Eurasia Specialized Veterinary Publication

Entomology Letters

ISSN:3062-3588

2021, Volume 1, Issue 1, Page No: 1-7

Available online at: www.esvpub.com/

Investigating the Insecticidal Properties of Titanium Oxide Nanoparticles against *Periplaneta Americana*

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ABSTRACT

The *Periplaneta Americana*, an important household insect, plays a crucial role in transmitting various pathogens in the environment. Traditional methods of controlling this pest have often been ineffective and have led to the development of resistance. This study aimed to evaluate the lethal effects of titanium oxide nanoparticles, both by contact and ingestion, on *Periplaneta Americana*. Different concentrations of nanoparticles were prepared for oral and contact applications. The insects were exposed to these treatments with varying nanoparticle concentrations across three repetitions. The mean mortality rates among the groups were analyzed using a one-way analysis of variance and Duncan's test. In addition, the mortality rates observed on different days were compared using repeated measures analysis of variance. The oral application of titanium oxide nanoparticles showed higher efficacy than the contact method at concentrations of 15% and 21% across all days, while the contact method was more effective than the oral method at a 25% concentration, except on the seventh day. Overall, the oral treatment showed better efficacy than the contact treatment. As the concentration increased and over time, the mortality rate of *Periplaneta Americana* increased significantly with both treatment methods. These findings suggest that the use of titanium dioxide nanoparticles, in combination with existing control methods, could provide a promising approach to managing *Periplaneta Americana* infestations.

Keywords: Insecticidal effect, Pathogens, Nanoparticles, *Periplaneta americana*, Titanium oxide

Received: 09 February 2021
Revised: 03 March 2021
Accepted: 05 March 2021

How to Cite This Article: Gutiérrez-Ramírez JA, Betancourt-Galindo R, Aguirre-Uribe LA, Cerna-Chávez E, Sandoval-Rangel A, Ángel ECD, et al. Investigating the Insecticidal Properties of Titanium Oxide Nanoparticles against *Periplaneta Americana*. Entomol Lett. 2021;1(1):1-7. https://doi.org/10.51847/KBGR8yk5MT

Introduction

Arthropods, particularly insects, are among the most significant pests affecting humans. These organisms have successfully adapted to a wide range of ecosystems due to their advantageous morphological and physiological traits, small size, rapid mobility, and high reproductive capacity. Arthropods create various problems for humans, both directly through stings and bites and indirectly by transmitting infectious diseases. Some, such as termites, are considered economic and structural pests, while others, including cockroaches, flies, mosquitoes, bedbugs, ticks, and scorpions, are classified as health-related pests [1, 2]. These pests are commonly found in areas with moderate to hot climates. One of the most recognized and widespread household pests, the *Periplaneta Americana*, or American cockroach, primarily inhabits sewage systems and spreads to residential areas, especially those linked to sewage networks. From an ecological perspective, cockroaches play an essential role in the nitrogen cycle of nature [3, 4].

Cockroaches are known to feed on unsanitary materials, decaying food, stored food, and various types of household food. Their feeding habits contribute to the spread of diseases including staphylococcal infections, salmonella, giardiasis, *Escherichia coli* infections, and trichomonas, among others. Multiple pathogenic species, including protozoa, bacteria, fungi, viruses, and parasitic worms, have been identified in the American cockroach. Research has found that 11 different types of bacteria inhabit the American cockroach's body. Cockroach control remains a significant challenge worldwide. These pests typically reside in sewage and waste collection systems, where they breed in large numbers—sometimes over 5,000 in a single inlet. Besides the nuisance they cause by spreading their feces, their connection to excrement and fecal diseases, as well as their ability to infiltrate human homes via sewage pipes, poses health risks in contaminated areas [5, 6].

In recent years, the use of nanoparticles has become increasingly prevalent in both domestic and industrial applications. These particles exhibit unique physical and chemical properties because of their small size, high surface-to-volume ratio, and size-dependent visual traits. Metal nanoparticles have diverse uses in biotechnology, clinical diagnostics and treatments, water and wastewater treatment, food safety, and as insecticides and pesticides. Before predicting the toxicity of nanoparticles, it is crucial to understand the factors and characteristics that influence their toxicity. The interaction of these particles with the animals, the human body, and the environment determines their effects. As nanoparticles decrease in size, their surface-to-volume ratio increases exponentially, making them more toxic and active. Smaller particles also have a higher likelihood of penetrating plant and animal tissues. The ability of nanoparticles to cross cell barriers depends significantly on their size. It is noted that particles smaller than 35 nm can cross the blood-brain barrier, particles smaller than 41 nm can enter cell nuclei, and particles smaller than 111 nm can pass through cell membranes. Additionally, the size of the nanoparticles affects the stability of their binding to protein structures and their durability after binding [7-9].

