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Morphological, optical and thermal properties of α - and γ -aluminum nanoparticles: Assessment of their biological activities against storage mites and mycotoxin producing fungi

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ABSTRACT

Recently, using eco-friendly controlling methods against pests has been getting great attention. Therefore, this study aimed to assess synthesis of two different phases of nano aluminum oxide, alpha and Gamma, (α - and γ -Al₂O₃ NPs) from wastes. Additionally, evaluate them to control stored product mites, *Mycetoglyphus fungivorus*, and *Caloglyphus mycophagus*, and the fungi *Aspergillus flavus*, *Alternaria* sp., and *Fusarium oxysporum*. Two phases of Al₂O₃ NPs were synthesized in acidic and basic media and characterized using the field emission scanning electron microscope (FESEM), energy dispersive X-ray spectroscopy (EDX), ultraviolet-visible (UV-Vis) and thermo gravimetric analysis (TGA). Mortality percentages of mites were evaluated at concentrations of 10, 30 and 60 ppm after 1, 2, 3 and 4 days. The growth of fungi was assessed to determine the fungicidal concentrations in PDB cultures by Mycelium Dry Weights Method. The study confirmed the formation and thermal stability of α - and γ -Al₂O₃ NPs using FESEM, UV-Vis and TGA. The average particle size of α -Al₂O₃ is 4–10 nm and for γ -Al₂O₃ is 14–30 nm. Both phases were toxic to tested stages of mites. Increasing concentration and exposure time, results in increased mite mortality rates. γ -Al₂O₃ NPs were more efficient than α -NPs post initial exposure, then all through time both have the same effect. The female's fecundity was significantly influenced. Tested eggs did not hatch at all. The toxic activity remained strong after 1, 2, and 3 months of exposure. The SEM images revealed a strong adhesive of α - and γ -NPs which were visibly bonded to various areas of dead mite females. γ -Al₂O₃ showed antifungal activity more than the α -NPs. Concentrations of fungicides depended on the fungal strain sensitivity. It has been concluded that α - and γ -Al₂O₃ NPs are efficient environmentally friendly agents against the proliferation of storage mites and mycotoxin producing fungi.

KEYWORDS: Alpha and gamma aluminum oxide nanoparticles, aluminum cans, eco-friendly products, exposure time, stored product mites.

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INTRODUCTION

Cereal crops, such as maize, wheat, beans, rice, and soybeans, are necessary sources of carbohydrates,

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proteins, lipids, minerals, vitamins, and oils, which are necessary food and feed for maintaining the health of humans and animals worldwide (Abd-Elsamee *et al.* 2012; Liu *et al.* 2020; Elsherif *et al.* 2021, 2022; Oso and Ashafa 2021). These crops are vulnerable to a variety of pests during storage under unsuitable conditions, as the interaction between grains and pests mainly depends on environmental changes, such as storage locations, temperature, and humidity (Malik *et al.* 2018; Vogel *et al.* 2021).

Mycetoglyphus fungivorus Oudemans and *Caloglyphus mycophagus* (Mégnin) (Acari: Acaridae) are two of the most important pests infesting a wide range of stored grains (Li *et al.* 2015). Mites inflict damage through inhibiting grain germination, altering moisture and nutrient content (da Silva *et al.* 2019). Mites have an allergic reaction-like effect on human health (Fones *et al.* 2020). The small size of the mites and the difficulty of seeing them led to their rapid reproduction. Damage is typically not recognized until grains are removed from storage facilities (Hubert 2012). In addition, mites can encourage fungal growth (Vogel *et al.* 2021) and promote fungal dispersion via transporting spores on their body surface or through their feces (da Silva *et al.* 2019).

Mites-fungi interaction creates hazardous substances that render them inappropriate for animals (Kumar and Kalita 2017). Consumption of produced fungal mycotoxins substances in contaminated grains, causes various diseases proven to be toxic to human and animals (Kępińska-Pacelik and Biel 2021). In general, the Acaridae mites are becoming increasingly important as storage pests due to their increased prevalence, associations with insects, or interactions with fungus that cause rapid qualitative and quantitative loss of grains (Sushma *et al.* 2022).

Traditional pest control methods like synthetic pesticides are accompanied by worries about pest resistance and unfavorable effects on both humans and animals (Manu *et al.* 2021). Therefore, efficient alternative management techniques are required to eradicate these mite populations and lower the requirement for pesticides. Inert dusts like Diatomaceous Earth (DE) have become extremely popular in recent years as grain protectants (Baliota and Athanassiou 2023).

Nanotechnology holds significant promise for pest control and food protection in the current decade. Nanocides with their enormous surface-to-volume ratio are significantly more potent than traditional pesticides in far smaller concentrations (Ismail *et al.* 2019; Elbeheri *et al.* 2022; Ibrahim *et al.* 2022).

Nanomaterials show unique physical and chemical properties different from their bulk counterparts that enhances efficacy, reactivity, absorption enough to target pests (Hassan *et al.* 2016; Mohamed *et al.* 2016; Ismail *et al.* 2019; Samy *et al.* 2019, 2023). Metal and metal oxide-based nanoparticles (NPs) application is a new innovate strategy for storage pest control, (Sahayaraj 2017; Jasrotia *et al.* 2021). Several reports emanated the pesticide effect of some metal NPs against storage pests. Silica, zinc, and aluminum NPs showed high toxicity against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) (Keratum *et al.* 2015; Elbeheri *et al.* 2022; Ibrahim *et al.* 2022). Also, zinc, titanium, copper, magnesium and iron against *Rhizoglyphus robini* Claparède (Acari: Acaridae) (Korghon *et al.* 2021). Also, other studies showed metal nanoparticles' effect in inhibiting and eliminating plant pathogenic growth, mycotoxin producing fungi (Horky *et al.* 2018). For example, silver nanoparticle was used to control the growth of two species of *Alternaria* (*A. brassicicola* and *A. solani*) (Gupta and Chauhan 2016; Abdel-Hafez *et al.* 2016). Moreover, Suryavanshi *et al.* (2017) reported the antifungal activity of aluminum nanoparticles against both fungi *Fusarium oxysporum* and *Aspergillus flavus*.

The discovery of nanostructure alumina toxicity opens ways in pest management (Stadler *et al.* 2017). Recently, scientists have focused on environmentally acceptable nanoparticle production from waste, which has been used in a variety of applications (Ibrahim *et al.* 2022; Samy *et al.* 2023). Additionally, the performance of the recycling and repurposing operations is closely related to the financial value of the finished goods. Therefore, using technologies to produce value-added goods from trash seems to be a workable solution. The amount of rubbish produced by aluminum cans poses

a serious threat to the environment. As a result, recycling trash results in material returns from recycling and reusing it, as well as pollution reduction and cost and energy savings, (Menazea *et al.* 2021). Therefore, this study aimed to 1) synthesize two separate phases of nano aluminum oxide, alpha and gamma, (α - and γ -Al₂O₃ NPs), 2) evaluate their bioactivity against stored mites, *M. fungivorus*, and *C. mycophagus* and, 3) detect each α - and γ -Al₂O₃ NPs concentration, which can control and restrict the growth of those common *A. flavus*, *Alternaria* sp. and *F. oxysporum* fungal species and consequently eliminate the infections.

