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Alpha and Gamma Alumina Nanoparticles Synthesized from Aluminum Cans Wastes as Grain Protectant against Insects and Mycotoxin-Producing Fungi

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Abstract

This research focused on isolating and purifying the most frequent mycotoxin, aflatoxin-producing fungi from maize and soybean grains, which are of enormous economic worth as animal and human feed. Alpha and gamma aluminum oxide nanoparticles (α and γ-Al_2O_3 NPs) were also green generated from aluminum cans. XRD, HRTEM, and ATR-FTIR were utilized to analyze the structure and morphology of α and γ-Al_2O_3 NPs. Also, studied the antifungal and insecticidal activity of aluminum nanoparticles produced, antifungal activities of both nanoparticles types were evaluated on the fungal isolates' growth at starting concentration of 0.67mg/ml using agar well diffusion method. The potential toxicity of α and γ-Al_2O_3 NPs on Sitophilus oryzae and Oryzaephilus surinamensis adults was also evaluated at 50, 100, 150 and 200ppm. FESEM was used to examine the dead insects treated with 200 ppm of α or γ-AL_2O_3 NPs. Results confirmed the formation of α and γ -Al_2O_3 NPs using ATR –FTIR and XRD. As measured by HRTEM, the particle size of α and γ -Al_2O_3 NPs were found to be 4-8, 2-4nm, respectively. A wide variety of A. flavus, Fusarium sp, and Alternaria sp. fungi were found in the samples. Concerning, fungal isolates response, A. flavus was the most sensitive fungus against γ and α-Al_2O_3 nanoparticles with inhibition zone 9 and 13.5mm, followed by F. oxysporum with 7 and 8mm for both types respectively. Alternaria sp. was the less sensitive fungal isolate with 1.5mm inhibition zone for both aluminum nanoparticles types. Thus, Alpha-Al_2O_3 nanoparticles possess a higher antifungal activity than γ-Al_2O_3 type. Results of insect toxicity showed that the survival and progeny production of both insect species were affected by the phase of nanoparticles, the applied concentration and exposure time. Gamma alumina NPs exhibited significant influence on both insect species more than alpha phase. Additionally, FESEM micrographs showed greater distribution intensity of γ-AL_2O_3 NPs on treated insects more than that of alpha phase. This study suggests that the synthesized alpha and gamma alumina NPs can provide eco-friendly insecticide and fungicidal to protect stored products.

Keywords: Aluminum cans; Alumina nanoparticles; *S. oryzae; O. surinamensis*; Antifungal effect

1. Introduction

Maize, rice, wheat, and other stored legumes and grains are important food, feed, and industrial grain crops in the global economy and trade [1-3]. Storage insect pests wreak havoc on stored goods, with 10–40 percent of stored grains lost each year owing to infestation [4]. It is common for the yield of maize and soybeans to be contaminated with saprotrophic, mycotoxin-producing fungi until spoiling due to improper preservation unless the crops are treated with fungicide during the storage period. Aspergillus sp., Alternaria sp., and Fusarium sp. are among the most common fungi [5]. Also, Insects damage grains by feeding on endosperm and grain embryos, and the scratches that result enhance the grain's exposure to rot, rendering the product unpleasant to humans and animals [6]. Rice weevils, Sitophilus oryzae (L.) (Coleoptera: Curculionidae), and sawtoothed grain beetles, Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae), are two of the most common stored product pests in the world.

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Though rice is considered its principal feeding, S. oryzae may grow on a variety of cereals and cause severe losses, especially in warm temperature settings [7]. Adults and larvae of O. surinamensis infest a wide range of foods in both food storage facilities and home pantries [8]. To manage pests in stored goods, fumigants and chemical pesticides are commonly utilized. However, health risks, insect resistance, and residual toxicity of synthetic pesticides are all important issues that have prompted researchers to look into safer alternatives for controlling stored-product insects [9]. Using synthetic fungicides for an extended period of time is not recommended because of the high costs and residues, as well as the long-term effects on human and environmental health. Fungicides manufactured in a lab face additional challenges due to the pathogens they are meant to control developing resistance to the fungicides [10]. Previous research has been conducted to develop environmentally friendly alternatives to traditional chemical control approaches [11-13]. As an alternative to chemically manufactured alternatives, nanotechnology has recently gained increased interest for its widespread applicability in the control of the great majority of plant and human infection against various pathogenic microbes [14-15]. Thus, Diatomaceous earth, zeolite, and metal oxides of silica, zinc, titanium, and aluminum particles have all recently been studied for their potential as safe insecticides to replace conventional chemicals [3, 14-19]. In comparison to traditional pesticides, inert dusts have various advantages, including low production costs, long-term insecticidal effect, and lower human toxicity [20-21]. Nanotechnology has the potential to create environmentally safe and effective solutions for controlling insect pests in agriculture without harming the environment. The particle size, shape, and adsorptive capacity of nanomaterials are known to be critical factors in influencing their efficiency. [14-15, 22-23]. The most efficient inert dusts have very small, uniformly sized particles, a large specific surface area, and a high porosity. At lower concentrations, nanoparticles of silica and alumina, for example, have a greater insecticidal effect than their bulk equivalents [24]. Previously, researchers used nano pesticide dusts such as alumina nanoparticles and nanosilica to calculate lethal concentrations and insect mortality in the laboratory [25-26]. Scientists have recently concentrated on producing nanoparticles from garbage in an environmentally responsible manner, which have been employed in a range of applications. Nanoparticles have been employed in food and feed additives for preservation, medical equipment, water purification, and a variety of other products [3, 14-15, 23, 27-28]. Furthermore, the monetary value of the end-products is directly linked to the success of recycling and repurposing processes. As a result, employing "clean manufacturing" technology to create "value-added" commodities from waste appears to be a viable option for achieving both recycling and sustainability objectives [15]. Aluminum cans generate a lot of waste, which is a big problem for the environment. As a result, the trash recycling process reduces pollution while also conserving money and energy, in addition to the material return that comes from recycling and reusing it [29-31]. Because of its unique chemical and physical properties, alumina is employed in a wide range of applications.

