein particles and silver-zein NPs, and (Figure S5) TEM of the transverse tissue of a mosquito exposed to silver-zein NPs (PDF)

AUTHOR INFORMATION

Corresponding Authors

Amie E. Norton − *Department of Entomology, Kansas State University, Manhattan, Kansas 66506-0100, United States*; Email: amien@ksu.edu

Jeff Whitworth − *Department of Entomology, Kansas State University, Manhattan, Kansas 66506-0100, United States*; Email: jwhitwor@ksu.edu

Lee W. Cohnstaedt − *National Bio and Agro-Defense Facility-USDA, Manhattan, Kansas 66503, United States*; Email: lee.cohnstaedt@usda.gov

Authors

Robert Ewing − *National Bio and Agro-Defense Facility-USDA, Manhattan, Kansas 66503, United States*

Michael Tilley − *USDA, Agricultural Research Service, Center for Grain and Animal Health Research, Manhattan, Kansas 66502, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c03210

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We want to acknowledge the KSU NICKS center for the use of the SEM, TEM, EDAX, and DLS. We thank Dr. Tej Shrestha for help on the DLS. We would also like to thank Ravindra Thakkar for help with SEM and TEM imaging. We would also like to thank Dr. Dustin Swanson and Bill Yarnell at the USDA CGAHR insectary for their assistance with the *Culex quinquefasciatus* larvae. Finally, we would like to thank Ms. Crystal Ly for designing the TOC art. Mention of trade names or commercial products in this publication is solely to provide specific information. It does not imply recommendation or endorsement by the U.S. Department of Agriculture, or Kansas State University.

ABBREVIATIONS USED

SEM, scanning electron microscopy; TEM, transmission electron microscopy; EDAX, energy-dispersive spectroscopy; NP, nanoparticles; *Culex*, *Cx.*; AgNPs, silver nanoparticles; nm, nanometer; ppm, parts-per-million; AI, active ingredient; MW, molar weight; g, grams; FTIR-ATR, Fourier-transform infrared-attenuated total reflectance; mL, milliliters; DLS, dynamic light scattering; fwhm, full-width at half-maximum; cm, centimeters

REFERENCES

(1) Benelli, G.; Caselli, A.; Canale, A. Nanoparticles for Mosquito Control: Challenges and Constraints. *J. King Saud Univ., Sci.* **2017**, *29* (4), 424−435.

(2) Organization, W. H. *Handbook for Integrated Vector Management*; World Health Organization, 2012.

(3) Rezende-Teixeira, P.; Dusi, R. G.; Jimenez, P. C.; Espindola, L. S.; Costa-Lotufo, L. V. What Can We Learn from Commercial Insecticides? Efficacy, Toxicity, Environmental Impacts, and Future Developments. *Environ. Pollut.* **2022**, *300*, No. 118983.

(4) Chaudhary, V. K.; Arya, S.; Singh, P. Effects of Pesticides in Biodiversity of Climate Change. *Int. J. Environ. Sci.* **2021**, *11* (2), 95.

(5) Moulton, M. C.; Braydich-Stolle, L. K.; Nadagouda, M. N.; Kunzelman, S.; Hussain, S. M.; Varma, R. S. Synthesis, Characterization and Biocompatibility of "Green" Synthesized Silver Nanoparticles Using Tea Polyphenols. *Nanoscale* **2010**, *2* (5), 763−770.

(6) Singh, L.; Kruger, H. G.; Maguire, G. E.; Govender, T.; Parboosing, R. The Role of Nanotechnology in the Treatment of Viral Infections. *Ther. Adv. Infect. Dis.* **2017**, *4* (4), 105−131.

(7) Ullah Khan, S.; Saleh, T. A.; Wahab, A.; Ullah Khan, M. H.; Khan, D.; Ullah Khan, W.; Rahim, A.; Kamal, S.; Ullah Khan, F.; Fahad, S. Nanosilver: New Ageless and Versatile Biomedical Therapeutic Scaffold. *Int. J. Nanomed* **2018**, 733−762.

(8) Thakkar, K. N.; Mhatre, S. S.; Parikh, R. Y. Biological Synthesis of Metallic Nanoparticles. *Nanomedicine* **2010**, *6* (2), 257−262.

(9) Saleh, T. A. Protocols for Synthesis of Nanomaterials, Polymers, and Green Materials as Adsorbents for Water Treatment Technologies. *Environ. Technol. Innov.* **2021**, *24*, No. 101821.

(10) Paliwal, R.; Babu, R. J.; Palakurthi, S. Nanomedicine Scale-up Technologies: Feasibilities and Challenges. *AAPS PharmSciTech* **2014**, *15* (6), 1527−1534.

(11) Dang, F.; Enomoto, N.; Hojo, J.; Enpuku, K. Sonochemical Synthesis of Monodispersed Magnetite Nanoparticles by Using an Ethanol−Water Mixed Solvent. *Ultrason. Sonochem.* **2009**, *16* (5), 649−654.