Silver nanoparticles have been found to cause cytotoxicity in animal and human cells through the release of silver ions into the cellular environment, with the extent of toxicity being size-dependent. Studies indicate that silver nanoparticles can lead to various toxic effects, such as inflammation, cytotoxicity, genotoxicity, and developmental toxicity, depending on their size [10]. Titanium dioxide nanoparticles, particularly those smaller than eleven nm, have been shown to cause immunotoxicity in mice, with varying levels of pulmonary toxicity based on particle size. As nano-based products become more prevalent, there is a growing need to evaluate their potential toxicological effects on the environment, animals, and humans [11].

Zorlu *et al*. [12] examined the impact of various concentrations of titanium dioxide nanoparticles on total protein levels, biological markers, antioxidant enzyme activities, and malondialdehyde levels in the hemolymph of *Galleria mellonella* (L.). Their findings revealed that larval and pupal development times were significantly longer at concentrations of one hundred, five hundred, one thousand, and three thousand parts per million compared to the control, with the highest titanium dioxide dose having the most pronounced effect. At lower concentrations (one hundred, five hundred, and one thousand ppm), the adult lifespan was shortened. Additionally, exposure to titanium dioxide nanoparticles led to increased total protein and malondialdehyde levels, as well as enhanced glutathione S-transferase activity in hemolymph at the tested concentrations, compared to the control and other doses. The study suggests that nanoparticles could provide a novel and effective method for pest control, in addition to conventional approaches.

Titanium oxide, a highly reactive nanoparticle known for its ability to rapidly absorb and generate free radicals, can cause toxicity even in small amounts. This study, therefore, investigates the lethal effects of titanium nanoparticles on *Periplaneta Americana* through both oral and contact exposure.

Materials and Methods

Preparation of Periplaneta Americana samples

A total of 311 adult *Periplaneta Americana* cockroaches were obtained for the study. The cockroaches were maintained under controlled conditions, handled with disposable plastic gloves, and housed in plastic containers with sanitary lids to ensure proper care and cleanliness.

Preparation of titanium oxide nanoparticles

The nanoparticles were acquired from the Merck and Aldrich company.

Preparation of different concentrations of titanium oxide nanoparticles

To formulate various concentrations of nanoparticles for both oral and contact treatments in *Periplaneta Americana*, the following procedures were followed: A 10% solution for both applications was prepared by dissolving two grams of titanium oxide nanoparticle powder in 20 cubic centimeters of distilled water, ensuring thorough mixing. For a 15% contact treatment, 7.5 grams of the nanoparticle powder was blended with 42 cubic centimeters of distilled water. The 15% oral solution was created by combining fifteen grams of powder with 64 cubic centimeters of water and 21 grams of sugar, ensuring a homogeneous mixture. A 20% contact formulation required dissolving ten grams of powder in 40 cubic centimeters of distilled water. The corresponding 20% oral mixture was prepared using twenty grams of powder, sixty grams of water, and twenty grams of sugar. For the 25% contact concentration, thirteen grams of nanoparticle powder was combined with 40 cubic centimeters of distilled water. Lastly, the 25% oral preparation consisted of 25 grams of powder mixed with 60 cubic centimeters of water and 20 grams of sugar, all thoroughly blended.

Treatment of Periplaneta Americana by contact method

For the contact treatment of *Periplaneta Americana*, the aquarium was first thoroughly cleaned and left to air dry. To prevent the cockroaches from escaping, a layer of Vaseline oil was applied around the upper inner edges, approximately 2 to 3 centimeters from the top. Each prepared concentration of titanium oxide nanoparticles was individually sprayed onto the floor and walls of the aquarium. A total of 15 *Periplaneta Americana* cockroaches were then introduced into the treated environment. Care was taken to ensure that the nanoparticle solution was not applied to the food and water sources inside the aquarium.