MATERIAL AND METHODS

Synthesis and characterization of α - and γ -Al₂O₃ NPs

Synthesis of alpha and gamma nanoparticles with aluminum cans as a precursor

Alpha aluminum nanoparticles are produced by HCl₆N (Acidic approach), and gamma aluminum nanoparticles are produced by NaOH₆N (Basic approach), then neutralization of each solution. After that, centrifugation of the samples at 4000 rpm for 15 minutes to separate the nanoparticles, then dry them at 70 °C and calcine them at 350 °C.

Utilizing the SEM Model Quanta 250 FEG, a field emission scanning electron microscope (FESEM) with an EDX detector was conducted. At room temperature, Jasco V-630 UV/vis spectrophotometer data spanning the wavelength range of 200–1000 nanometers was acquired. A thermal analyzer, SDT Q600 V20.9 Build 20, was utilized to conduct thermogravimetric analysis (TGA) from room temperature to 1000 °C at a rate of 10 °C/min.

Bioassay of stored product mites

Rearing mites

Mycetoglyphus fungivorus and *C. mycophagus* originated from soybeans and cornmeal provided by an animal feed producer. The races were stocked for many generations at the Acarology laboratory at the National Research Centre (NRC). They were reared in Petri dishes (3 cm in diameter) under 26 ± 2 °C, 75 ± 5% RH and constant darkness. The base of each rearing unit contains a substrate of gypsum mixture and charcoal (1:9 ratios) (Abbatiello 1965). Each rearing unit was filled up with wheat bran (80 g) mixed with baker's yeast (*Saccharomyces cerevisiae*) (20 g) which was used as a diet for mites.

Toxicity assessment

To determine the cumulative toxic effect of α - and γ -Al₂O₃ NPs, females of the same age were moved to experiment units. Each unit is a cylinder (2cm in diameter by 5 cm in height) with holes at its cover for ventilation. Each cylinder was provided with 10 g of (wheat bran +baker's yeast) mixed with α - and γ -Al₂O₃ NPs. Three concentrations of α - and γ -Al₂O₃ NPs were prepared (10, 30 and 60 ppm) of rates, 0.01, 0.03 and 0.06 mg/g of (wheat bran+ baker's yeast). Five replicates were used for each concentration. Each replicate had twenty adult females.

Before transferring the examined females, the cylinder was shaken for a few minutes to make sure that α - and γ -Al₂O₃ NPs distributed well. Mortality of females was calculated daily post-treatment. To check and count the hatched eggs, the dead females were removed, and the eggs remained for another six days. Untreated experiment units served as controls were included. The same procedure was done for larvae and nymphs. All treated and untreated experiment units were kept under controlled conditions at 26 ± 2 °C, 75 ± 5% R.H.

α - and γ -Al₂O₃ NPs evaluation on mites after some time

After 1, 2 and 3 months from the previous final assessment on adult females, the same treated experiment units with the same replicates were used again to verify the residual activity of each tested

material on them and the mortality was recorded after four days.

Scanning Electron Microscope (SEM)

The adherence of each tested Al₂O₃ NP on females of *M. fungivorus* and *C. mycophagus* mites were verified via SEM. The females were air dried before scanning and stuck to adhesive stubs. The evaluated tested females were exposed to 60 ppm (TESCAN, Vega III/Czech Republic). SEM was done in Central Laboratory, NRC, Electronic Microscope Unite.

Bioassay of mycotoxin producing fungi

Fungal species isolation and identification

Aspergillus flavus, *Alternaria* sp. and *F. oxysporum* were isolated from rotted soybeans and cornmeal provided by an animal feed producer. The isolation methodology and identification followed Elbehery *et al.* (2022).

Fungicidal assessment

Fungal growth was determined by measuring the fungal dried mycelium according to Carlile (1970). Potato Dextrose Agar (PDA) media was used to incubate fungi for five days. A growing 7 mm disc was transferred to 100 ml PDA broth culture in a 250 ml conical flask. α - and γ -Al₂O₃ NPs concentrations were determined according to the growth inhibition results obtained from Elbehery *et al.* (2022) which depend on the fungal strain type as follows: (0, 0.5, 1, 2 and 3 mg/100ml) for *A. flavus* (0, 0.5, 2, 4, 5 and 6mg/100ml) for *F. oxysporum* and (0, 5, 10, 20, 30 and 50 mg/100ml) for *Alternaria* sp. for both Al₂O₃ NPs types.

Fungal liquid cultures were incubated at 30 °C for 5 days under 180 rpm. The fungicide activity of Al₂O₃ NPs at different concentrations was measured by filter collecting the fungal mycelia (pre-desiccated, weighted No.1 Whatman filter paper). The mycelia were dried overnight at 70 °C then weighed for two consecutive nights till reached a constant weight.

Statistical analysis

The statistical software program SPSS 28.0 was utilized to conduct all statistical analyses. One-way ANOVA test ($P \leq 0.05$) was used for analyzing the mortality percentages of larvae, nymphs and females, of both mites at various concentrations and exposure times as well as for fungicidal treatments. Duncan multiple range tests were used to compare means. Corrected mortality was not calculated as the control mortality was zero. Treatments that caused 100% mortality of both mites were omitted from the analyses. Independent sample T-test was used to compare the efficacy of Al₂O₃ NPs phases at different concentrations and exposure times, groups with null-variance were also omitted.

RESULTS

Synthesis and characterization of α - and γ -Al₂O₃NPs

Field emission scanning electron microscope (FESEM)

Figure 1 illustrates the FESEM for two different Al₂O₃ NPs that have been synthesized from aluminum Pepsi cans in two distinct procedures. α -Al₂O₃ nanoparticles, which were synthesized in an acidic medium, have an irregular form with sharp edges. γ -Al₂O₃ produced using a basic medium, however, displayed a spongy shape with porous and homogenous aggregates.

Energy dispersive X-ray spectroscopy (EDX)

Figure 2 shows the EDX analysis results. The results of the EDX analysis showed that aluminum and oxygen were present in both treatments, indicating the formation of aluminum oxide because these elements and this ratio were present in aluminum oxide. Additionally, certain elements like Na

and Cl found in alpha aluminum nanoparticles suggest that the sample needs to be washed over and over because the initial washing procedure took a long time.

