NON-LETHAL EFFECTS *N sativa* SILVER NANOPARTICLES ON EARTHWORMS: A BASIC TOXICITY STUDY USING *Eisenia fetida*

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Abstract
This study evaluates the basic physiological responses of Eisenia fetida to Nigella sativa-derived silver nanoparticles (AgNPs) in soil, with emphasis on visible morphology, body coloration, and weight change. Earthworms were exposed to varying concentrations of N. sativa AgNPs under controlled conditions to simulate realistic agricultural soil amendments. Results showed no significant morphological deformities across all treatment groups, indicating physical tolerance to the nanoparticle exposure. Body coloration remained consistent, with no bleaching, darkening, or lesion development observed, suggesting minimal external stress indicators. These findings suggest that at sublethal levels, AgNPs may not induce immediate harmful effects on E. fetida's basic health indicators. The study supports the cautious use of AgNPs in agricultural contexts while encouraging further investigation into long-term and sublethal effects.
Keywords: *Silver nanoparticles; Perplexity Assessment; Eisenia fetida; Complex toxicity; Nanoecotoxicology*

Introduction

The advancement of nanotechnology has led to the widespread application of silver nanoparticles (AgNPs) in agriculture due to their potent antimicrobial and antifungal properties (Pulit-Prociak & Banach, 2016). Among various synthesis methods, the green synthesis of AgNPs using plant extracts such as *Nigella sativa* has gained increasing attention for its eco-friendly, cost-effective, and biocompatible nature (Ahmed et al., 2016). These biologically synthesized nanoparticles are being investigated for use in crop protection, fertilizer enhancement, and pathogen control (Mishra et al., 2017). However, their release into agricultural soils raises important ecological concerns, particularly regarding their interactions with soil-dwelling organisms (Tourinho et al., 2012).

Eisenia fetida, commonly known as the red wiggler earthworm, is a well-established model organism in soil ecotoxicology due to its high sensitivity to pollutants and critical role in soil health and nutrient cycling (OECD, 2008; Spurgeon et al., 2003). As such, understanding its response to AgNP exposure is essential for evaluating environmental safety. While many studies have focused on biochemical or genotoxic endpoints (Garcia-Velasco et al., 2011; Schlich et al., 2013), basic physiological indicators such as morphology, skin coloration, and body weight offer valuable early insight into nanoparticle-induced stress (Shoults-Wilson et al., 2011).

This study investigates the effects of *N. sativa*-derived AgNPs on *E. fetida* by assessing external morphology, body pigmentation, and changes in biomass under controlled soil exposure conditions. By focusing on these fundamental yet informative parameters, the research aims to provide a straightforward assessment of sublethal nanoparticle toxicity relevant to real-world agricultural applications. The findings contribute to the broader understanding of nanoecotoxicology and inform guidelines for the safe use of nanomaterials in sustainable farming systems.

Methodology

a) Synthesis and Characterization of *N. sativa* AgNPs

Synthesis: Silver nanoparticles (AgNPs) were synthesized using *Nigella sativa* seed extract as a green reducing and stabilizing agent; briefly, 10 mL of aqueous *N. sativa* extract was added to 90 mL of 1 mM silver nitrate ($AgNO_3$) solution under continuous stirring at 60°C for 2 hours. The color change from pale yellow to dark brown indicated the reduction of Ag^+ ions and the formation of AgNPs.

Characterization

UV-Vis Spectroscopy: The synthesized AgNPs exhibited a distinct surface plasmon resonance (SPR) peak at approximately 420 nm, confirming the successful formation of nanoparticles.

Scanning Electron Microscopy (SEM): SEM analysis revealed that the AgNPs possessed a spherical morphology with a narrow size distribution ranging from 28 to 40 nm

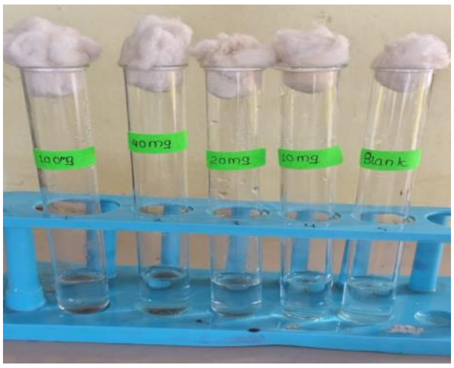
b) Preparation of Soil Substrate

A standardized soil substrate was prepared by thoroughly mixing the following components per treatment unit: cocopeat (10 g), cow dung cake (dried and powdered, 50 g), calcium carbonate ($CaCO_3$, 1 g), and used tea powder (1 g). Moisture content was adjusted to 60–70% using distilled water, following soil preparation methods described by Suthar, (2009) and Sharma et al., 2017. The mixture was homogenized thoroughly and placed into individual plastic containers to form the experimental bedding.

c) Experimental Design and Treatment Setup

Each container was treated with silver nanoparticles (AgNPs) at varying concentrations. Five treatment groups were established as follows:

The required amount of AgNPs was dissolved in 5 mL of distilled water and uniformly added to each container according to its treatment group. The control (blank) group received only 5 mL of distilled water without nanoparticles. Each container was appropriately labeled. The method was adapted from nanoparticle soil exposure protocols described by Schlich et al., (2013) and Tourinho et al., (2012).