As a result, the goal of this research was to isolate and purify the most common mycotoxin, synthesize and analyze aluminum oxide NPs made from aluminum cans. In addition to study their antifungal activity against the isolated fungal, Also, the insecticidal activity of aluminum oxide NPs produced on adult survival and production of S. oryzae and O. surinamensis.

2. Materials and methods

Synthesis of α and γ-Al_2O_3 NPs

Aluminum can waste was used to make alpha and gamma aluminum oxide nanoparticles using 6 N HCl or NaOH, respectively. To obtain it, neutralize the aluminum hydroxide. Before being calcined at 350°C for 3h, the samples were dried at 70°C overnight.

Measurements

High-resolution transmission electron microscopy (HRTEM) was performed using a JEM-2100F electron microscope and a 200 kV accelerating voltage. With secondary monochromatic Holland radiation running at 45 kV and 0.1540 nm wavelength, X'Pert Pro target Cu-K was used for XRD in a range of 5°–80°.A Vertex 80 Bruker (made in Germany) is used to obtain ATR-FTIR spectral data in the range 4000-400 cm-1 at ambient temperature.

Identification and isolation of fungal species:

A grocery store in Cairo, Egypt, sold us maize and soybean grains. For fungal isolation, the rotting grains were collected individually. Research on plants is carried out in accordance with all applicable institutional, national, and international norms and legislation. Prior to drying under aseptic conditions, the grains are first surface-sterilized using a solution of 0.5 percent sodium hypochlorite, 1 gm of each grain type, and three washes of sterile, distilled water. It was then crushed in a mortar, suspended in 9ml of water, and vortexed until homogeneity was obtained. Rosebengal agar Petri dishes with 1 ml of the 2nd and 3rd dilutions were kept at 30oC for 5 days until the 3rd factor was achieved. Colonies of fungal spores that formed throughout the incubation period were transferred to potato dextrose agar dishes and cultivated as before. Colony morphology, form, colour, and medium pigmentation, as well as microscopic analysis of the spores and mycelium, were used to identify the fungal isolates grown on PDA Petri dishes.

Fungicidal effect

0.6mg/ml DDH2O of aluminum nanoparticle solution was used to investigate their fungicidal effect on A. flavus, Fusarium sp, and Alternaria sp. In order to obtain 105-106 homogenous fungal spore suspensions, the 7mm fungal discs of each strain were sliced and injected into 10ml of sterilized distilled H2O. Sterilized Petri dishes, covered with warm PDA-sterilized medium, and thoroughly mixed with 1ml of fungal suspension were then allowed to solidify. Using a sterilizing tip end, a 7mm agar well was made, and 50l (33.3 g/50l) of the prepared nanoparticle solution was inoculated into it. Plates of PDA were incubated at 30°C for five days after 30 minutes of cooling in the refrigerator to allow the solution to diffuse. Antifungal activity was measured by tracing a circle around the well with a millimeter-long ruler after the well had been incubated for 24 hours.

Insects rearing

S. oryzae and O. surinamensis were cultured for numerous generations at 30±2°C, 60-65 percent relative humidity, and a 13:11 light: dark photoperiod in the Pests and Plant Protection Department of the National Research Centre in Egypt. S. oryzae insects were reared on whole wheat, while O. surinamensis insects were reared on oat flakes. Adults of both species were released into two-liter glass jars containing uninfected food, which was used in the assays. They were taken out after 10 days and stored for an additional 60 days. The experiment was carried out on the F1 adults that had been developed (1-7 days old). For the studies, researchers bought clean wheat grains from a local market. The grains were kept in cold storage at -18 °C for at least 5 days before usage to sterilize the product.

Insecticidal effect

A dry dust application approach was used to proceed the toxicity bioassay. Adults of S. oryzae and O. surinamensis were subjected to four concentrations: 50, 100, 150, and 200 mgkg-1 α or γ-AL_2O_3 NPs equaling 50, 100, 150, and 200 ppm. Experiment was carried out in five duplicates, with 10 unsexed insects placed in separate plastic vials (120 ml) containing 40 g of wheat grains in each replication. Wheat grains were dusted with various quantities of α or γ-AL_2O_3 NPs before insects being released, and vials were shaken for 2 minutes to ensure uniform distribution of nanoparticles. Vials were filled with untreated wheat grains in the control groups. For both S. oryzae and O. surinamensis, the experiment was maintained under the same set of previous circumstances, and dead insects were collected and counted every 24 hours until the completion of the experiment; for 15 days. After 1, 3, 7, and 15 days of exposure to varied concentrations of two phases of AL_2O_3 NPs, the percentage of death was assessed. Adult insects that were carefully probed with sharp tip forceps and showed no movement were considered dead. The effect of α or γ-Al_2O_3 NPs on the development of offspring in treated adults was also investigated. After 15 days of exposure, all treatments' live and dead insects were taken from the vials, and the vials were preserved under the same conditions as before. The number of generated adults in F1 was counted 60 days later in the vials.

Field Emission Scanning Electron Microscope (FESEM)

The dead S. oryzae and O. surinamensis insects were examined using FESEM after being exposed to wheat grains treated with 200 ppm of a or γ-AL_2O_3 NPs for seven days. The insects were dried in the open air for five days. Imaging and mapping of the insect body's Al relative fraction.

Data analysis

The Kaplan-Meier survival curve was used to compare the survival distributions of S. oryzae and O. surinamensis insects treated with and without AL2O3 NPs, and statistical analysis was performed with the Log-rank (Mantel-Cox) test in SPSS to compare the survival distributions of the experimental groups. Because treatment with 200 ppm resulted in 100 percent mortality of both insects after one day, it was excluded from the test. To compare the toxicity of each phase of AL_2O_3 utilizing varied concentrations at all tested exposure intervals, the one-way analysis of variance (ANOVA) by

Tukey test (P<0.05) was employed to examine the effect of exposure duration on the resultant mortality data. The same test was used to compare the progeny production of treated and untreated groups in F1. The toxic activity of two phases of AL_2O_3 NPs was investigated using an independent sample T-Test with varied doses at specific exposure intervals; groups with null-variance were excluded from the analysis. After three days of exposure for S. oryzae and one day for O. surinamensis, the effective concentrations of a or γ-AL_2O_3 NPs that killed 50 percent (LC50) and 95 percent (LC95) of treated insects were calculated using Probit analysis (Finney, 1971). Because control mortality was zero in all treatments, no correction was made. For all statistical studies, SPSS version 14.0 was utilized.