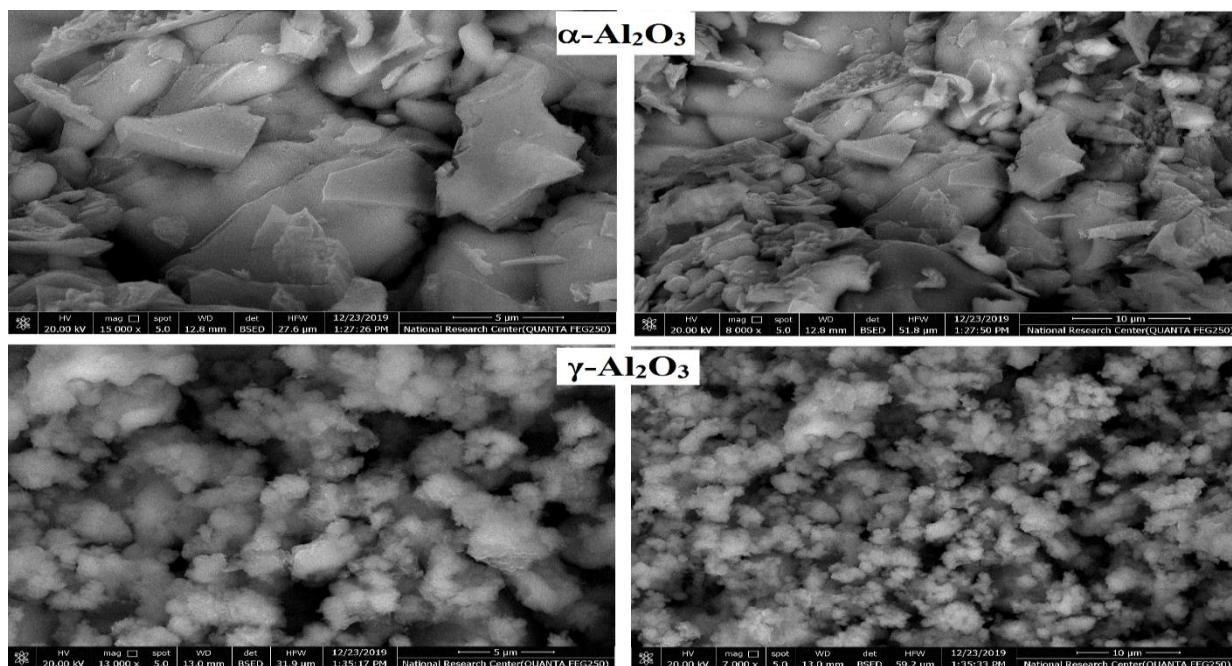


Figure 1. Field emission scanning electron microscope (FESEM) of α - and γ - Al_2O_3 NPs.

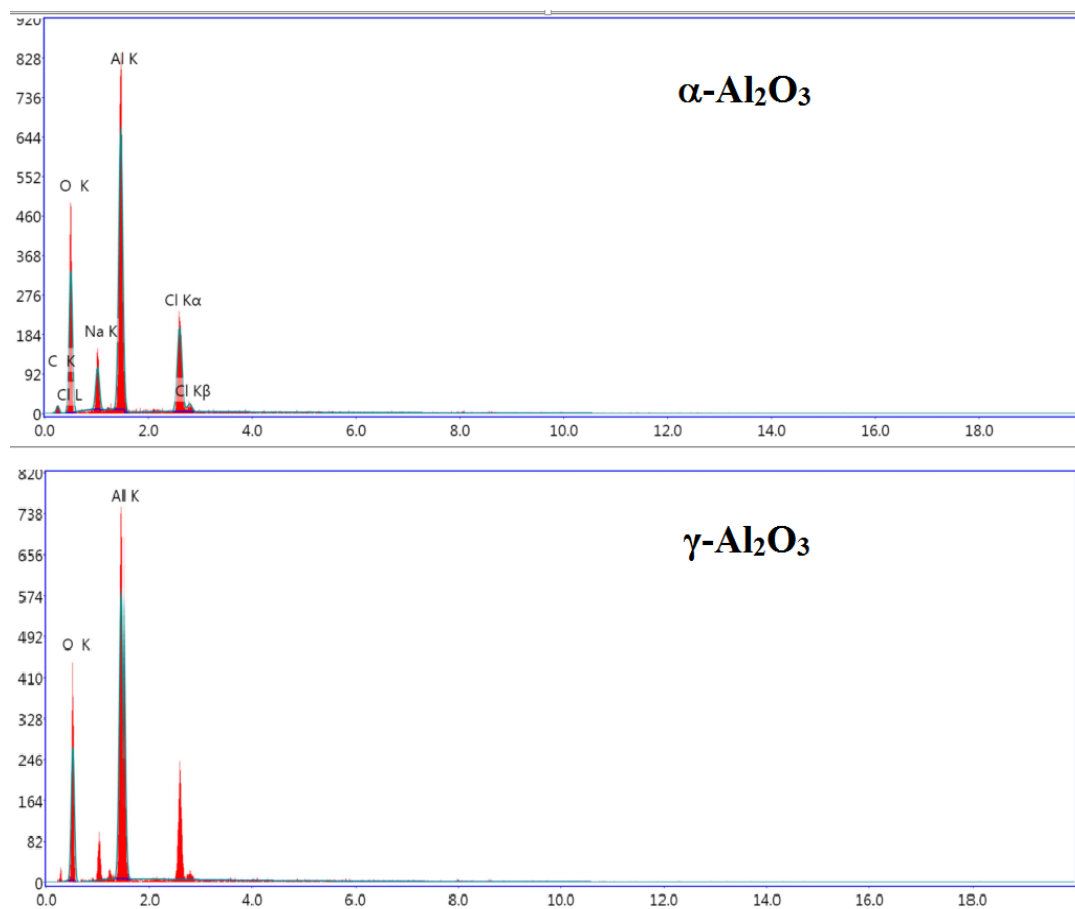


Figure 2. Energy dispersive X-ray spectroscopy of α - and γ - Al_2O_3 NPs.

Optical properties

UV-Vis absorption spectra and optical band gap energy of α -Al₂O₃ NPs (3a) and γ -Al₂O₃ NPs (3b) are depicted in Figure 3. Strong absorption peaks can be seen in the spectra for α -Al₂O₃ NPs and γ -Al₂O₃ NPs at 245 nm and 235 nm, respectively (Prashanth *et al.* 2015; Yuan *et al.* 2015). This is due to electron photo excitation from the valence band to the conduction band.