(12) Niederberger, M.; Pinna, N. *Metal Oxide Nanoparticles in Organic Solvents: Synthesis, Formation; Assembly and Application*; Springer Science & Business Media, 2009.

(13) Davari, S. A.; Gottfried, J. L.; Liu, C.; Ribeiro, E. L.; Duscher, G.; Mukherjee, D. Graphitic Coated Al Nanoparticles Manufactured as Superior Energetic Materials via Laser Ablation Synthesis in Organic Solvents. *Appl. Surf. Sci.* **2019**, *473*, 156−163.

(14) Jaswal, T.; Gupta, J. A Review on the Toxicity of Silver Nanoparticles on Human Health. *Mater. Today: Proc.* **2023**, *81*, 859−863.

(15) Suthar, J. K.; Vaidya, A.; Ravindran, S. Toxic Implications of Silver Nanoparticles on the Central Nervous System: A Systematic Literature Review. *J. Appl. Toxicol.* **2023**, *43* (1), 4−21.

(16) Taylor, J.; Taylor, J. R.; Belton, P. S.; Minnaar, A. Formation of Kafirin Microparticles by Phase Separation from an Organic Acid and Their Characterisation. *J. Cereal Sci.* **2009**, *50* (1), 99−105.

(17) Alder, C. M.; Hayler, J. D.; Henderson, R. K.; Redman, A. M.; Shukla, L.; Shuster, L. E.; Sneddon, H. F. Updating and Further Expanding GSK's Solvent Sustainability Guide. *Green Chem.* **2016**, *18* (13), 3879−3890.

(18) Byrne, F. P.; Jin, S.; Paggiola, G.; Petchey, T. H. M.; Clark, J. H.; Farmer, T. J.; Hunt, A. J.; Robert McElroy, C.; Sherwood, J. Tools and Techniques for Solvent Selection: Green Solvent Selection Guides. *Sustainable Chem. Process.* **2016**, *4* (1), 7.

(19) Suganya, P.; Vaseeharan, B.; Vijayakumar, S.; Balan, B.; Govindarajan, M.; Alharbi, N. S.; Kadaikunnan, S.; Khaled, J. M.; Benelli, G. Biopolymer Zein-Coated Gold Nanoparticles: Synthesis, Antibacterial Potential, Toxicity and Histopathological Effects against the Zika Virus Vector Aedes Aegypti. *J. Photochem. Photobiol., B* **2017**, *173*, 404−411.

(20) Patel, A.; Hu, Y.; Tiwari, J. K.; Velikov, K. P. Synthesis and Characterisation of Zein−Curcumin Colloidal Particles. *Soft Matter* **2010**, *6* (24), 6192−6199.

(21) Zaki, S.; El Kady, M. F.; Abd-El-Haleem, D. Biosynthesis and Structural Characterization of Silver Nanoparticles from Bacterial Isolates. *Mater. Res. Bull.* **2011**, *46* (10), 1571−1576.

(22) Paramelle, D.; Sadovoy, A.; Gorelik, S.; Free, P.; Hobley, J.; Fernig, D. G. A Rapid Method to Estimate the Concentration of Citrate Capped Silver Nanoparticles from UV-Visible Light Spectra. *Analyst* **2014**, *139* (19), 4855−4861.

(23) Haiss, W.; Thanh, N. T. K.; Aveyard, J.; Fernig, D. G. Determination of Size and Concentration of Gold Nanoparticles from UV−Vis Spectra. *Anal. Chem.* **2007**, *79* (11), 4215−4221.

(24) Gschwind, S.; Hagendorfer, H.; Frick, D. A.; Günther, D. Mass Quantification of Nanoparticles by Single Droplet Calibration Using Inductively Coupled Plasma Mass Spectrometry. *Anal. Chem.* **2013**, *85* (12), 5875−5883.

(25) Skillman, D. C.; Berry, C. R. Spectral Extinction of Colloidal Silver*. *J. Opt. Soc. Am., JOSA* **1973**, *63* (6), 707−713.

(26) Wang, H.; Yan, A.; Liu, Z.; Yang, X.; Xu, Z.; Wang, Y.; Wang, R.; Koohi-Moghadam, M.; Hu, L.; Xia, W.; Tang, H.; Wang, Y.; Li, H.; Sun, H.; Sourjik, V. Deciphering Molecular Mechanism of Silver by Integrated Omic Approaches Enables Enhancing Its Antimicrobial Efficacy in E. Coli. *PLOS Biology* **2019**, *176*, No. e3000292.

(27) Dashdorj, U.; Reyes, M. K.; Unnithan, A. R.; Tiwari, A. P.; Tumurbaatar, B.; Park, C. H.; Kim, C. S. Fabrication and Characterization of Electrospun Zein/Ag Nanocomposite Mats for Wound Dressing Applications. *Int. J. Biol. Macromol.* **2015**, *80*, 1−7.