3. Results and discussion

Characterization of α and γ-Al_2O_3 NPs

XRD of the synthesized two phases of alumina using cans wastes treated in different media acid and base medium is shown in Fig. 1. Fig. 1a which is treated in acid medium shows crystalline diffraction peaks at 2q =27.3°, 31.6°, 44.6°, 45.2°, 56.2°, 66.1° and 75.3° which corresponded to alpha alumina according to JCPDS card 01-075-0278. For Fig. 1b which treated cans in basic medium shows also crystalline diffraction peaks at 2q = 31.7°, 45.2°, 56.4°and 66.5° which corresponded to boehmite-derived gamma alumina according to JCPDS card 98-009-9836. HRTEM images of Aluminum oxide nano particles synthesized from aluminum cans treated in acid and base medium are shown in Fig. 2. Figure 2a shows a sponge-like mesoporous structure with an average particle size of 4-8 nm for cans treated in acid medium. Whereas, the average particle size of 2-4 nm may be seen in figure 2b, which depicts cans treated with basic medium. ATR-FTIR of Aluminum oxide NPs (4a) and γ Aluminum (4b) are shown in Figure 3. The bands at 3355 cm-1 and 1629 cm-1 for Aluminum oxide and at 3393 cm-1 and 1638 cm-1 for γ Aluminum. The bands at 872 cm-1, 726 cm-1, 532 cm-1 and 477 cm-1 are related to pseudo boehmite structure vibration for α or γ-AL2O3 NPs [32-33]

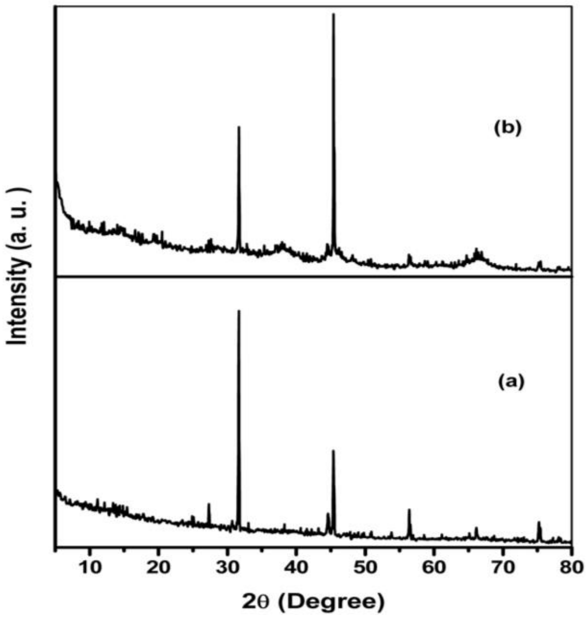

Fig. 1. XRD patterns of (a) α-Al_2O_3 and (b) γ- Al_2O_3

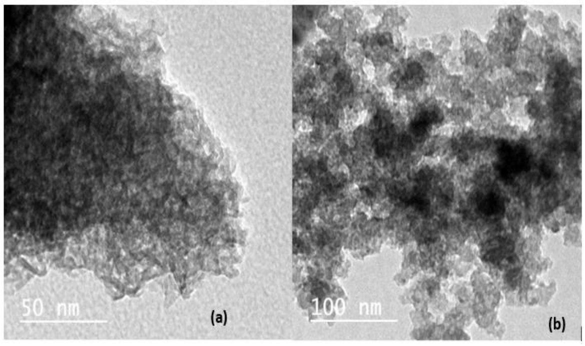

Fig. 2. HRTEM of (a) α-Al_2O_3 and (b) γ- Al_2O_3

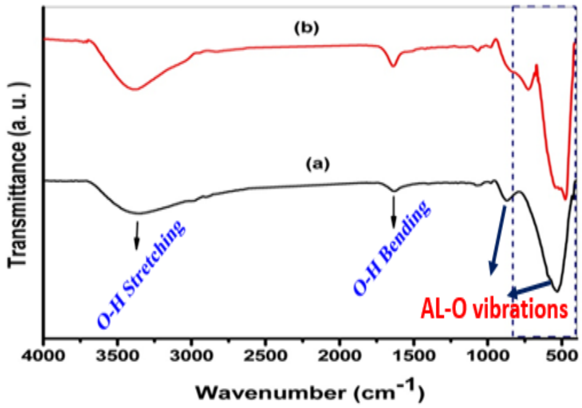

Figure 3: ATR-FTIR (Transmittance mode) spectra of α and - γ Al_2O_3

Identification of fungal isolates

Colony shape, texture, and medium pigmentation differed significantly among 15 fungal colonies cultivated on Rosaebengal Agar media (Figure 4). According to the taxonomy provided by Ainsworth, [34] and Alexopoulos et al., [35].

Fig.1 shows the three isolates as Fusarium oxysporum (Fig.4.a), Aspergillus flavus (Fig.4.b), and Alternaria sp. (Fig.4.c). Gulbis et al., [5] found that Fusarium, Alternaria, and Aspergillus sp., which produce Aflatoxin and Mycotoxin, were the most common fungi species in damaged maize and soybean grains, which is consistent with our findings from the isolation and identification experimental method.

Evaluation of antifungal activity.