The direct optical band gap energy (E_g) of both phases Aluminum is calculated using Tauc relation (Tauc 1969):

$$(\alpha h\nu)^2 = \beta (h\nu - E_g) \quad (3)$$

Where β is constant and α is the absorption coefficient and it is determined using the following relation (Siddaiah *et al.* 2018):

$$\alpha(\lambda) = \frac{2.303}{T} A \quad (4)$$

The absorbance and thickness of the synthesized samples are denoted as A and T, respectively. Figure 3 (a and b) represent the plot of $(\alpha h\nu)^2$ against $h\nu$ of the two phases of Aluminum. The energy of the direct optical band gap E_g is determined from the extrapolating the linear portion of the plot with photon energy ($h\nu$). As seen values of E_g are 5.25 eV and 3.5 eV for α -Al₂O₃ NPs and γ -Al₂O₃ NPs, respectively.

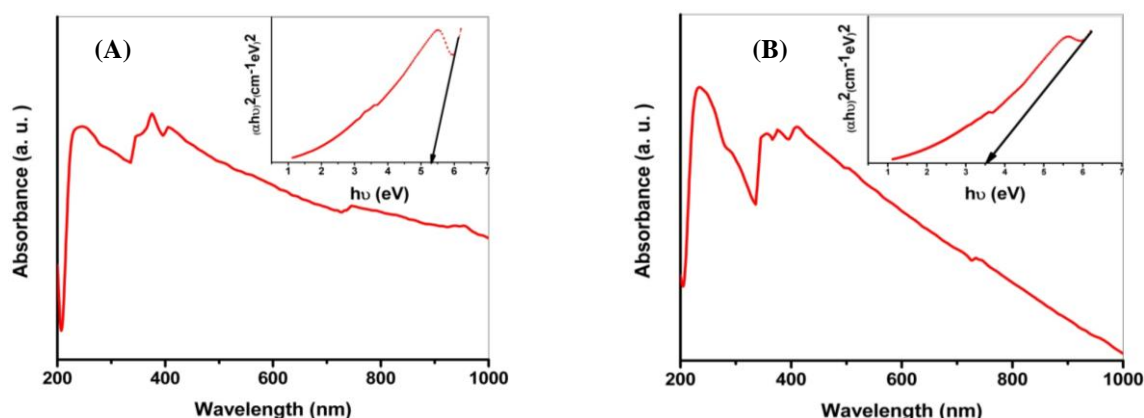


Figure 3. UV-Vis and direct optical band gap spectra of (A) α -Al₂O₃NPs and (B) γ -Al₂O₃NPs.

Thermogravimetric analysis

The thermal characteristics of α -Al₂O₃ NPs and γ -Al₂O₃ NPs are examined by thermogravimetric analysis (TG) over a temperature range spanning from room temperature to 1000 °C, as depicted in Figure 4. A weight loss of 10% was observed at temperatures of approximately 400 °C or 150 °C for α - or γ -Al₂O₃NPs, respectively. It demonstrates the robust interconnections present in the alumina structure. When the temperature approaches 1000 °C, the total weight loss is only 20% or 32% for α or γ -Al₂O₃ NPs, indicating that they have excellent thermal stability—a crucial quality for upcoming industrial applications like high-temperature catalysis and the production of biomaterials.

Bioassay of α - and γ -Al₂O₃ NPs on stored product mites

Acaricidal activity of on females, nymphs, and larvae of M. fungivorus

Mortality–exposure time relationship

Data presented in Figure 1 concluded the mortality percentages of *M. fungivorus* mite stages in relation to exposure time at three tested concentrations. As shown, the highest mortality was 100% by α - and γ -Al₂O₃ NPs on larval stages after one day post-treatments at all tested concentrations. At the same time, the highest percentage mortality of females and nymphs was recorded at 60 ppm, and

it was 61.00 ± 3.32 and 63.00 ± 2.00 by α - Al_2O_3 NPs, respectively and 84.00 ± 1.87 and 92.00 ± 2.55 by γ - Al_2O_3 NPs, respectively. Data in Figure 5 illustrated that the mortality percentages of females and nymphs significantly increased by exposure time and tested concentrations increase ($P = 0.000$). After four days of exposure, phases of Al_2O_3 NPs at 10, 30, and 60 ppm caused mortality to all *M. fungivorus* females and nymphs.

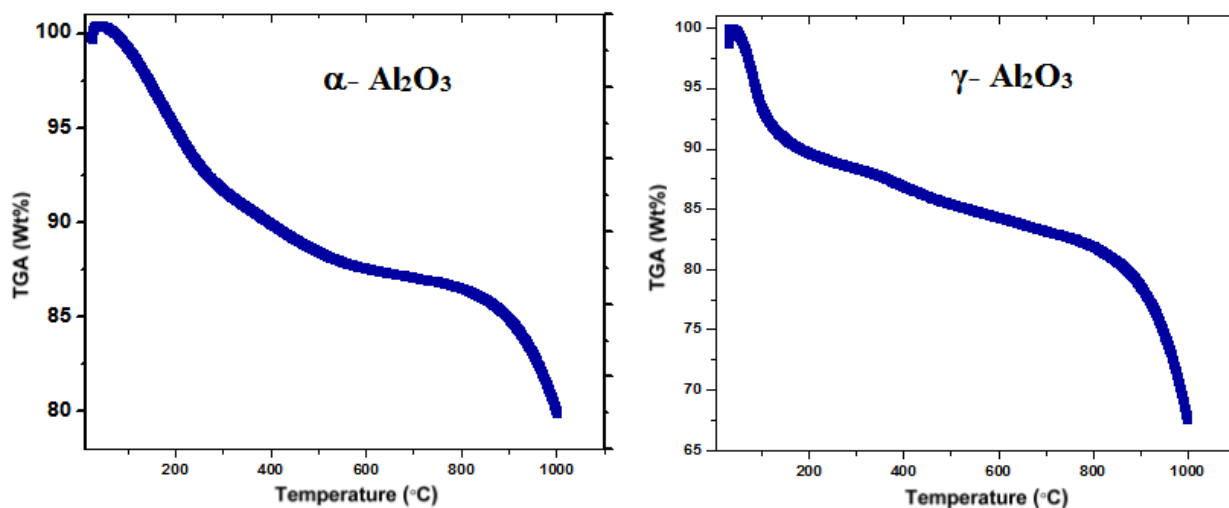


Figure 4. Thermogravimetric analysis curve of α - and γ - Al_2O_3 .

