ISSN: 2644-2957
DOI: 10.33552/OJCAM.2019.02.000531
Online Journal of Complementary & Alternative Medicine
Iris Publishers

Short Communication

Potential Nematicidial Activity of Silver Nanoparticles Against the Root-Knot Nematode (Meloidogyne Incognita)

Gregory C. Bernard[1]*, Jacob Fitch[1], Byungjin Min[2], Naresh Shahi[2], Marceline Egnin[1], Inocent Ritte[1], Willard E Collier[3], and Conrad Bonsi[1]

[1]*Department of Agriculture & Environmental Sciences, USA*

[2]*Department of Food and Nutritional Sciences, Tuskegee University, USA*

[3]*Department of Chemistry, Tuskegee University, USA*

***Corresponding author:** Gregory C Bernard, Department of Agriculture & Environmental Sciences, USA.

Received Date: August 16, 2018

Published Date: September 11, 2019

Abstract

Plant-parasitic nematodes (PPNs) are incredibly damaging pests, which cause significant losses in crop yields worldwide. One of the most prevalent PPNs is the root-knot nematode (*Meloidogyne* spp.) ranks number one on the most economically devastating list of pests and thus scientifically important PPNs. Recently, the use of chemical nematicides for root-knot nematode management has decreased due to governmental restrictions; which necessitates the development and identification of alternative pest management procedures. In this study, we evaluated the use of silver nanoparticles (AgNPs) as a potential biopesticide under *in-vitro* conditions. AgNPs were synthesized utilizing a naturally occurring biopolymer (chitosan) as a reducing agent through microwave irradiation. When J2-stage nematodes were exposed to 0.0005 µg of AgNPs for 1 min, significant mortality ($P \leq 0.01$) was observed and approximately 100% of nematodes became inactive within 24 and 48 hrs. Our preliminary study has demonstrated a potential environmentally friendly alternative for the management of the root-knot nematodes.

Keywords: Root-knot nematode, Silver nanoparticles, Biopesticide, Disease, Plants

Introduction

Each year, plant-parasitic nematodes (PPNs) cause over $100 billion in damages to crops [1]. The most scientifically and economically essential PPNS include cyst nematodes (*Heterodera and Globodera* spp.) root-lesion nematodes (*Pratylenchus* spp.); the burrowing nematode *Radopholus similes*; Ditylenchus dipsaci, and root-knot nematodes (*Meloidogyne* spp.) which ranks number one [2]. The use of chemical pesticides typically maintains nematode control; however, the overuse of such chemical agents has resulted in a negative environmental impact Sande D, et al. [3] thus, alternative methods of nematode control which are environmentally friendly and cost-effective are of great interest to researchers and plant breeders. Silver nanoparticles (AgNPs) are a well-known example of nano-sized materials that have been applied as a means of controlling human pathogenic microbes. AgNPs possess antimicrobial effects and have been frequently used in the field of medicine [4]. Recently, nanotechnology has been successfully applied to pest management in crops Chhipa H [5] and the use of silver nanoparticles (AgNPs) has been shown to demonstrate anti-nematode effects Cromwell WA, et al. [6] Silver nanoparticles are often synthesized employing harsh chemicals including sodium borohydride (NaBH4) as a primary reductant [7]. In this objective, we seek to develop AgNP pesticides from reducing agents which are natural biological polymers to potentially mitigate damages caused by root-knot nematodes. Overall, the development of such biopesticides may provide an alternative means of nematode control that are environmentally friendly and as efficacious as the conventional high–risk synthetic pesticides commonly used.

Materials and Methods

Silver nanoparticle synthesis:

Method: AgNPs were synthesized by microwave technique Seku K, et al. [8] using low molecular weight chitosan as a reducing agent. Chitosan is derived from chitin, a naturally occurring polymer of N-acetylglucosamine , found in the exoskeleton of crustaceans and in the cell wall of fungi. Field Emission Scanning Electron Microscopy (FE-SEM) images analysis showed that the

size and shape of AgNPs in nanoscale and spherical, which were homogeneously dispersed into the biopolymer matrix (chitosan) (Figures 1&2). Chitosan-derived AgNPs were homogenized in 10 ml 1% Lactic acid for a final working solution of 1mL of 1% lactic acid containing 0.1 ug of AgNPs and 0.01gram of chitosan Chitosan-derived AgNPs were solubilized in 10 ml 1% Lactic acid for treatment.

