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Studying the Insecticidal Effect of Titanium Oxide Nanoparticles on the *Periplaneta Americana*

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ABSTRACT

One of the important domestic insects that transmit many pathogens to the environment is the *Periplaneta Americana*. The control methods of this insect are often ineffective and have caused resistance in these insects. The purpose of the present study was to investigate the effects of contact and feeding lethality of titanium nanoparticles on *Periplaneta Americana*. In this study, different concentrations of nanoparticles were prepared for oral and contact treatments of *Periplaneta Americana*. *Periplaneta Americana* was treated by contact and oral methods with different concentrations of titanium oxide nanoparticles with three repetitions. The average percentage of insect mortality in different groups was compared by one-way analysis of variance and Duncan's test. Also, the average percentage of insect mortality on different days in each group was compared by analysis of variance with repeated observations. Oral treatment of *Periplaneta Americana* was more effective than contact treatment in concentrations of 15 and 21 percent on all days, but contact treatment was more effective than oral treatment on all days except the seventh day at a concentration of 25 percent. In general, oral treatment was more effective than contact treatment. With increasing doses and the passage of time, the mortality percentage of *Periplaneta Americana* due to contact and oral treatment with titanium dioxide nanoparticles increased significantly. According to the obtained results, the use of different nanoparticles, including titanium dioxide nanoparticles, can create a significant effect in controlling the *Periplaneta Americana* as a new method along with the previous methods.

Keywords: Pathogens, *Periplaneta Americana*, Nanoparticles, Titanium oxide, Insecticidal effect.

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INTRODUCTION

Among the pests that disturb humans, arthropods, especially insects, are very important. These organisms have been able to adapt to most ecosystems due to having suitable morphological and physiological capabilities, small size with the ability to move quickly, and high reproduction power. Arthropods directly (sting and bite) and indirectly (transmission of infectious diseases) cause many problems for humans. A part of arthropods are considered economic and construction pests like termites and some like cockroaches, mosquitoes, flies, bedbugs, ticks, scorpions, etc. are health pests [1, 2] and they usually live in areas with moderate to hot climates. It is considered one of the most well-known and probably the most common household pests that are found when the weather warms up. In general, cockroaches have different species and each of them has their characteristics. The *Periplaneta Americana* or the American bathroom cockroach mostly lives and reproduces in the sewage network and spreads to residential buildings, especially in the areas covered by the sewage collection networks. From the point of view of ecology, cockroaches play a very important role in establishing the nitrogen cycle of nature [3, 4].

Cockroaches feed on dirty materials, rotten food, stored food, and all kinds of household food. Food causes the transmission of diseases such as staphylococcal diseases, salmonella, giardiasis, escherichia infection, and trichomonas, etc. Several pathogenic species from different groups including bacteria, viruses, fungi, protozoa, and parasitic worms have been isolated and identified from the American cockroach species. Studies have shown that 11 types of bacteria live in the body of the American cockroach. Cockroach control is still an important problem in many parts of the world. American cockroaches often live in collection systems and sewer networks, where they breed in great numbers, where more than 5,000 may be found in a single inlet. Apart from the problem of disturbing and dispersing their feces in the environment, their relationship with excrement and fecal diseases as well as their ability to enter human residential places through sewage pipes have caused health hazards in polluted areas [5, 6].

The use of nanoparticles has increased significantly in domestic and industrial processes in recent years. These particles show special physical and chemical behavior due to their high surface-to-volume ratio, small size, and visual characteristics related to their size. Metal nanoparticles have wide applications in the fields of biotechnology, bioassays, clinical diagnosis and treatment, food safety, water and wastewater treatment, and insecticides and pesticides. The most important point before predicting the toxicity of nanoparticles is to obtain information about the factors and characteristics that increase their toxicity. The way these particles interact with the human body, animals, and the environment determines their effects. As the size of nanoparticles decreases, their surface-to-volume ratio increases exponentially, which makes these particles more active and toxic. Also, by reducing their size, the possibility of their penetration into plant and animal tissues increases. Penetration of nanoparticles through cell barriers is highly dependent on their size. It is said that particles with a size less than 35 nm can penetrate the protective blood-brain barrier, and particles with a size less than 41 nm can enter the nucleus of cells, while particles with a size less than 111 nm can pass through the cell membrane and enter the cell. The binding stability and post-binding durability of protein structures depend on the size of the nanoparticles [7-9].