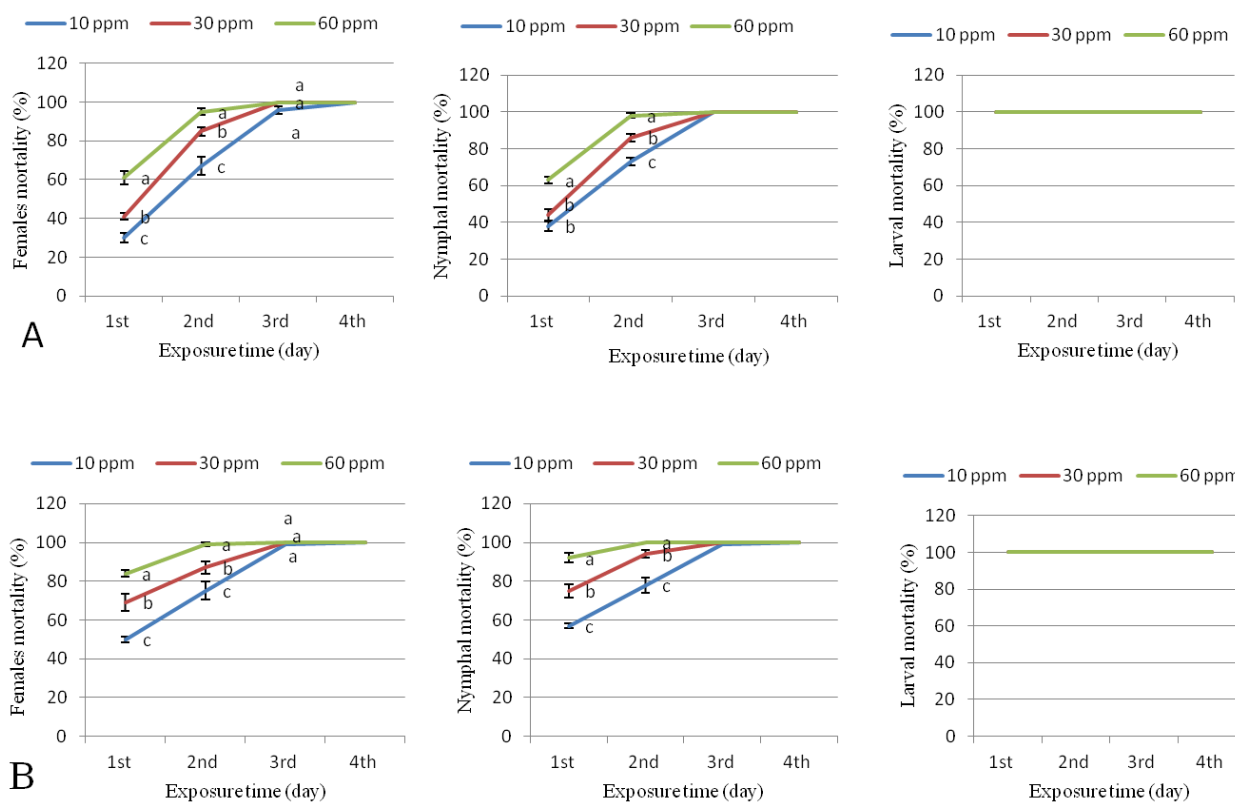


Figure 5. Females, nymphal and larval mortality (means \pm SE) of *M. fungivorus* mites, subjected to synthesized α - and γ - Al_2O_3 NPs at different concentrations and exposure time – **A.** α - Al_2O_3 NPs; **B.** γ - Al_2O_3 NPs. Different letters within the same exposure time are significantly different (Duncan test, $P \leq 0.05$).

Mortality – α - and γ -Al₂O₃ NPs relationship

Independent sample T-test configured the efficacy of α - or γ -Al₂O₃ NPs, and showed significance in the toxic effect on females and nymphs mortalities at 10, 30, 60 ppm concentrations ($P = 0.000$) just after the 1st day of treatments. As illustrated in Figure 6, γ -Al₂O₃ NPs were more toxic than α -Al₂O₃ NPs on both *M. fungivorus* mite stages.

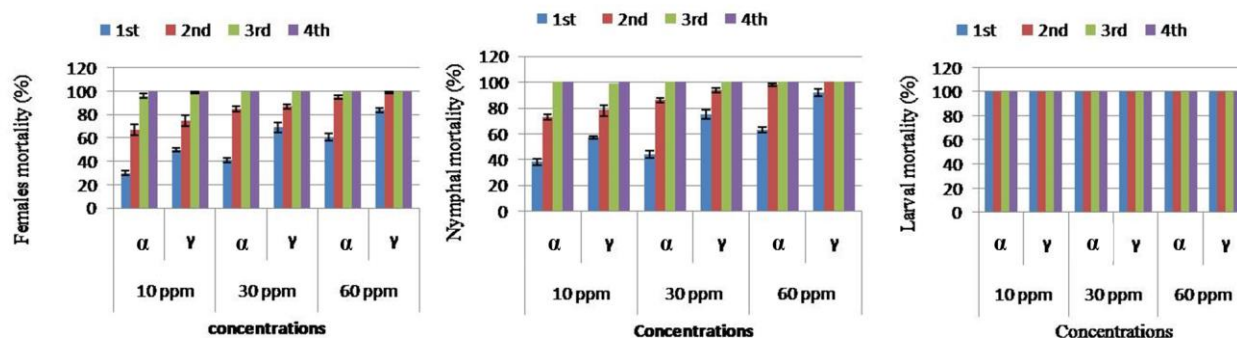


Figure 6. Mortality (means \pm SE) of *M. fungivorus* females, nymphs and larvae, concerning α - and γ -Al₂O₃ NPs at tested concentrations and exposure time.

Acaricidal activity on females, nymphs, and larvae of *C. mycophagus*

Mortality – exposure time relationship

The data shown in Figure 7 indicated that the mortality of females, nymphs and larvae of *C. mycophagus* mites increased with longer exposure times to α and γ -Al₂O₃ NPs. Greatest mortality in shortest time was caused by 60 ppm of α - or γ -Al₂O₃ NPs. For females' mortality, α -Al₂O₃ NPs caused percentage mortality ranging from 20.00 ± 3.50 to 56.00 ± 2.90 , 48.00 ± 1.20 to 90.00 ± 2.70 , and 88.00 ± 2.60 to 100.00 ± 0.00 at 10, 30, and 60 ppm during exposure times of 1–3 days, respectively. At the same times, the mortality percentages of females brought on by γ -Al₂O₃ NPs at 10, 30, and 60 ppm varied from 40.00 ± 2.24 to 68.00 ± 4.64 , 67.00 ± 2.55 to 97.00 ± 2.00 , and 94.00 ± 1.87 to 100.00 ± 0.00 , respectively. The same trend of mortality on females was observed on nymphs and larvae at 10, 30, and 60 ppm, α - and γ -Al₂O₃ NPs killed all nymphs after 3 and 4 days of exposure, respectively. As illustrated in Figure 7, larvae stage was the most sensitive as all died after 3 days post-exposure to α -Al₂O₃ NPs, and after 2 days post-exposure to γ -Al₂O₃ NPs.