Concentration of nanoparticle

Vermicompost bed at different concentration

Earthworm Exposure

Adult *Eisenia fetida* earthworms of similar size and physiological condition were selected based on OECD guidelines for soil organism testing OECD (2008). Each earthworm was weighed individually prior to exposure and introduced into the containers (10 worm per container unless stated otherwise).

Incubation and Monitoring

All containers were incubated at room temperature (20–25°C) under controlled laboratory conditions for a period of 7 days, in accordance with methods outlined in Shoults-Wilson et al., (2011). Moisture content was monitored daily and maintained at 60–70%. No supplementary feeding was provided during the experimental period.

Post-Exposure Analysis

After 7 days of exposure, earthworms were carefully removed, rinsed with distilled water, and reweighed to assess any weight change. Morphological and physiological assessments were conducted, which included: Observation of external deformities or lesions, Changes in body coloration (e.g., bleaching, darkening), Activity level and responsiveness, Survival status.

These health indicators were documented and compared across all treatment groups to assess sublethal effects of AgNPs, based on parameters used by Garcia-Velasco et al., (2011) and Loureiro et al., (2010).

Result:

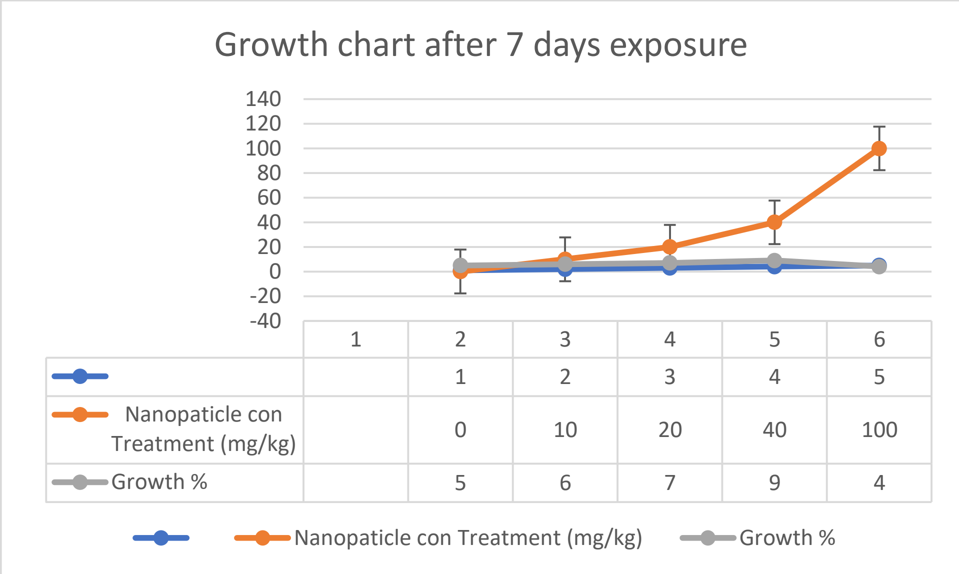
Weight Gain and Growth Rate

Earthworms (*Eisenia fetida*) exhibited varying degrees of weight gain across all treatment groups following 7 days of exposure to *Nigella sativa*-derived silver nanoparticles (AgNPs). The weight gain and percentage growth are summarized in Table 1.

Table 1. Weight changes of *E. fetida* exposed to different concentrations of *N. sativa* AgNPs in soil.

Treatment (mg/kg)	Volume of AgNP Solution	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Growth (%)
0 (Blank)	5 mL	8.40	8.82	0.42	5%
10	5 mL	9.27	9.83	0.56	6%
20	5 mL	9.23	9.88	0.65	7%
40	5 mL	9.13	9.96	0.83	9%
100	5 mL	8.88	9.24	0.36	4%

Weight gain was observed in all groups, indicating general physiological tolerance. The highest growth rate (9%) was recorded in the 40 mg/kg group, while the lowest (4%) was in the 100 mg/kg group, suggesting a possible decline in nutrient assimilation or stress at the highest concentration.



Coloration, Activity, and Morphological Health

Physical and behavioural responses of the earthworms were assessed post-exposure. Observations are presented in **Table 2**.

Table 2. Morphological and behavioural responses of *E. fetida* to AgNP treatments.