It has been observed that silver nanoparticles can cause cytotoxicity in human and animal cells due to the release of silver in the cellular environment, which depends on the size of the particles. Laboratory studies show that silver nanoparticles cause a wide range of toxicities such as inflammation, genotoxicity, cytotoxicity, and developmental toxicity based on their particle size [10]. Titanium dioxide nanoparticles with a size smaller than 11 nm caused immunotoxicity in the pulmonary system of mice. Different sizes of titanium dioxide nanoparticles have different degrees of toxicity in the pulmonary system of mice. With the widespread production of nano-based products, there is an urgent need to investigate their potential toxic effects on the human body, animals, and the environment [11].

In the study of Zorlu *et al*. [12], the effects of different concentrations of titanium dioxide nanoparticles on biological parameters and total protein amount, antioxidant enzyme activity, and malondialdehyde levels in the hemolymph of the *Galleria mellonella* (L.) were investigated. The results of their investigation showed that the development time of larvae and pupae increased significantly at 100, 500, 1000, and 3000 ppm compared to the control and the highest dose of titanium dioxide nanoparticles. However, the lifespan of adults at low concentrations of titanium dioxide nanoparticles 100, 500, and 1000 ppm was shortened. Exposure to titanium dioxide nanoparticles caused a significant increase in the amount of total protein and malondialdehyde content and activity of glutathione S-transferase in hemolymph at concentrations of 100, 500, and 1000 ppm compared to the control and other doses of nanoparticles. It seems that during the research conducted, the use of nanoparticles can create a significant effect in controlling these pests as a new method along with the previous methods.

One of the most important nanoparticles is titanium oxide with reactivity and fast absorption power, which can be used in very small amounts by creating free radicals and causing poisoning. Therefore, the present study was conducted to investigate the contact and oral lethality effects of titanium nanoparticles on *Periplaneta Americana*.

MATERIALS AND METHODS

Preparation of Periplaneta Americana samples

311 adult American cockroaches were purchased. The mentioned samples were kept under completely standard conditions (disposable plastic gloves) in plastic containers with sanitary lids.

Preparation of titanium oxide nanoparticles

Nanoparticles were purchased from .... company.

Preparation of different concentrations of titanium oxide nanoparticles

To prepare different concentrations of nanoparticles for oral and contact treatments of American cockroaches, it was done as follows: To prepare a concentration of 10 percent of contact and oral treatment, 2 grams of titanium oxide nanoparticle powder was mixed with 20 cc of distilled water and mixed well. To prepare a 15 percent contact concentration, 7.5 grams of powder was mixed with 42 cc of distilled water and mixed well. In the case of 15 percent edible concentration, 15 grams of powder was mixed with 64 cc of water and 21 grams of sugar and mixed well. To prepare a 20 percent contact concentration, 10 grams of powder was mixed with 40 cc of distilled water and mixed well. In the case of 20 percent edible concentration, 20 grams of powder was mixed with 60 grams of water and 20 grams of sugar and mixed well. To prepare a 25 percent contact concentration, 13 grams of powder was mixed with 40 cc of distilled water and mixed well. In the case of the 25 percent edible concentration, 25 grams of powder was mixed with 60 cc of water and 20 grams of sugar and mixed well.

Treatment of Periplaneta Americana by contact method

To treat American cockroaches by contact method, the interior of the aquarium was washed and then exposed to air to dry. About 2 to 3 cm from the upper edges of the aquarium was smeared with Vaseline oil to prevent cockroaches from leaving the aquarium. Each of the prepared titanium oxide nanoparticle concentrations was sprayed separately on the floor and walls of the aquarium. 15 American cockroaches were placed inside the aquarium. The solution containing titanium oxide nanoparticles should not be sprayed on cockroaches' food and water.

Treatment of Periplaneta Americana by oral method

To treat American cockroaches by oral method, a solution containing titanium oxide nanoparticles prepared with specific concentrations was prepared. After cleaning the aquarium, 15 American cockroaches were placed inside the aquarium and the solution containing titanium oxide nanoparticles prepared with a certain concentration was mixed with sugar, cockroach feed, and dry bread powder. The cockroaches' food was soaked in the solution and the solution was not poured into any part of the aquarium. The aquarium door was closed with a net cloth. The time of the start of the experiment was written down exactly along with the hour and minute and it was pasted on the outer wall of the aquarium.

Record results

The results (number of dead insects) in the present study at different hours and days after contact and oral treatment were recorded in the respective tables. The contact and oral treatments of American cockroaches with titanium dioxide nanoparticles were repeated three times.