Mortality – α - and γ -Al₂O₃ NPs relationship

As illustrated in Figure 8, a significant difference was seen in the efficacy of α - and γ -Al₂O₃ NPs on *C. mycophagus* mite stages at 10, 30, 60 ppm concentrations after 1st and 2nd days of treatment on females and nymphs and after 1st day of treatment on larvae (Sig 2-tailed < 0.05). Other than mentioned exposure times, α - and γ -Al₂O₃ NPs displayed similar toxicity on mite stages.

Acaricidal activity on females' oviposition during four days of exposure

According to data in Figure 9, there was a highly significant reduction in the number of eggs laid by females of both tested mites treated with α - and γ -Al₂O₃ NPs compared to that of control ($P \leq 0.05$). As shown, the number of eggs deposited by *M. fungivorus* females treated with α -Al₂O₃ NPs was reduced from approximately 208 ± 10.27 to 25.00 ± 3.12 , 11.20 ± 1.16 and 1.40 ± 0.60 eggs at 10, 30 and 60 ppm, respectively and it was 5.00 ± 0.71 , 2.00 ± 0.54 and 0.00 eggs at 10, 30 and 60 ppm of γ -Al₂O₃ NPs, respectively. While, the reduced number of eggs deposited by *C. mycophagus* was 116.80 ± 5.13 , 77.8 ± 3.95 and 35.8 ± 3.35 eggs at 10, 30 and 60 ppm of α -Al₂O₃ NPs, respectively and 56.2 ± 0.71 , 10.8 ± 0.54 and 0.0 eggs at 10, 30 and 60 ppm of γ -Al₂O₃ NPs,

respectively compared to that of control, 846.00 ± 24.00 . It was noted that none of the deposited eggs from the treated females of either mite hatched.

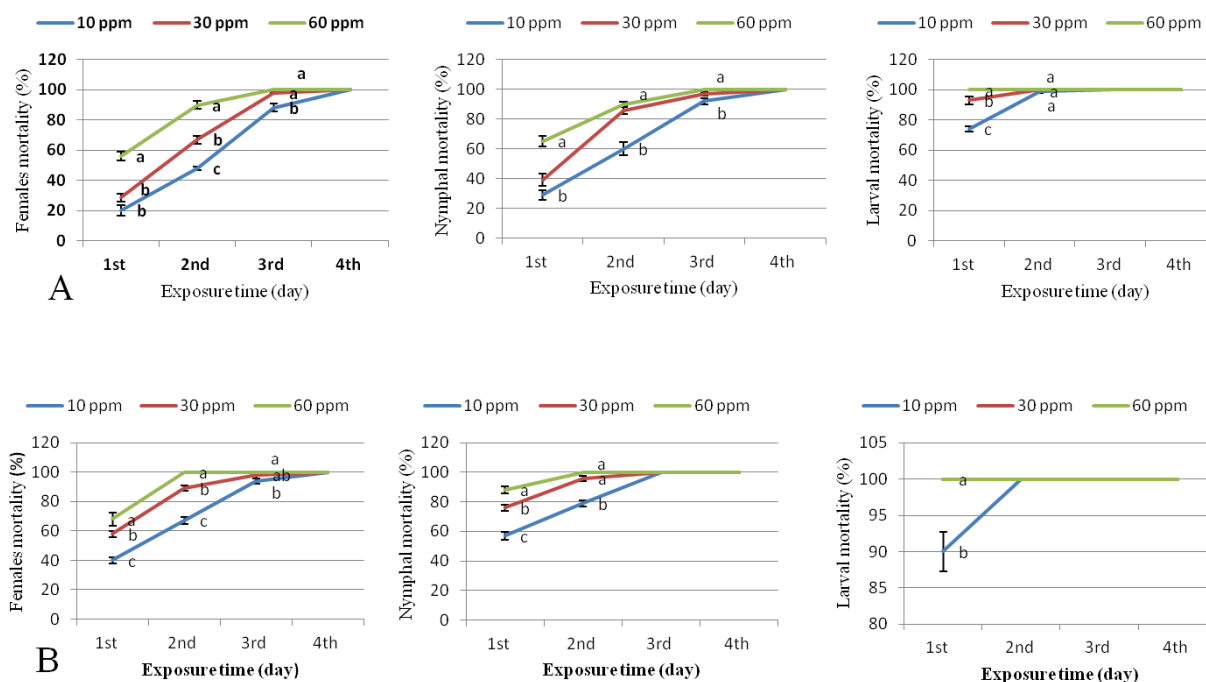


Figure 7. Females, nymphal and larval mortality (means \pm SE) of *C. mycophagus* mites, subjected to synthesized α - and γ -Al₂O₃ NPs at different concentrations and exposure time – **A.** α -Al₂O₃ NPs; **B.** γ -Al₂O₃ NPs. Different letters within the same exposure time are significantly different (Duncan test, $P \leq 0.05$).

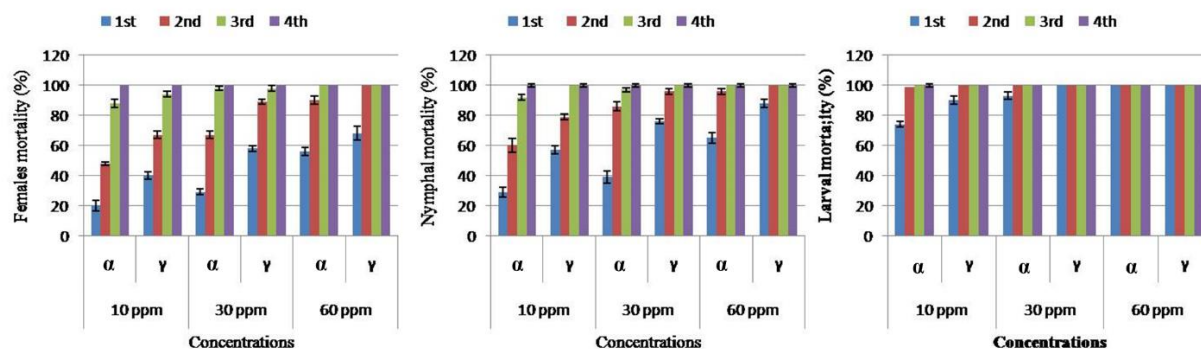


Figure 8. Mortality (means \pm SE) of *C. mycophagus* females, nymphs and larvae, concerning α - and γ -Al₂O₃ NPs at tested concentrations and exposure time.

The effect of α and γ -Al₂O₃ NPs on females after some time

Data presented in Figure 10 proved the efficiency of α - and γ -Al₂O₃ NPs on females of *M. fungivorus* and *C. mycophagus* mites after one, two and three months of treatment. Each phase at each tested concentration showed non-significant difference in mortality after 4th day of detected months. The mortality was $\geq 97\%$ by both phases on *M. fungivorus* mite at 60 ppm; while, at the same concentration ranged from 91.00 ± 4.85 to $99.00 \pm 1.00\%$ and $\geq 99\%$ by α - and γ -Al₂O₃ NPs respectively, in *C. mycophagus* mite. By comparing the data in Figure 5 and 8 on females after 4th day of treatment, the mortality percentage after a period of time was closely related.