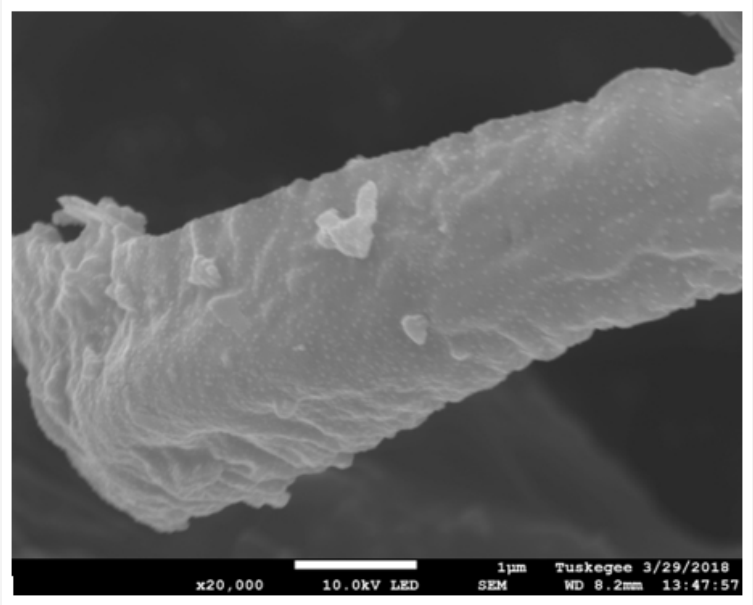

Figure 1: FE-SEM images of homogeneously dispersed AgNPs into Chitosan matrix at 20, 000 X.

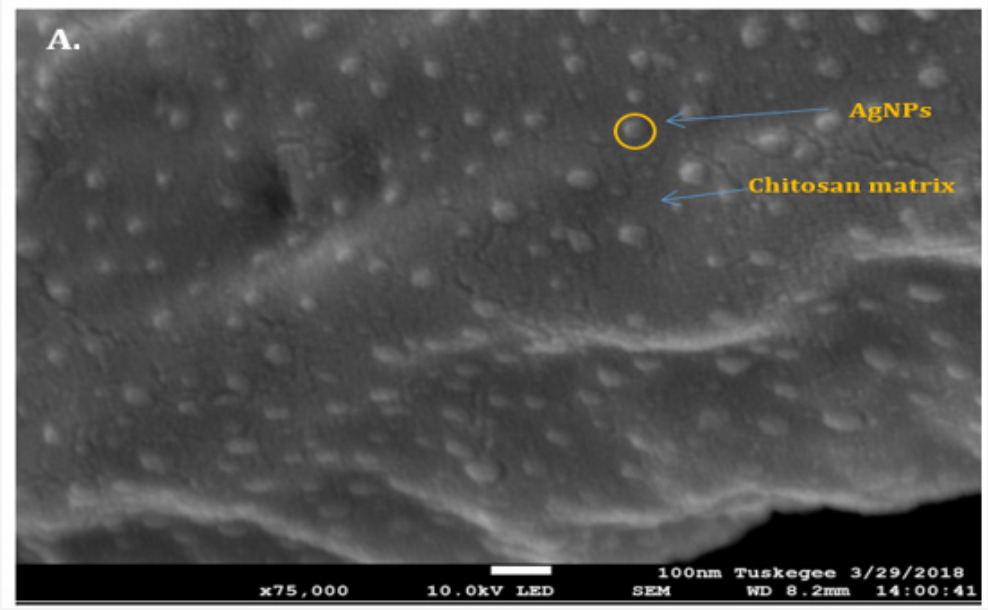

Figure 2: FE-SEM images of homogeneously dispersed AgNPs into Chitosan matrix at 75, 000 X.

Nematode collection and preparation

Meloidogyne incognita race three juveniles were collected from infested soil and suspended in 1ml of sterile tap water. Approximately 100-160 nematodes were placed in 1 ml microcentrifuge tubes and treated with 0.0005 ug AgNPs suspended in 0.05 mg chitosan/0.005% lactic acid at 1min, 24 hr and 48 hr intervals, incubated at 28°C. Nematodes treated with 0.05 mg ug of chitosan and 0.005% lactic acid served as controls. The total number of living and dead nematodes from treated and control groups were quantitated using a gridded nematode counting dish and each time interval replicated three independent times. Dead nematodes were confirmed by fine needle probing [1]. Due to the dramatic difference in nematode numbers between treated control groups, an ANOVA Single Factor analysis ($P \leq 0.05$) was used to identify significant differences in the mean number of living and dead nematodes between groups.