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

One-way analysis of variance with Duncan's One Way-ANOVA test. Duncan Test showed that there was no significant difference between the groups in the oral method on the first and second day. Although insect mortality occurred only at high doses (20 and 25 percent). On the third day, the percentage of insect mortality was significant at concentrations of 20 and 25 percent, and no mortality was observed at lower concentrations. On the fourth day, mortality occurred in all doses, but it was significant only in doses of 20 and 25 percent compared to other groups. On the fifth and sixth day, insect mortality was observed in all doses, which was significant only in doses higher than 10 percent compared to the control. Also, on the seventh day, the percentage of insect mortality in all doses was significant compared to the control. So that the lowest mortality was observed in the dose of 10 percent and the highest in the dose of 25 percent. Also, the death rate in the 25 percent dose was significantly higher than other doses.

The results showed that in the doses of 10 and 15 percent, insect mortality was observed from the fourth day of treatment onwards. In the dose of 15 hundred percent, a significant difference was created from the fifth day and in the dose of 10 hundred percent only on the seventh day. In general, the increase in the percentage of insect mortality due to oral treatment with titanium oxide nanoparticles increased significantly in a dose-dependent manner and with time.

One Way-ANOVA, Duncan Test showed that no mortality was observed on the first day at a concentration of 10 percent. From a concentration of 15 hundred percent to above, insect mortality occurred, which was significantly different from other groups only at the highest concentration (25 percent). On the second day, mortality occurred in all experimental groups, but it was significant only in the dose of 25 percent compared to other groups. On the third and fourth day, the percentage of insect mortality was significant in doses higher than 10 percent compared to the control, and also in the dose of 25 percent compared to the doses of 15 and 20 percent, the percentage of mortality increased significantly. On the fifth and sixth days, there was a significant increase in the percentage of insect mortality in all groups compared to the control, the lowest percentage of which was related to the dose of 10 percent and the highest percentage of death was related to the dose of 25 percent, which was significantly different from other doses. Also, on the seventh day, a significant increase in the percentage of insect mortality was observed in all groups compared to the control, which was significantly different in dose 25 compared to other doses. In general, the findings of the research showed that at a concentration of 10 hundred percent, the death rate from the fifth day onwards, at a concentration of 15 and 20 hundred percent from the third day onwards, and a concentration of 25 hundred percent from the first day, compared to the control. Significant was observed. In general, with the increase of the dose and the passage of time, the mortality percentage of American cockroaches due to contact treatment with titanium oxide nanoparticles increased significantly.

The percentage of *Periplaneta Americana* mortality on different days and doses of oral treatment is presented in **Figure 1**.

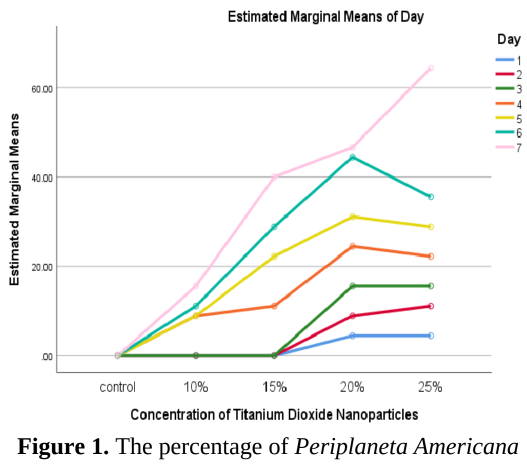

Figure 1. The percentage of *Periplaneta Americana* mortality on different days and doses of oral treatment.

Analysis of Repeated Measure (ANOVA) and Bonferroni and Huynh-Feldt tests showed a significant increase in the percentage of American cockroach mortality in each dose for 7 days of oral treatment ($p<0.001$, $F= 42.558$). In other words, with time for 7 days, the percentage of insect mortality in each concentration of titanium dioxide nanoparticles increased significantly.

Repeated Measure (ANOVA) and Bonferroni and Huynh-Feldt tests showed a significant increase in the percentage of American cockroach mortality in each dose for 7 days of contact treatment (F = 2.494; p<0.01). In other words, with time for 7 days, the percentage of insect mortality in each concentration of titanium dioxide nanoparticles increased significantly.

The percentage of American cockroach mortality on different days and doses of contact treatment is presented in **Figure 2**.