Treatment of Periplaneta Americana by oral method

For the oral treatment of *Periplaneta Americana*, a solution containing titanium oxide nanoparticles was prepared at specific concentrations. Following the cleaning of the aquarium, 15 *Periplaneta Americana* cockroaches were introduced. The nanoparticle solution was then combined with cockroach feed, sugar, and finely ground dry bread. The prepared food was soaked in this solution, ensuring thorough absorption, while no part of the aquarium itself was directly exposed to the liquid. A mesh cloth was used to securely cover the aquarium. The exact start time of the experiment, including the hour and minute, was recorded and affixed to the outer wall of the aquarium.

Record results

The recorded data on insect mortality at various time intervals and days following both contact and oral treatments were documented in the corresponding tables. Each treatment using titanium dioxide nanoparticles on *Periplaneta Americana* was conducted in three separate trials.

Statistical analysis

The average percentage of insect mortality in different groups was compared by one-way analysis of variance and Duncan's test. Also, a comparison of the average percentage of insect mortality on different days in each group was done by analysis of variance with repeated observations.

Results and Discussion

A one-way analysis of variance (ANOVA) using Duncan's test was performed. The Duncan test indicated no statistically significant difference among groups in the oral treatment method on the 1st and 2nd days, although mortality was observed only at higher concentrations (20 and 25%). By the 3rd day, insect mortality at these concentrations became statistically significant, while no deaths occurred at lower doses. On the 4th day, mortality was recorded across all concentrations, but only the 20 and 25% doses showed a significant difference compared to other groups.

By the 5th and 6th days, mortality was evident at all concentrations, but only doses exceeding 10% exhibited a significant increase relative to the control. On the 7th day, mortality rates at all concentrations were significantly higher than the control, with the lowest mortality recorded at 10% and the highest at 25%. The mortality rate at 25% was notably greater than at any other concentration.

Findings demonstrated that at 10 and 15% concentrations, insect mortality was first observed from the 4th day onward. A significant increase in mortality occurred at 15% from the 5th day, whereas at 10%, a notable effect was detected only on the 7th day. Overall, mortality because of oral exposure to titanium oxide nanoparticles increased significantly in a dose-dependent manner and over time.

A One-Way ANOVA followed by Duncan's test revealed that at a 10% concentration, no mortality was recorded on the 1st day. However, at concentrations of 15% and higher, insect mortality was observed, with a statistically significant difference only at the highest concentration (25%). On the 2nd day, mortality was noted across all treatment groups, though significance was only observed at the 25% concentration compared to other groups.

By the 3rd and 4th days, mortality rates were significantly higher in concentrations exceeding 10% relative to the control. Additionally, at 25%, mortality increased significantly compared to the 15 and 20% doses. On the 5th and 6th days, all treatment groups exhibited a significant rise in mortality compared to the control, with the lowest mortality recorded at 10% and the highest at 25%, the latter showing a statistically significant difference from other doses.

On the 7th day, mortality in all treatment groups showed a significant increase compared to the control, with the 25% dose demonstrating the highest mortality rate, significantly differing from other concentrations. Overall, findings indicated that mortality became statistically significant from the 5th day onward at a 10% concentration, from the 3rd day onward at 15 and 20%, and from the 1st day at 25%, relative to the control. In general, as both concentration and exposure duration increased, the mortality rate of American cockroaches because of contact exposure to titanium oxide nanoparticles rose significantly.

Figure 1 presents the mortality percentage of *Periplaneta Americana* across different days and oral treatment doses.

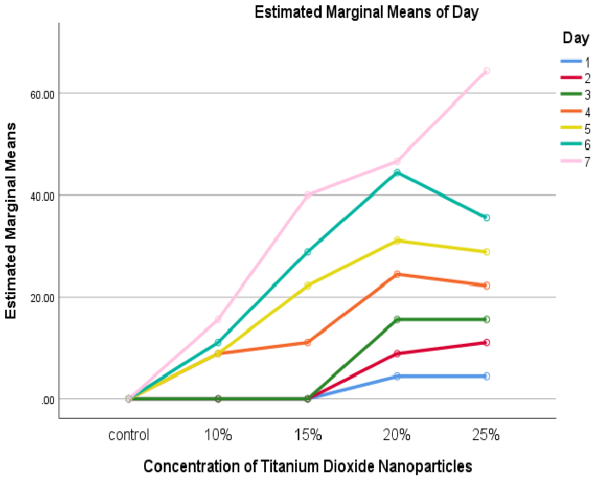

Figure 1. The mortality rate of *Periplaneta Americana* across various days and concentrations following oral treatment.