Figure 5 and 6 shows the influence of and-type aluminum nanoparticles on the growth inhibition of different fungal strains in millimeters. All of the fungal strains had varying levels of sensitivity to different types of Al nanoparticles. The acquired results revealed that both types of aluminum nanoparticles have equal antifungal activity, however the - nanoparticles type had a stronger destructive activity against the selected fungal isolates, as evidenced by the growth clearance zone formed after incubation. In general, A. flavus was the most impacted strain, as evidenced by the highest inhibitory formed after development, followed by F. oxysporum, with inhibition zones of 13.5 and 8 mm for -nanoparticles type, respectively. Alternaria sp. has a lower sensitivity response when exposed to nanoparticles with a 1.5 mm inhibitory zone. Similarly, when antifungal activity of $-Al_2O_3$ nanoparticles was tested, the most impacted strain with the highest inhibitory activity was A. flavus, followed by F. oxysporum with inhibition zones of 9 and 7mm, respectively, while Alternaria sp. had an inhibition zone of 1.5mm. This results agreed with the results of Suryavanshi et al., [37] studied the antifungal efficacy of aluminium nanoparticles against diverse food borne pathogenic fungi such as Fusarium Oxysporum and Aspergillus flavus and found that they were effective. Aluminum nanoparticles were found to have antifungal action against F. oxysporum and A. flavus at MIC values of 250 and 150 g/ml, respectively. In addition, Shenashenet al., [38] investigated the antifungal activity of mesoporous aluminum nanoparticles against Fusarium Oxysporum and discovered that the highest antifungal activity was observed at a concentration of 400mg/L, with a maximum inhibition percentage of 78.57 percent after growth on PDA plates, compared to the control treatment.

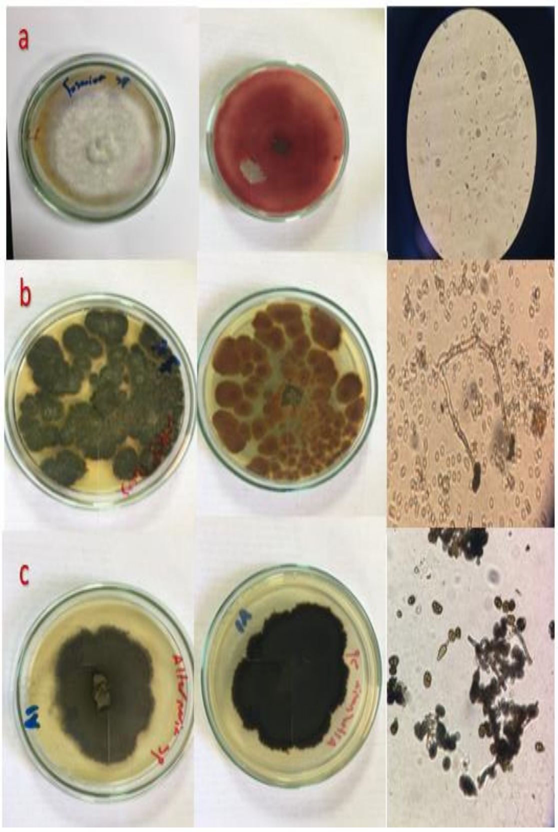

Figure 4: Morphological features of fungi isolated from maize and soy grains (*Fusarium Sp.* (Fig.4.a) *A. flavus* (Fig.4.b), and *Alternaria sp* (Fig.4.c).

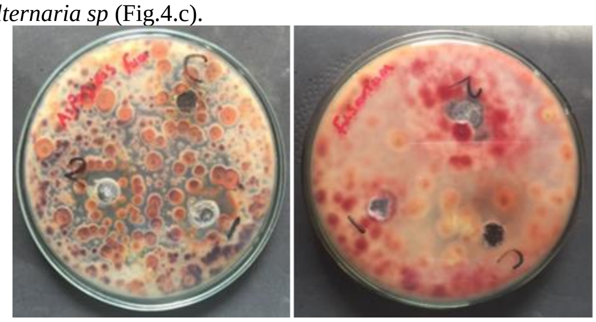

Figure 5: Inhibition zone for the selected fungi

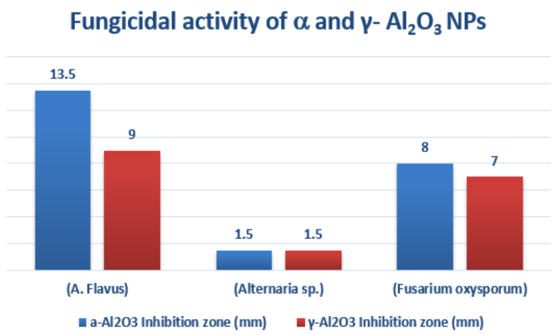

Figure 6. Inhibition zone measurements in millimeters for the selected fungi

Evaluation of insecticidal activity.

Survival time test

The log-rank test of survival time with AL_2O_3 NPs phase and concentration performed by Kaplan–Meier estimators revealed significant relationships of survival time with all α or γ-AL_2O_3 NPs treatments in adults of *S. oryzae* and *O. surinamensis* (Fig. 7). Individuals in both species' control groups survived to the end of the experiment (15 days). In comparison to control groups, survival time was considerably reduced after application of α or γ-AL_2O_3 NPs in various concentrations to *S. oryzae* and *O. surinamensis*. Gamma AL_2O_3 NPs had a greater impact on both insect species than alpha phase NPs. *S. oryzae* treated with 150 ppm of γ-AL_2O_3 NPs followed by 100 ppm of the same phase had the shortest survival duration (Fig. 7a). Adult *O. surinamensis* treated with 150 and 100 ppm of γ-AL_2O_3 NPs had a similar effect (Fig. 7b). α-AL_2O_3 NPs at 50 ppm showed minimal efficacy in both insect species, as shown in the survival curve in Fig (7). Also, mortality was delayed at lower concentrations of α or γ-AL_2O_3 NPs, however only few insects of *O. surinamensis* and *S. oryzae* had survived to the third and fifteenth days, respectively.

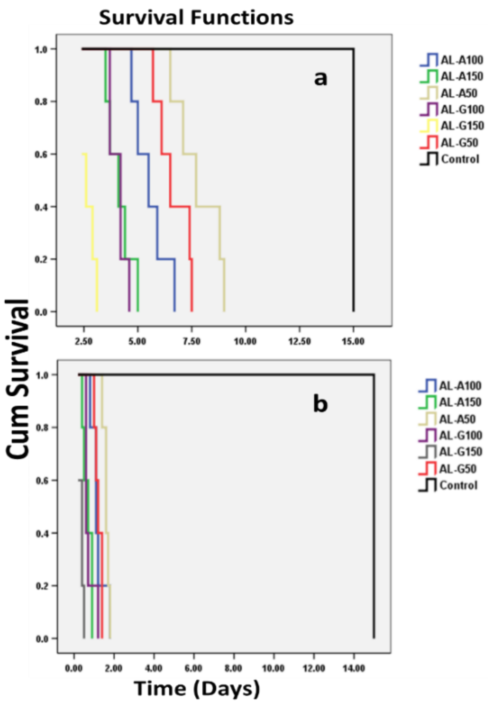

Fig. 7. Survival of a) *Sitophilus oryzae* and b) *Oryzaephilus surinamensis* adults visualized using Kaplan-Meier survival curve (Log-rank (Mantel- Cox) test: Chi2 value 84.348 (a), 64.955 (b), df 6, *P*< 0.0001). The concentration of 200 ppm was omitted. AL-A= α-AL_2O_3 NPs and AL-G= γ-AL_2O_3 NPs.