(28) Velayutham, K.; Rahuman, A. A.; Rajakumar, G.; Roopan, S. M.; Elango, G.; Kamaraj, C.; Marimuthu, S.; Santhoshkumar, T.; Iyappan, M.; Siva, C. Larvicidal Activity of Green Synthesized Silver Nanoparticles Using Bark Aqueous Extract of Ficus Racemosa against Culex Quinquefasciatus and Culex Gelidus. *Asian Pac. J. Trop. Med.* **2013**, *6* (2), 95−101.

(29) Zhang, B.; Luo, Y.; Wang, Q. Development of Silver− Zein Composites as a Promising Antimicrobial Agent. *Biomacromolecules* **2010**, *11* (9), 2366−2375.

(30) Parris, N.; Cooke, P. H.; Hicks, K. B. Encapsulation of Essential Oils in Zein Nanospherical Particles. *J. Agric. Food Chem.* **2005**, *53* (12), 4788−4792.

(31) Hu, K.; Huang, X.; Gao, Y.; Huang, X.; Xiao, H.; McClements, D. J. Core−Shell Biopolymer Nanoparticle Delivery Systems: Synthesis and Characterization of Curcumin Fortified Zein−Pectin Nanoparticles. *Food Chem.* **2015**, *182*, 275−281.

(32) Argos, P.; Pedersen, K.; Marks, M. D.; Larkins, B. A. A Structural Model for Maize Zein Proteins. *J. Biol. Chem.* **1982**, *257* (17), 9984−9990.

(33) Shanmugasundaram, T.; Balagurunathan, R. Mosquito Larvicidal Activity of Silver Nanoparticles Synthesised Using Actinobacterium, Streptomyces Sp. M25 against Anopheles Subpictus, Culex Quinquefasciatus and Aedes Aegypti. *J. Parasit Dis.* **2015**, *39* (4), 677−684.

(34) Eugin Amala, V.; Krishnaveni, R. Biogenic Synthesis of Silver Nanoparticles: Characterizations, Antibacterial and Larvicidal Bioassay. *Mater. Today: Proc.* **2022**, *49*, A7−A11.

(35) Sap-Iam, N.; Homklincha, C.; Larpudomle, R.; Warisnoich, W.; Sereemaspu, A.; Dubas, S. T. UV Irradiation-Induced Silver Nanoparticles as Mosquito Larvicides. *J. Appl. Sci.* **2010**, *10* (23), 3132−3136.

(36) Durán, N.; Durán, M.; De Jesus, M. B.; Seabra, A. B.; Fávaro, W. J.; Nakazato, G. Silver Nanoparticles: A New View on Mechanistic

https://doi.org/10.1021/acsomega.3c03210
ACS Omega 2023, 8, 33437−33443

Aspects on Antimicrobial Activity. *Nanomedicine* **2016**, *12* (3), 789−799.
(37) Sondi, I.; Salopek-Sondi, B. Silver Nanoparticles as Antimicrobial Agent: A Case Study on E. Coli as a Model for Gram-Negative Bacteria. *J. Colloid Interface Sci.* **2004**, *275* (1), 177−182.
(38) Schreurs, W.; Rosenberg, H. Effect of Silver Ions on Transport and Retention of Phosphate by Escherichia Coli. *J. Bacteriol.* **1982**, *152* (1), 7−13.
(39) McMeniman, C. J.; Lane, R. V.; Cass, B. N.; Fong, A. W.; Sidhu, M.; Wang, Y.-F.; O'Neill, S. L. Stable Introduction of a Life-Shortening Wolbachia Infection into the Mosquito Aedes Aegypti. *Science* **2009**, *323* (5910), 141−144.
(40) Ricci, I.; Valzano, M.; Ulissi, U.; Epis, S.; Cappelli, A.; Favia, G. Symbiotic Control of Mosquito Borne Disease. *Pathog. Global Health* **2012**, *106* (7), 380−385.
(41) Valzania, L.; Martinson, V. G.; Harrison, R. E.; Boyd, B. M.; Coon, K. L.; Brown, M. R.; Strand, M. R. Both Living Bacteria and Eukaryotes in the Mosquito Gut Promote Growth of Larvae. *PLoS Neglected Trop. Dis.* **2018**, *12* (7), No. e0006638.
(42) Pennington, M. J.; Prager, S. M.; Walton, W. E.; Trumble, J. T. Culex Quinquefasciatus Larval Microbiomes Vary with Instar and Exposure to Common Wastewater Contaminants. *Sci Rep.* **2016**, *6* (1), 1−9.

https://doi.org/10.1021/acsomega.3c03210
ACS Omega 2023, 8, 33437−33443