Morphological and Physiological Observations

Concentration (mg/kg)	Colour Consistency	Burrowing Score (0-5)	Morphological Abnormalities
0 (Control)	Normal	4	None
10	Normal	4	None
20	Normal	3	None
40	Normal	2	None
100	Slight pale discoloration	1	Mild segmental swelling

- Colour consistency was maintained across most treatments, with only the 100 mg/kg group showing slight pale discoloration, suggesting early signs of physiological stress.
- Burrowing activity declined with increasing concentration, from a score of 4 (active burrowing) in the control and 10 mg/kg groups to 1 (minimal burrowing) in the 100 mg/kg group, indicating possible sublethal behavioral effects.
- Morphological abnormalities were only observed at 100 mg/kg, including mild segmental swelling, potentially due to irritation or nanoparticle accumulation.

Conclusion:

The study demonstrates that short-term (7-day) exposure of *Eisenia fetida* to *Nigella sativa*-derived silver nanoparticles in soil resulted in no significant mortality or severe morphological damage across concentrations up to 100 mg/kg. All groups showed positive weight gain, with the 40 mg/kg treatment resulting in the highest growth. However, behavioural changes (reduced burrowing) and mild morphological effects (discoloration, swelling) were noted at 100 mg/kg, suggesting that higher concentrations may induce early sublethal stress responses.

These results indicate that biosynthesized AgNPs at low to moderate levels (≤40 mg/kg) can be tolerated by *E. fetida* without major physiological disruption, supporting their cautious use in agricultural soil amendments. However, at higher concentrations (100 mg/kg), signs of physiological stress emerge, highlighting the need for further long-term, chronic, and reproductive toxicity studies to evaluate ecological safety.

References

1. Shoults-Wilson, W. A., Reinsch, B. C., Tsyusko, O. V., Bertsch, P. M., Lowry, G. V., & Unrine, J. M. (2011). Role of particle size and soil type in toxicity of silver nanoparticles to earthworms. Soil Biology and Biochemistry, 43(8), 1831–1836.
2. Garcia-Velasco, N., Gandariasbeitia, M., & Soto, M. (2011). Avoidance behavior and oxidative stress in earthworms (Eisenia fetida) exposed to silver nanoparticles. Ecotoxicology and Environmental Safety, 74(3), 530–537.
3. Tourinho, P. S., van Gestel, C. A., Lofts, S., Svendsen, C., Soares, A. M. V. M., & Loureiro, S. (2012). Metal-based nanoparticles in soil: Fate, behavior, and effects on soil invertebrates.

Environmental Toxicology and Chemistry, 31(8), 1679–1692.
4. Schlich, K., Klawonn, T., Terytze, K., & Hund-Rinke, K. (2013). Hazard assessment of a silver nanoparticle in soil applied via sewage sludge. Environmental Sciences Europe, 25(1), 17.
5. Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *Journal of Advanced Research*, 7(1), 17–28.
6. Garcia-Velasco, N., Gandariasbeitia, M., & Soto, M. (2011). Avoidance behavior and oxidative stress in earthworms (*Eisenia fetida*) exposed to silver nanoparticles. *Ecotoxicology and Environmental Safety*, 74(3), 530–537.
7. Mishra, V., Sharma, R., & Baranwal, D. (2017). Plant-mediated green synthesis of silver nanoparticles and their applications. In R. Baranwal & S. Srivastava (Eds.), *Green nanomaterials* (pp. 23–45). Springer.
8. OECD. (2008). *Earthworm reproduction test (Eisenia fetida/Eisenia andrei)*. OECD Guidelines for the Testing of Chemicals, Section 222.
9. Pulit-Prociak, J., & Banach, M. (2016). Silver nanoparticles – A material of the future...? *Open Chemistry*, 14(1), 76–91.
10. Schlich, K., Klawonn, T., Terytze, K., & Hund-Rinke, K. (2013). Hazard assessment of a silver nanoparticle in soil applied via sewage sludge. *Environmental Sciences Europe*, 25(1), 17.
11. Shoults-Wilson, W. A., Reinsch, B. C., Tsyusko, O. V., Bertsch, P. M., Lowry, G. V., & Unrine, J. M. (2011). Role of particle size and soil type in toxicity of silver nanoparticles to earthworms. *Soil Biology and Biochemistry*, 43(8), 1831–1836.
12. Spurgeon, D. J., Svendsen, C., Weeks, J. M., Hankard, P. K., Stubberud, H. E., Kammenga, J. E., & Krogh, P. H. (2003). Quantifying copper and cadmium impacts on intrinsic rate of population increase in the terrestrial oligochaete *Eisenia fetida*. *Environmental Toxicology and Chemistry*, 22(6), 1465–1472.
13. Tourinho, P. S., van Gestel, C. A., Lofts, S., Svendsen, C., Soares, A. M. V. M., & Loureiro, S. (2012). Metal-based nanoparticles in soil: Fate, behavior, and effects on soil invertebrates. *Environmental Toxicology and Chemistry*, 31(8), 1679–1692.