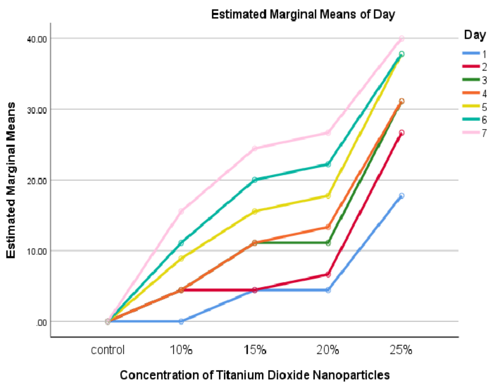

Figure 2. The percentage of American cockroach mortality on different days and doses of contact treatment.

Comparison of the lethality percentage of titanium dioxide nanoparticles at a concentration of 10 percent on different days between the oral and contact methods showed that the lethal effect of the contact treatment appeared earlier than the oral treatment (the second day compared to the fourth day). But in general, there was no significant difference between these two methods on different days of treatment. Even on days 5 to 6 of treatment, the percentage of oral and contact lethality was the same. One-way analysis of variance showed that the lethal effects of titanium dioxide nanoparticles at a concentration of 15 percent on different days were not significantly different between the contact and oral methods. Although the effects of contact lethality started on the first day and oral on the fourth day. In the concentration of 20 percent titanium oxide nanoparticles, the oral lethality effects were higher than contact on all days of treatment (except the first day), but only on the fifth, sixth, and seventh days, there was a significant increase. In both methods, lethal effects started from the first day of treatment. In the concentration of 25 percent of titanium dioxide nanoparticles, the percentage of lethality by contact method was higher on all days except the seventh day, which had a significant difference only on the first and second day. But on the seventh day, the lethality of the oral method was significantly higher than the contact method.

The results of comparing the mortality percentage of insects on the first day of treatment in different concentrations of titanium dioxide nanoparticles between the oral and contact methods showed a significant increase (p<0.05) in the percentage of mortality in the contact method at a concentration of 25 hundred percent. The results of comparing the mortality percentage of insects on the second day of treatment in different concentrations of titanium dioxide nanoparticles between the oral and contact methods showed a significant increase (p<0.05) in the percentage of mortality in the contact method at a concentration of 25 hundred percent. The results of comparing the percentage of insect mortality on the third day of treatment in different concentrations of titanium dioxide nanoparticles between oral and contact methods did not show any significant difference. However, in all doses except 20 percent, the death rate was higher in the contact method.

The results of comparing the percentage of insect mortality on the fourth day of treatment in different concentrations of titanium dioxide nanoparticles between oral and contact methods did not show any significant difference. But in the dose of 15 hundred percent, the mortality rate was the same, and in the dose of 10 and 20 percent, the mortality rate was higher in the oral method and in the 25 hundred percent dose, in the contact method. The results of comparing the percentage of insect mortality on the fifth day of treatment in different concentrations of titanium dioxide nanoparticles between the oral and contact method showed a significant increase (p<0.05) in the percentage of mortality in the oral method at a concentration of 20 percent. The results of comparing the percentage of insect mortality on the sixth day of treatment in different concentrations of titanium dioxide nanoparticles between the oral and contact method showed a significant increase (p<0.01) in the percentage of mortality in the oral method at a concentration of 20 percent. The results of comparing the percentage of insect mortality on the seventh day of treatment in different concentrations of titanium dioxide nanoparticles between the oral and contact method showed a significant increase ($p<0.05$) in the percentage of mortality in the oral method at concentrations of 20 and 25 percent.

As it was said, nanoparticles as insecticides are a new strategy that insects have been exposed to in different researches in different ways. However, further research in field trials is needed to achieve suitable efficacy for use in an insect management system. In line with the current study and the effect of nanoparticles on insects, the study by Gutiérrez-Ramírez *et al*. [13] aimed to investigate the insecticidal effect of zinc oxide nanoparticles, titanium dioxide nanoparticles, and their combination on *Bactericera cockerelli* nymphs in laboratory and greenhouse conditions. Their results showed that both zinc oxide and titanium dioxide nanoparticles showed significant toxicity to *B. cockerelli* nymphs in the laboratory and greenhouse. Laboratory results showed that nanoparticles significantly increased mortality after 96 hours after treatment with zinc oxide nanoparticles, TiO_2 nanoparticles, and their combinations. Their results were consistent with the current study in terms of the toxicity characteristics of titanium dioxide nanoparticles.