Results

After AgNPs treatment, dead nematodes exhibited the typical "banana shape" curvature predominately displayed in treated groups (Figures 3,4). At the 1-min exposure interval, a significant difference($P < 0.01$) in living and dead nematodes between treated and control groups was observed(Figure 5). The 24 and 48 hr AgNPs exposure groups showed a 100% mortality in comparison to the controls (Figures 6,7).

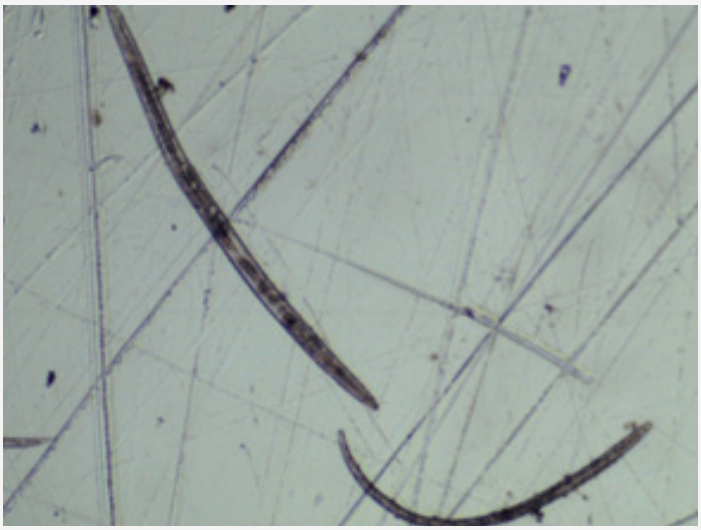

Figure 3: Second-stage *M. incognita* root-knot nematodes from AgNPs exposed groups..

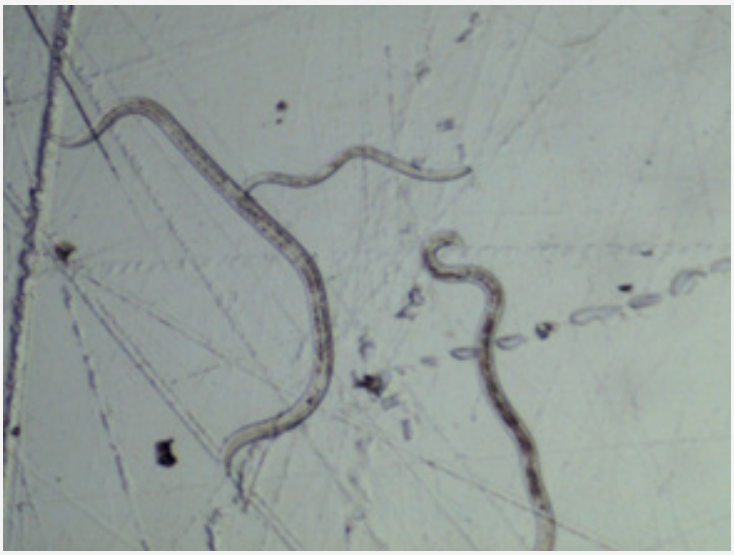

Figure 4: Second-stage *M. incognita* root-knot nematodes from control groups.