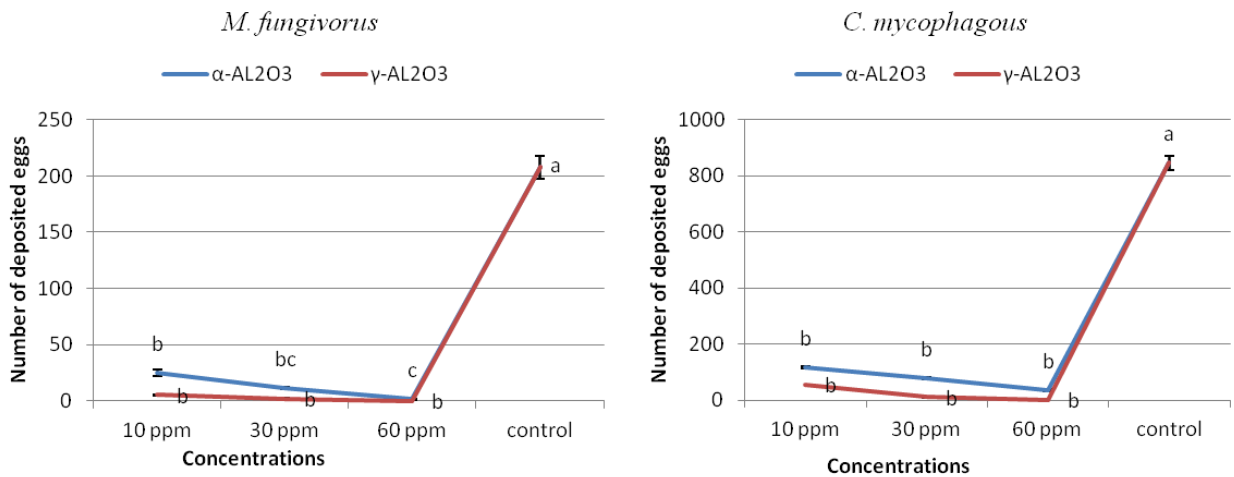


Figure 9. Means number of deposited eggs by females of tested mites after 4 days post-exposure to α - and γ -Al₂O₃ NPs at tested concentrations. Different letters denote to significant differences in means at tested concentrations (Duncan test, $P \leq 0.05$).

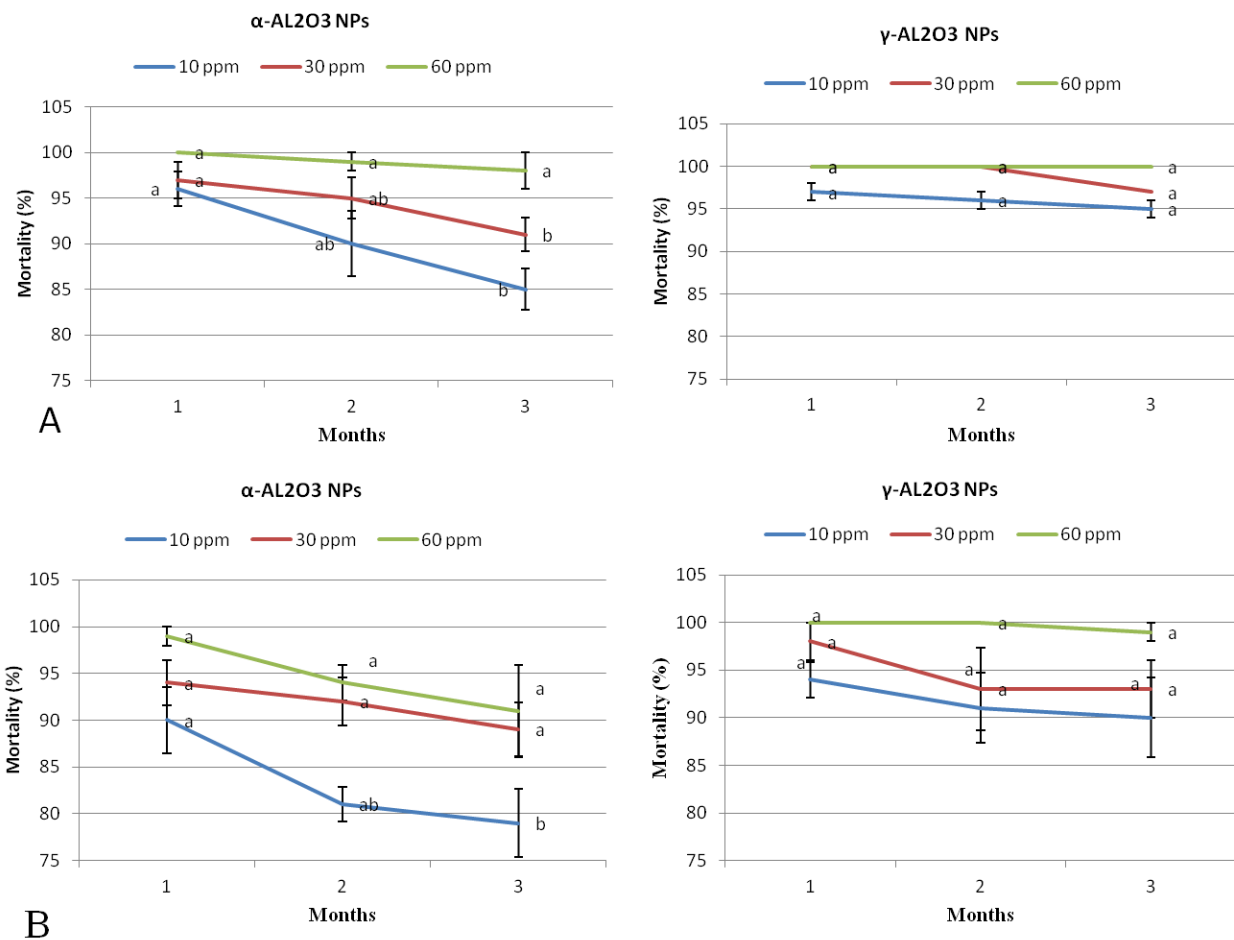


Figure 10. Females' mortality (means \pm SE) of (A) *M. fungivorus* and (B) *C. mycophagous* mites subjected to synthesized α and γ -Al₂O₃ NPs at different concentrations and exposure time. different letters within the same concentrations are significantly different, Duncan test ($P \leq 0.05$).