AbdEl-Raheem and Eldafrawy [14] conducted a study to investigate the effect of silver nanoparticles on the German cockroach *Blattella germanica* (L). Silver nanoparticles were applied to both the third and adult stages of nymphs by contact and oral methods. The obtained results showed that with the increase in the concentration of nanoparticles, the percentage of mortality increased in both the larval and adult stages. In the oral method, the percentage of losses in purees treated after 72 hours with a concentration of 300 ppm reached its highest level, which was 93.33%, while it reached 73.33% in the contact test with the same concentration. The percentage of mortality in adults treated after 72 hours with a concentration of 300 ppm reached its highest level, which was 96.67% in the feeding method and 83.33% in the contact test with the same concentration.

The results of the present study show that the oral treatment was more effective than the contact treatment on all days and at concentrations of 15 and 20 percent, but the contact treatment was more effective than the oral treatment on all days except the seventh day at a concentration of 25 percent. In general, in the present study, oral treatment is more effective than contact treatment because the nanoparticles directly enter the diet of American cockroaches and enter the digestive system. The results of AbdEl-Raheem and Eldafrawy [14] study were consistent with the current study in that the percentage of death also increases with the increase in the concentration of nanoparticles. It seems that in the present study when American cockroaches are exposed to contact treatment with titanium dioxide nanoparticles, the mechanism of titanium dioxide nanoparticles relies on the physical disturbance of American cockroaches instead of affecting the biochemical performance. In this regard, Stadler *et al*. [15] also showed that the charged particles of aluminum nanoparticles are attracted to the insect by attaching to their cuticle through triboelectric forces and by the surface effect of the insect's wax layer. These abrasive particles along with their hydrophobic behavior cause cracks and scratches on the body of insects. Also, in the present study, in addition to physical damage through contact, nanoparticles can change the biochemical activity after ingestion or inhalation, and in this way, they can even go to different organs of the American cockroach, including the brain, after inhalation. In this regard, Raj *et al*. [16] stated that silver nanoparticles cause stress in insects, which appears as an increase in cytokines, reactive oxygen species, and pro-inflammatory mediators, as well as changes in membrane potential and mitochondrial respiratory chain. Also, their results showed that the consumption of silver nanoparticles in the early larval stages of Drosophila melanogaster is associated with impaired crawling ability, and with an increase in the oral dose of silver nanoparticles during the larval stage, metabolic changes in protein, carbohydrate, and lipid levels as well as a decrease in the presence of fat droplets and the increase of reactive oxygen species occurs in larval tissue. Benelli [17] also stated that when using insecticide dust, parameters such as particle morphology, surface area, and particle size should be considered to affect the efficacy of insecticide activity. In the present study, the percentage of mortality of American cockroaches due to contact and oral treatment with titanium dioxide nanoparticles increased significantly with increasing doses and the passage of time. Therefore, according to the present study as well as other studies in the field of the effect of various types of nanoparticles on cockroaches, it shows their adverse effect on reproduction, growth of non-target organs, and even non-target organisms.

Considering the environmental problems and costs caused by the consumption of large amounts of conventional pesticides and insecticides in nature, as well as the problems caused by the resistance of insects to these insecticides, research, and development in the field of nano-insecticides is a necessity. Increasing the effectiveness of nano insecticides reduces their consumption compared to conventional insecticides. In addition, the targeted transfer and absorption of nano-insecticides not only increases their effectiveness but also reduces their adverse effects on non-target organisms. Usually, in the production of conventional insecticide formulations, various organic substances are used, which are dangerous for the environment and wildlife in addition to the insecticide molecule itself [18-20]. But on the other hand, a new problem related to the use of nanoparticles and nano-based insecticides is their longer shelf life and higher toxicity. Small droplet size may also lead to premature evaporation of nanoparticles before reaching the target. The interaction of nanoformulations with microorganisms, plants, and other animals at different nutritional levels is another main field related to the use of nano insecticides that needs to be investigated. In addition, the environmental fate of insecticide nanoformulations on soil, groundwater, and non-target organisms is unknown and needs to be investigated.

CONCLUSION

In the present study, the oral treatment of American cockroaches was more effective than the contact treatment on all days and at concentrations of 15 and 20 percent, but the contact treatment was more effective than the oral treatment on all days except the seventh day at a concentration of 25 percent. In general, in the present study, oral treatment was more effective than contact treatment, and in this study, with the increase of dose and passage of time, the mortality percentage of American cockroaches due to contact and oral treatment with titanium dioxide nanoparticles increased significantly.

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