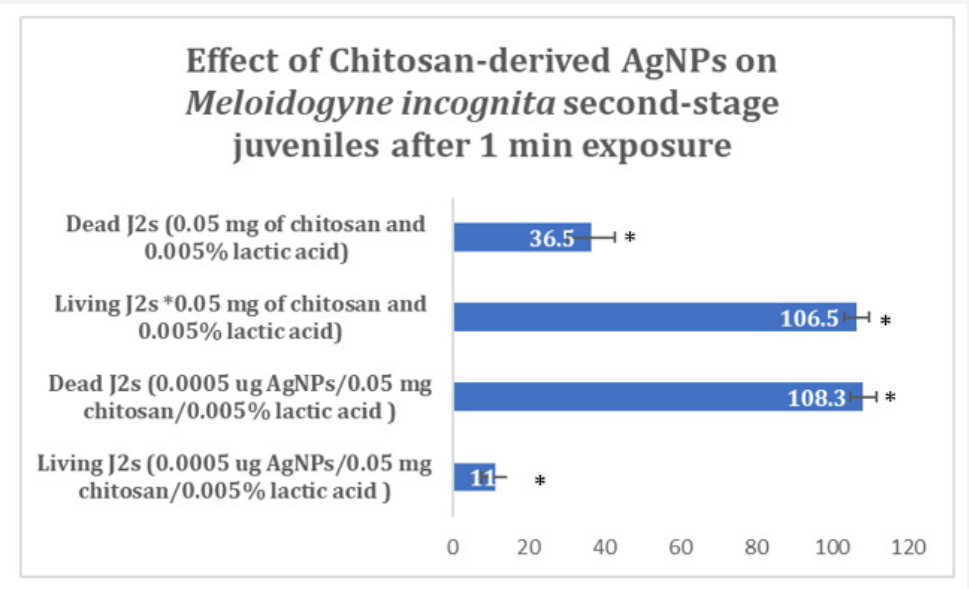

Figure 5: Effect of Chitosan-derived AgNPs on *Meloidogyne incognita* second stage juveniles after 1 min exposure.

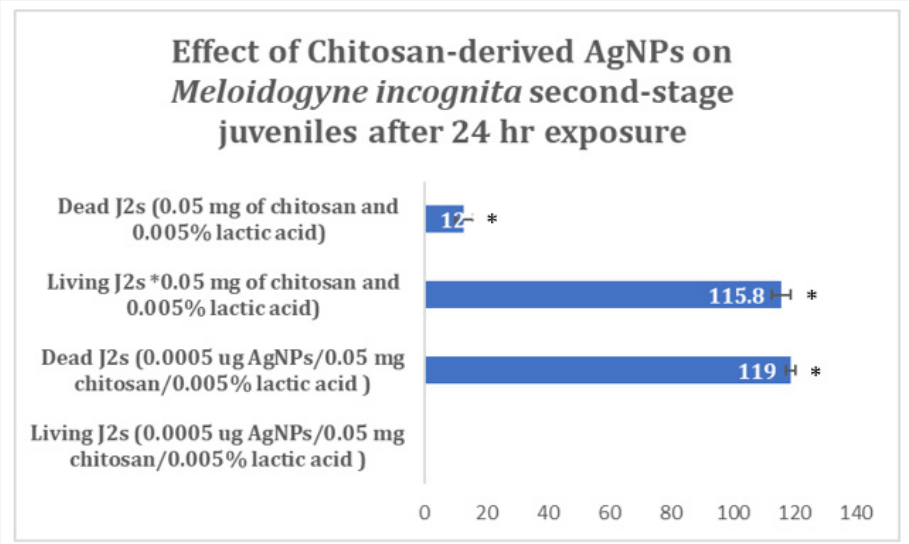

Figure 6: Effect of Chitosan-derived AgNPs on *Meloidogyne incognita* second stage juveniles after 24 hr exposure.