Nanoparticles have the potential to aid in the development of new insecticides. Nanoparticle insecticides are a cutting-edge technology for controlling pests that have gained resistance to standard pesticides. Previous researches have reported the insecticidal efficiency of AL_2O_3 NPs on various insect species [9, 25, 37-39]. The quick action of larger doses (150, 100 ppm) of α or γ-AL_2O_3 NPs in this study may explain the short survival period of *S. oryzae* and *O. surinamensis* treated adults. Because of the negative effects of AL_2O_3 NPs on water balance, the formation of oxidative stress, and interference with insect movement and mating activity, insect performance is lowered [40-41]. Furthermore, the electrically charged particles formed by aluminum oxidation are likely to be responsible for AL_2O_3 NPs' insecticidal activity. The dipole-dipole interaction of these electrically charged particles promotes the formation of aggregates that attach securely to the insect's cuticular wax and generate electric charges via the triboelectric effect [42-43]. As a result of the charged surface of AL_2O_3 NPs, the NPs stick to the insect cuticle, causing harm via enhancing water diffusion through the cuticle [39]. As a result, desiccation stress appears to be the primary cause of death in pests treated with AL_2O_3 [40], as evidenced by DEs, ZnO, and zeolite NPs [3, 44- 47].

Effect of exposure time

The mortality of both insect species was significantly influenced by exposure time and α or γ-AL_2O_3 NPs concentration ($P<0.05$) according to a one-way ANOVA. The greatest concentration of α or γ-AL_2O_3 NPs (200 ppm) induced 100 percent mortality of *S. oryzae* adults after one day of treatment, as shown in Fig. 8. α-AL_2O_3 NPs at lower concentrations of 50 and 100 ppm demonstrated equivalent toxicity at the same exposure duration. While, the number of dead insects increased dramatically when the exposure period was increased. Long-term exposure to both phases of AL_2O_3 NPs resulted in considerable adult mortality at all doses evaluated. Insect mortality increased to 76±2.4, 80±3.1, and 86±2.4 after 15 days of exposure to α-AL_2O_3 NPs at 50, 100, and 150 ppm, respectively. Similarly, after 1-15 days of exposure, mortality of insects exposed to γ-AL_2O_3 NPs ranged from zero to 84±2.4, 16±2.4 to 92±2.0, and 26±2.4 to 100±0.0 at 50, 100, and 150 ppm, respectively.

The data in Fig. 9 also showed that increasing the time of exposure and the concentration of α or γ-AL_2O_3 NPs increased the mortality of *O. surinamensis*. Maximum mortality caused by 200 ppm of α or γ-AL_2O_3 NPs in the shortest period (one day interval). At 50, 100, and 150 ppm, α-AL_2O_3 NPs induced mortality ranging from 16±2.4 to 82±3.7, 16±6.0 to 94±2.4, and 52±5.8 to 96±4, respectively, after 1-3 days of exposure. The mortality of *O. surinamensis* caused by γ-AL_2O_3 NPs at 50, 100, and 150 ppm varied from 26±5.1 to 88±2.0, 50±5.4 to 98±2, and 72±3.7 to 100±0.0, respectively, throughout this time period. Both phases of AL_2O_3 NPs at 50, 100, and 150 ppm killed all *O. surinamensis* treated insects after 7 days of exposure.

To avoid the spread of infection to clean items, an efficient pesticide should kill pests as rapidly as possible. Inert dusts are known to be slow-acting control agents that need a long period of exposure time to be effective [46]. Adults of *S. oryzae* and *O. surinamensis* were killed after just one day of exposure to wheat grains treated with 200 ppm (200 $mgkg^{-1}$) produced AL_2O_3 NPs in both phases in this study. *O. surinamensis* mortality was >80% in both phases at the lowest dosage of nanoparticles (50 ppm). After three days of exposure. Our findings suggest that controlling both insect species can be accomplished with a lower dosage and in the least amount of time. Belhamel et al. (2020) found that after sixteen days, *Stegobium paniceum* had 100% insect mortality at the highest concentration tested (400 mgkg-1) of alumina NPs, followed by *O. surinamensis* (80.64 percent) and *Tribolium confusum* (79.41 percent).

Mortality in relation to phase of nanoparticles

Figure 10 and 11 shows the mortality of *S. oryzae* and *O. surinamensis* in relation to the phase of AL_2O_3 NPs at different exposure times. On *S. oryzae* adults, 50, 150, and 200 ppm concentrations of α or γ-AL_2O_3 NPs displayed equivalent toxicity after one day of treatment (Fig. 10a). During this time period, just 100 ppm of γ-AL_2O_3 NPs was substantially more hazardous than alpha phase. The mortality of *S. oryzae* caused by the gamma phase was higher, but not considerably, than that produced by the alpha phase after 3 days of exposure (Fig. 10b). After 7 days of treatment γ -AL_2O_3 NPs at 50, 100, and 150 ppm were substantially more harmful to *S. oryzae* adults than alpha phase, as shown in Fig. 10c. Similarly, after 15 days of exposure, the same concentrations of γ-AL_2O_3 NPs had a remarkably harmful effect on adult insects, which was higher than that of α-AL_2O_3 NPs (Fig. 10d).

Figure 11 depicts the influence of nanoparticles phase on *O. surinamensis*. The mortality of insects increased dramatically by gamma phase treatment (Fig. 11a) at 50, 100, and 150 ppm after one day of exposure. After 3 days of exposure, *O. surinamensis* mortality was higher in the gamma phase at the same doses (Fig. 11b). Both phases of nanoparticles had a similar harmful effect on *O. surinamensis* on the seventh day of exposure, resulting in 100% mortality.