Statistical analyses using repeated measures ANOVA, along with Bonferroni and Huynh-Feldt tests, revealed a significant rise in the mortality rate of *Periplaneta Americana* at all concentrations over the seven-day oral treatment period ($P < 0.001$, $F = 42.558$). This indicates that as time progressed, the mortality percentage at each titanium dioxide nanoparticle concentration increased notably.

Similarly, results from repeated measures ANOVA and Bonferroni and Huynh-Feldt tests demonstrated a statistically significant increase in mortality rates across all concentrations over seven days of contact treatment ($F = 2.494$; $P < 0.01$). This suggests that mortality rates rose progressively with time at each applied concentration of titanium dioxide nanoparticles.

Figure 2 presents the mortality percentage of *Periplaneta Americana* across various days and concentrations following contact treatment.

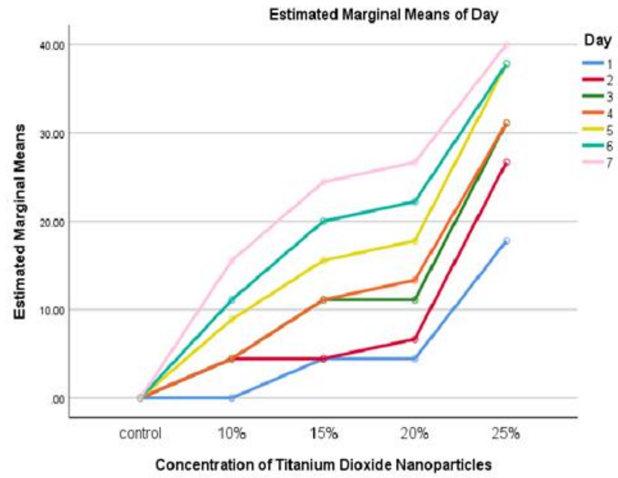

Figure 2. The mortality rate of *Periplaneta Americana* at various concentrations and time points following contact treatment.

A comparison of the mortality rate of *Periplaneta Americana* at a 10% concentration of titanium dioxide nanoparticles showed that the contact method had a quicker lethal effect than the oral method (observed on the second day for contact and the fourth day for oral treatment). However, no significant differences were found between the two methods across different treatment days. In fact, from day 5 to day 6, the mortality rates for both methods were the same. One-way analysis of variance revealed that at a 15% concentration, there were no significant differences in mortality between the oral and contact methods, although the contact method showed lethality from the first day, while the oral method showed effects starting on the 4th day. At a 20% concentration, oral lethality effects were observed to be higher than contact lethality on all treatment days except the first, with significant increases on the 5th, 6th, and 7th days. Both methods showed lethality from day one at this concentration. For the 25% concentration, the contact method produced higher mortality rates on all days except the 7th, where the oral method was significantly more lethal.

The comparison of mortality percentages on the 1st day at varying concentrations between the oral and contact methods revealed an important increase ($P < 0.05$) in the contact method at the 25% concentration. Similarly, on the 2nd day, an important increase in mortality ($P < 0.05$) was found in the contact method at 25%. However, on the 3rd day, no significant difference in mortality between the two methods was observed, though the contact method resulted in higher mortality at all doses except for the 20% concentration.

The comparison of insect mortality percentages on the 4th day of treatment at different titanium dioxide nanoparticle concentrations between oral and contact methods revealed no significant differences. However, for the 15% concentration, the mortality rates were identical between the two methods, while at 10 and 20%, the oral method resulted in higher mortality, and at 25%, the contact method was more effective. On the 5th day, the oral method at a 20% concentration displayed an important increase ($P < 0.05$) in mortality compared to the contact method. An important increase ($P < 0.01$) in mortality for the oral method was also observed on the 6th day at the same 20% concentration. On the 7th day, the oral method exhibited a significant increase in mortality ($P < 0.05$) at both 20 and 25% concentrations compared to the contact method.