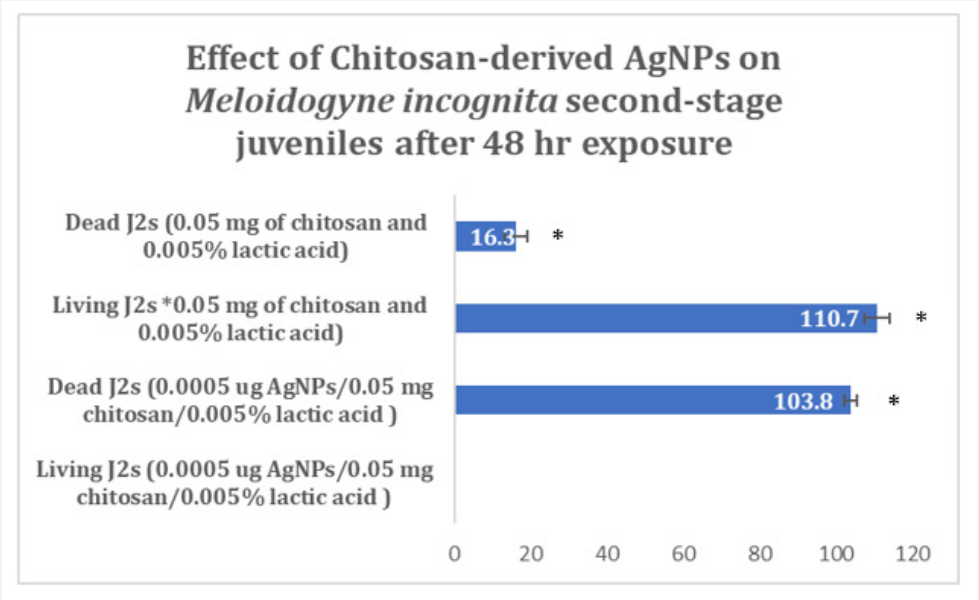

Figure 7: Effect of Chitosan-derived AgNPs on *Meloidogyne incognita* second stage juveniles after 48 hr exposure.

Conclusion

Our study has shown a potential nematicidial effect of Chitosan-derived AgNPs on root-knot nematodes. Interestingly, different molecular weights of chitosan has shown nematicidal activity on root-knot nematodes [10]. Although the antimicrobial mechanism of AgNPs on microorganisms has been poorly elucidated, the harmful impact of AgNPs may be correlated to the development of free radicals from the silver surface leading to an increase in oxidative stress and membrane damage [11]. Interestingly, in response to the pathogen invasion, plants regulate the expression of free radicals during defense signaling pathway activation [12]. Future experiments will include concentration optimizations of AgNPs and its reducing agent potentials prior to applying to greenhouse and field trials for efficacy determination on nematode burdens during plant cultivation [13].

Acknowledgment

SupportedbyUSDAUSDA-NIFA1890Grant#2014-38821-22448; Tuskegee University George Washington Carver Agricultural Experiment Station (GWCAES), and USDA NIFA-EVANS-ALLEN.

Conflict of interest

No conflict of interest.

References

1. Abad P, Gouzy J, Aury J, Castagnone Sereno P, Danchin E, et al. (2008) Genome sequence of the metazoan plant-parasitic nematode Meloidogyne incognita. Nat Biotechnol 26(8): 909-15.
2. Jones J, Haegeman A, Danchin E, Gaur H, Helder J, et al. (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14(9): 946-961.
3. Sande D, Mulle J, Wetzstein M, Houston J (2011) Environmental Impacts from Pesticide Use: A Case Study of Soil Fumigation in Florida Tomato Production. Int J Environ Res Public Health 8(12): 4649-4661.

4. Xiang Y, Li J, Liu X, Cui Z, Yang X et al. (2017) Construction of poly (lactic-co-glycolic acid)/ZnO nanorods/Ag nanoparticles hybrid coating on Ti implants for enhanced antibacterial activity and biocompatibility. Mater Sci Eng C Mater Biol Appl 79: 629-637.

5. Chhipa H (2017) Nanofertilizers and nanopesticides for agriculture. Environmental Chemistry Letters 15(1): 15-22.

6. Cromwell WA, Yang J, Starr JL, Jo YK (2014) Nematicidal Effects of Silver Nanoparticles on Root-knot Nematode in Bermudagrass. J Nematol 46(3): 261-266.

7. Bernard Gregory C, Egnin Marceline, Bonsi Conrad, Mortley Desmond, Witola H William, et al. (2017) Evaluation of root-knot nematode resistance in sweetpotato. African Journal of Agricultural Research. 12(16): 1411-1414.

8. Agnihotri S, Mukherji, Mukherji S (2014) Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. RSC Advances 4(8): 3974–3983.

9. Seku K, Gangapuram BR, Pejjai B (2018) Microwave-assisted synthesis of silver nanoparticles and their application in catalytic, antibacterial and antioxidant activities. J Nanostruct Chem 8: 179.

10. Abbasi WM, Ahmed N, Zaki JM, Shaukat SS (2008) Effect of *Barleria acanthoides* Vahl. on root-knot nematode infection and growth of infected okra and brinjal plants. Pak J Bot 40(5): 2193-2198

11. Khalil MS, Badawy MEI (2012) Nematicidal activity of a biopolymer chitosan at different molecular weights against root-knot nematode, Meloidogyne incognita. Plant Protect Sci 48(4): 170-178.

12. Phaniendra A, Jestadi D, Periyasamy L, (2015) Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem 30(1): 11-26.

13. Jose AO Brien, Arsalan Daudi, Vernon S Butt, Paul Bolwell G (2012) Reactive oxygen species and their role in plant defence and cell wall metabolism. Planta 236(3): 765-779.