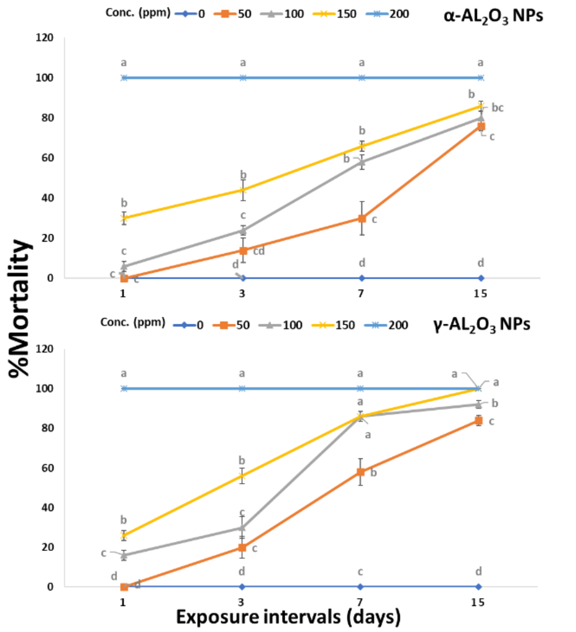

Fig. 8. Mortality of *Sitophilus oryzae* adults exposed to different concentrations of α or γ-AL_2O_3 NPs at different time intervals. Means (±SE) followed by different letters within the same exposure interval are significantly different, Tukey test (P<0.05).

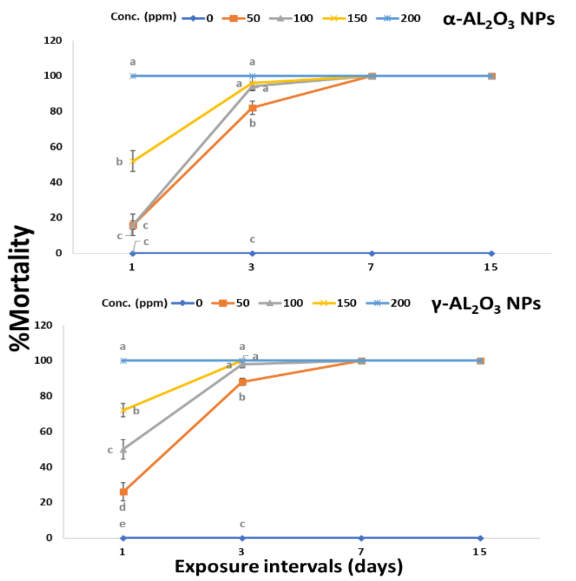

Fig. 9. Mortality of *Oryzaephilus surinamensis* adults exposed to different concentrations of α or γ-AL_2O_3 NPs at different time intervals. Means (±SE) followed by different letters within the same exposure interval are significantly different, Tukey test (P<0.05).

A concentration-response test was used to investigate the toxicity of α or γ-AL_2O_3 NPs to *S. oryzae* and *O. surinamensis* (Table 1). After one day of exposure, γ-AL_2O_3 NPs were shown to be more harmful to *O. surinamensis*, with LC_{50} and LC_{95} of 97.392 and 199.037 ppm, respectively, compared to LC_{50} and LC_{95} of 131.140 and 219.343 ppm, respectively, for α -AL_2O_3 NPs. As shown in Table 1, γ-AL_2O_3 NPs had a stronger harmful effect on *S. oryzae* adults after 3 days of exposure, with LC_{50} and LC_{95} values of 120.031 and 218.960 ppm, respectively, compared to 132.578 and 224.825 ppm for alpha phase treatment.

Environmental conditions, insect species, and the phase of AL_2O_3 NPs (α and γ) are all well-known factors that influence the percentage of insect mortality [17, 37]. By boosting the mortality of tested insect species, the γ-AL_2O_3 NPs proved to be more effective. The substantial toxicity of NPs against adults of *S. oryzae* and *O. surinamensis* may be related to NP features that expedite the adsorption of cuticular lipids, resulting in cuticular injury. Similarly, Lazarevic et al. [41] ascribed alumina powder's considerable toxicity against *Acanthoscelides obtectus* to the powder's huge specific surface area and porosity. After three and one days of exposure, the obtained data revealed that just 218.96 and 199.037 ppm of γ-AL_2O_3 are necessary to kill 95 percent of *S. oryzae* and *O. surinamensis* adults, respectively. Previously, 1000 ppm of synthetic alumina killed all *A. obtectus* after seven days of exposure duration in earlier research [40]. On the second day of exposure, a 2 gkg-1 dose of ANP- γ induced 100 percent mortality of *S. oryzae* [45].

Progeny production

Results in Fig. 12 describe the association between phase of nanoparticles and the number of emerging adults in the F1 generation at varied concentrations. When compared to untreated groups, the post hoc Tukey test indicated that both AL_2O_3 NPs phases and tested concentrations significantly reduced the number of adults in F1 of *S. oryzae* and *O. surinamensis*. The effect of nanoparticles on *S. oryzae* progeny production revealed that at 50 ppm gamma phase, the number of adults in F1 was reduced to 12.60±1.54 (Fig. 12a), whereas it was 26.0±3.74 after alpha phase treatments. The highest concentration; 200 ppm of both phases entirely inhibited the production of F1 adults.

The progeny count of *O. surinamensis* was clearly impacted by two stages of AL_2O_3 NPs treatment, as shown in Fig.12b, and it was more vulnerable than *S. oryzae*. After treatment with 150 and 200 ppm of α-AL_2O_3, no adults appeared, and all concentrations of γ-AL_2O_3 NPs decreased the development of adults in the F1 generation when compared to the number of adults counted in the untreated group (97.0±1.71).