As mentioned, nanoparticles represent a novel approach as insecticides, with various research efforts exposing insects to them in various ways. However, further studies, particularly field trials, are necessary to determine their practical effectiveness in pest management systems. In support of this, research by Gutiérrez-Ramírez *et al*. [13] investigated the insecticidal properties of zinc oxide nanoparticles, titanium dioxide nanoparticles, and their combination on *Bactericera cockerelli* nymphs in both laboratory and greenhouse environments. Their findings demonstrated significant toxicity of both titanium dioxide and zinc oxide nanoparticles to *B. cockerelli* nymphs under these conditions. Laboratory results indicated that the nanoparticles substantially increased mortality 96 hours after treatment with zinc oxide, TiO_2 nanoparticles, and their combinations. These results align with the current study's findings regarding the toxic effects of titanium dioxide nanoparticles.

AbdEl-Raheem and Eldafrawy [14] explored the impact of silver nanoparticles on the German cockroach *Blattella germanica* (L). The study applied silver nanoparticles to both the third-stage nymphs and adult cockroaches using oral and contact methods. The findings indicated that an increase in nanoparticle concentration led to a higher mortality rate in both adult and larval stages. After 72 hours, the highest mortality rate of 93.33% was observed in nymphs treated with a 300 ppm concentration using the oral method, while the contact method resulted in a mortality rate of 73.33% at the same concentration. For adults, the highest mortality rate of 96.67% was observed with the oral method at a 300 ppm concentration after 72 hours, compared to 83.33% in the contact treatment at the same concentration.

The findings of this study indicate that oral treatment was more effective than contact treatment on all days at concentrations of 15 and 20%. However, at a concentration of 25%, contact treatment was more effective than oral treatment on all days, except for the seventh day. Overall, oral treatment proved to be more effective, as the nanoparticles are directly ingested by the American cockroaches and enter their digestive system. These results align with those of AbdEl-Raheem and Eldafrawy [14], who also observed that mortality increased as nanoparticle concentration increased. In this study, exposure to titanium dioxide nanoparticles via contact treatment likely leads to physical disruption of the American cockroach's body rather than affecting its biochemical processes. This idea is supported by Stadler *et al*. [15], who found that aluminum nanoparticles attach to insects' cuticles via triboelectric forces and the insect's wax layer, causing physical damage through abrasions and cracks. Additionally, in this study, the nanoparticles may cause biochemical changes when ingested or inhaled, potentially affecting various organs, including the brain. Raj *et al*. [16] also noted that silver nanoparticles induce stress in insects by increasing reactive oxygen species, cytokines, and pro-inflammatory mediators, as well as altering membrane potential and mitochondrial activity. Their research further indicated that silver nanoparticles consumed during the early larval stages of Drosophila melanogaster resulted in impaired crawling ability, metabolic changes, and increased reactive oxygen species in the larvae. Benelli [17] emphasized the importance of factors including surface area, particle morphology, and size in influencing the effectiveness of insecticide dust. In this study, both contact and oral treatments with titanium dioxide nanoparticles led to significantly higher mortality rates in American cockroaches as the dose and exposure time increased. This, along with other studies on nanoparticles' effects on cockroaches, suggests that nanoparticles can have detrimental effects on the growth of non-target organs, reproduction, and even impact non-target organisms.

The growing environmental issues and costs associated with the heavy use of traditional pesticides and the rising resistance of insects to these chemicals highlight the need for advancements in nano-insecticides. Nano-insecticides offer the potential to be more effective while reducing the need for large quantities of conventional pesticides. Their targeted delivery and absorption increase effectiveness and help limit harmful impacts on non-target organisms. Traditional insecticide formulations often involve harmful organic substances, which pose risks to the environment and wildlife, aside from the insecticide itself [18-20]. However, the use of nanoparticles in insecticides raises concerns, such as their longer shelf life and greater toxicity. Additionally, the small particle size of nanoparticles can lead to early evaporation before they reach their target. The effects of nanoformulations on microorganisms, plants, and animals across different food chains also require further study. Moreover, there is limited knowledge about how insecticide nanoformulations behave in the environment, particularly in soil, and groundwater, and their impact on non-target organisms, which calls for more research.

Conclusion

The findings of this study indicate that oral treatment was generally more effective than contact treatment in controlling American cockroaches at concentrations of 15 and 20% across all days. However, at a concentration of 25%, contact treatment was more effective, except on the seventh day. In summary, oral treatment had a higher effectiveness, and both treatments resulted in a notable increase in mortality with higher doses and extended exposure times.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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