Our findings show that the efficacy of α or γ-AL_2O_3 NPs against the insect species examined was dose-dependent. Furthermore, we discovered that treatment with α or γ-AL_2O_3 NPs dramatically reduced progeny generation in both insect species after two months of incubation when compared to untreated groups. The significant toxicity of nanoparticles resulted in higher mortality of parental adults of *S. oryzae* and *O. surinamensis*, resulting in a decrease in F1 progeny. These findings are consistent with those of [19], who found that increasing the exposure period and the concentration of Al_2O_3 and ZnO NPs enhanced the mortality of *S. oryzae* adults significantly. In addition, the two materials dramatically reduced the number of offspring. In a prior work, treatment of *S. oryzae* with 250 and 500 ppm of nanostructured alumina resulted in considerable adult mortality and progeny suppression, depending on exposure period and concentration [49].

Field emission scanning electron microscopy (FESEM)

As shown in Fig. 13, AL_2O_3 nanoparticles adhere to the body surface of adults of *S. oryzae* and *O. surinamensis* treated with 200 ppm of both phases. The intensity of gamma phase nanoparticle distribution on treated insects was larger than that of alpha phase, as shown in the images. In contrast to untreated insects, both phases of nanoparticles were uniformly dispersed and strongly adhered to the body surface of treated insects (Fig. 14). Adults of *O. surinamensis* were shown to be more vulnerable to nanoparticles than adults of *S. oryzae* in this study. Particle adhesion to insect cuticle is influenced by cuticle composition, insect movement, and feeding behaviour [3, 50]. As a result, the efficiency of nanoparticles used in different species varied. The robust attachment of nanoparticles, particularly gamma phase, to the insect body is confirmed in this study by imaging and mapping the relative fraction of *Al* on the surface of treated insects. The very small size of AL_2O_3 NPs boosted particle adhesion in the cuticle, according to our findings. Thus, changes in NP attachment may explain the efficiency of NPs among different stored product beetle species [51]. Furthermore, certain flat-bodied species, such as *O. surinamensis* and *Cryptolestes ferrugineus*, have been discovered to be the most sensitive insect species to DEs at the adult stage

[51-52]. *O. surinamensis* was shown to be oversensitive to zeolites of various particle sizes and kaolin than *S. oryzae* and *S. granarius* in a prior study [8, 47, 52].

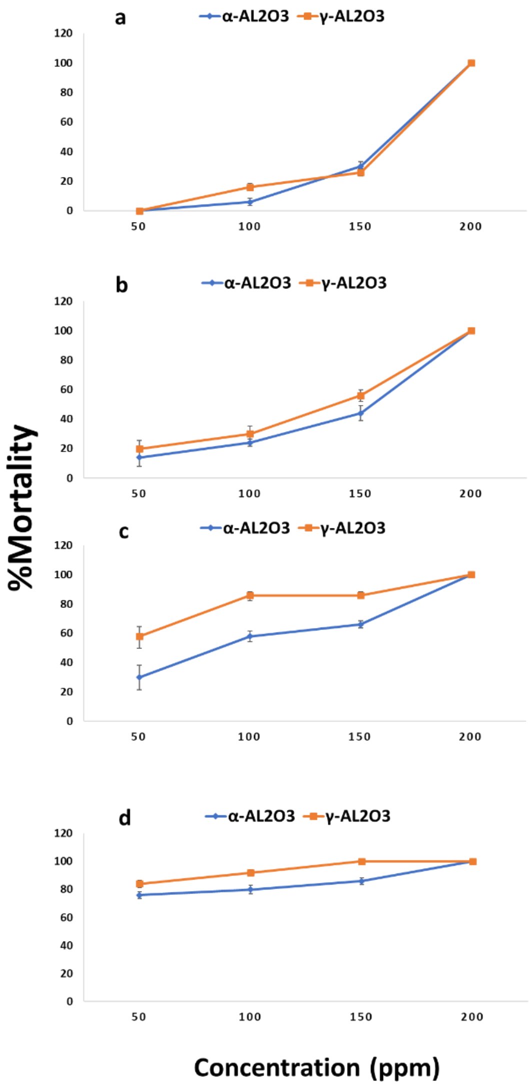

Fig. 10. Concentration-mortality data of *Sitophilus oryzae* adults (M±SE) in relation to phases of AL_2O_3 NPs at exposure time of; a) one day b) 3 days, c) 7 days, and d) 15 days.

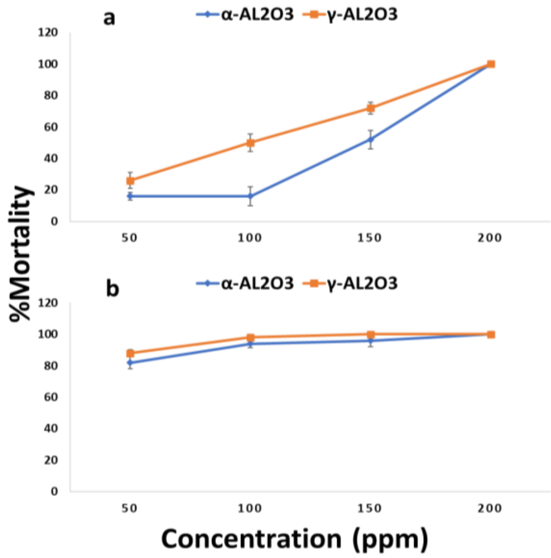

Fig. 11. Concentration-mortality data of *Oryzaephilus* adults (M±SE) in relation to phases of AL_2O_3 NPs at exposure time of; a) one day and b) 3 days.

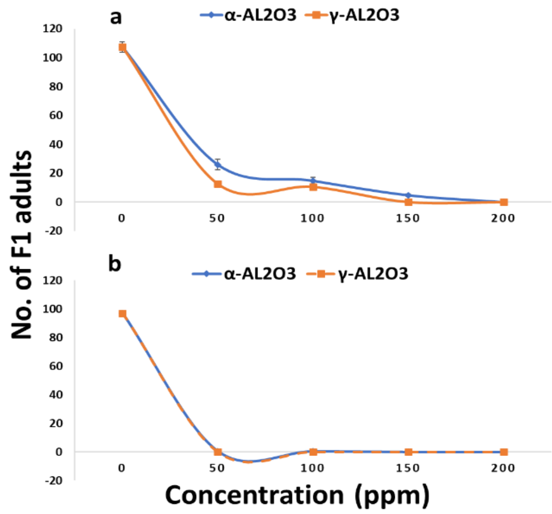

Fig.12. Mean number (±SE) of F1 adults of *Sitophilus oryzae* (a) and *Oryzaephilus surinamensis* (b) in relation to concentration of α or γ-AL2O3 NPs.

Table 1 Toxicity of α and γ-AL2O3 NPs on *Sitophilus oryzae* and *Oryzaephilus surinamensis* adults

	Nanomaterial	LC_{50} (95% CLs)	LC_{95} (95% CLs)	Slope±SE	Chi-Square
O. surinamensis (After one day exposure)	α-AL2O3	131.140 (115.963-147.654)	219.343 (193.451-265.047)	0.019±0.001	279.939
	γ-AL2O3	97.392 (84.828-108.541)	199.037 (179.396-228.863)	0.016±0.001	123.739
S. oryzae (After 3 days exposure)	α-AL2O3	132.578 (115.942-150.942)	224.825 (196.121-278.717)	0.018 ±0.001	314.191
	γ-AL2O3	120.031 (104.920-135.276)	218.960 (192.867-264.043)	0.017±0.001	225.991

Concentrations (in ppm) that killed 50% (LC_{50}) and 95% insects (LC_{95}) are presented with confidence limits (95%CLs) in parentheses, $P<0.05$.

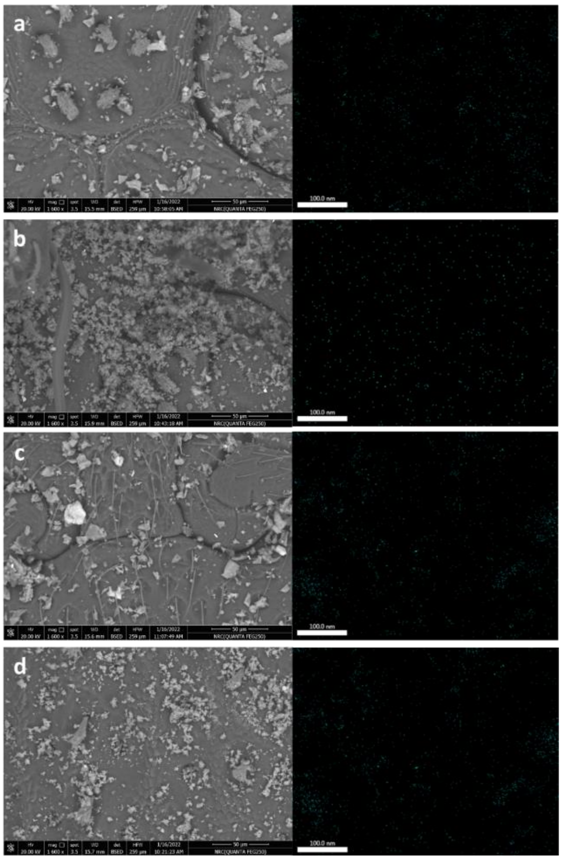

Fig. 13. FESEM images of insect cuticle ventral view exposed to 200 ppm of AL_2O_3 NPs for 7 days showing abrasion and impregnation of NPs adhere to hairy areas of insects' body. Aluminum (*Al*) counts from Energy Dispersive Spectroscopy (EDS) show high degree of gamma phase presence more than alpha phase; a) *Sitophilus oryzae* treated with α-AL_2O_3 NPs, b) *Sitophilus oryzae* treated with γ-AL_2O_3 NPs, c) *Oryzaephilus surinamensis* treated with α-AL_2O_3 NPs and d) *Oryzaephilus surinamensis* treated with γ-AL_2O_3 NPs.

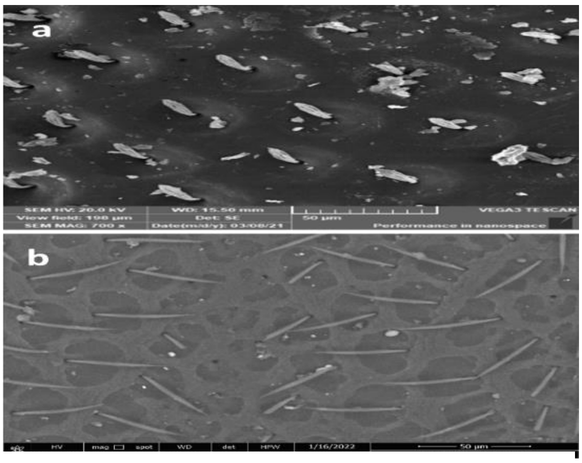

Fig. 14. FESEM images of untreated insect cuticle ventral view; a) *Sitophilus oryzae* and b) *Oryzaephilus surinamensis.*

Conclusion

The structure, morphology, and insecticidal action of α or γ-AL_2O_3 NPs were investigated. The production of Al_2O_3 was confirmed by HRTEM, FESEM and XRD. The typical particle size of α-Al_2O_3 is 4-8 nm, whereas γ-AL_2O_3 NPs are 2-4 nm, according to HRTEM. The FESEM structure of α-Al_2O_3 is spongy and porous. *S. oryzae* and *O. surinamensis* were both affected by nanoparticles that were created. Isolation of *A. flavus*, *Fusarium sp, and Alternaria sp*. fungi form the grain samples. Concerning, fungal isolates response, *A. flavus* was the most sensitive fungus against γ and α-Al_2O_3 nanoparticles with inhibition zone 9 and 13.5mm, followed by *F. oxysporum* with 7 and 8mm for both types respectively. *Alternaria sp.* was the less sensitive fungal isolate with 1.5mm inhibition zone for both aluminum nanoparticles types. Thus, Alpha-Al_2O_3 nanoparticles possess a higher antifungal activity than γ-Al_2O_3 type. The survival and progeny of treated adults were significantly harmed by both phases of alumina NPs. The effectiveness of α or γ-AL_2O_3 NPs against the insect species tested was dose-dependent. By increasing the concentration and exposure time, the mortality of both insect species rose. The physical mode of action of inert dusts such as AL_2O_3 NPs prevents grain pests from developing physiological resistance. Furthermore, because of the low dose of AL_2O_3 NPS utilized in this work (50-200 ppm), the effect of applied nanoparticles on grains is expected to be minimal.

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5. Declarations

Conflict of interest: The authors declare no conflict of interest in this work.

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