Scanning Electron Microscope (SEM)

Images of SEM showed the aggregation of α - and γ -Al₂O₃ NPs on the ventral side of *M. fungivorus* and *C. mycophagus* mites compared to that of control. As shown in Figures 11 and 12, the adherence of each phase particles to dead bodies and γ -Al₂O₃ NPs impregnation was higher than α -Al₂O₃ NPs.

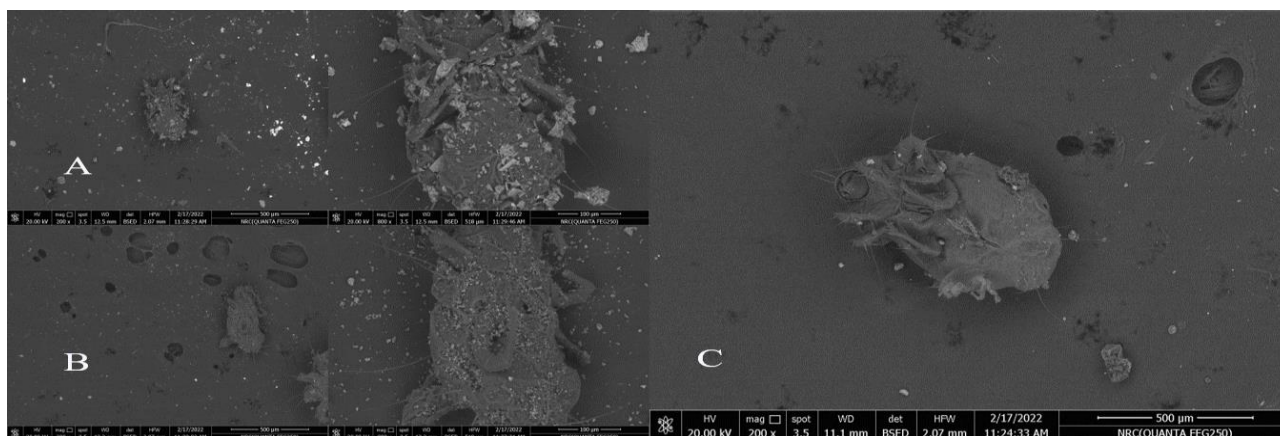


Figure 11. SEM visualization of α - and γ -Al₂O₃ NPs aggregation on ventral side of *M. fungivorus* mite. A = treated female by α -Al₂O₃ NPs, B = treated female by γ -Al₂O₃ NPs, C = untreated female.

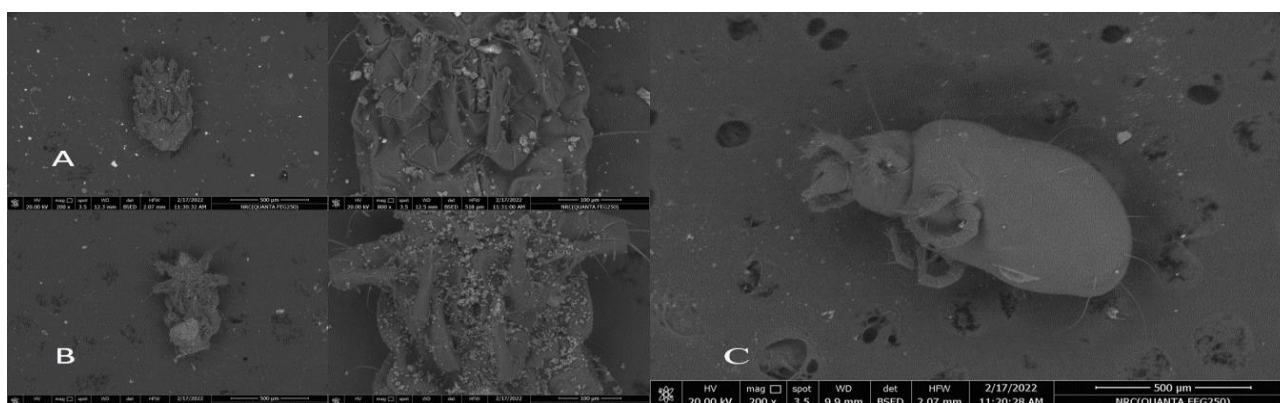


Figure 12. SEM visualization of α - and γ -Al₂O₃ NPs aggregation on ventral side of *C. mycophagus* mite. A = treated female by α -Al₂O₃ NPs, B = treated female by γ -Al₂O₃ NPs, C = untreated female.

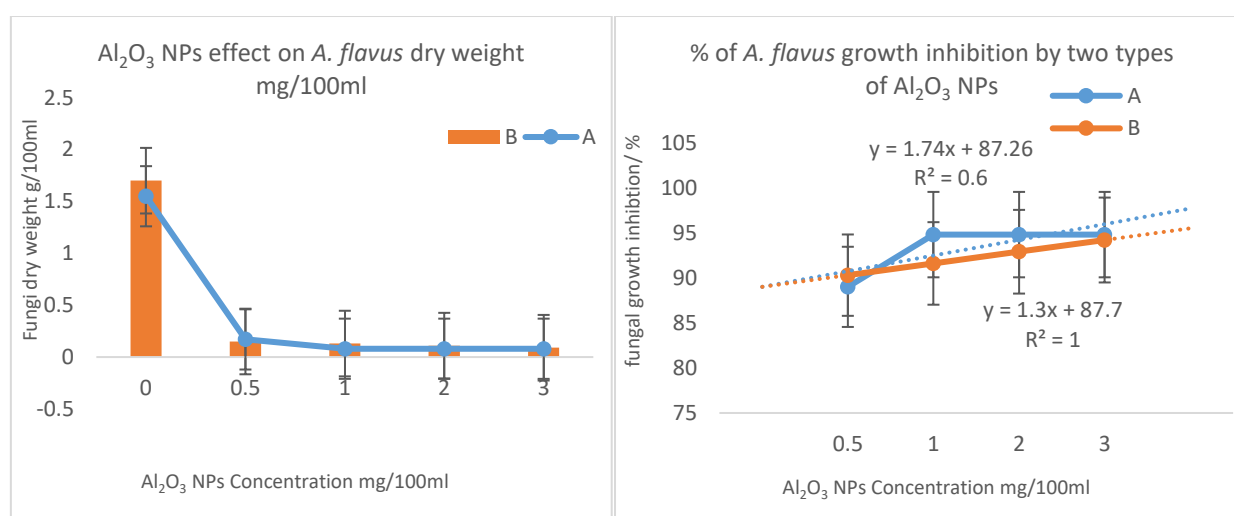
Bioassay of mycotoxin producing fungi

Tables 1–3 demonstrate that the fungal strains' responses to varying doses of α - or γ -Al₂O₃ NPs vary depending on the fungal isolate. Gamma aluminum nanoparticles (A) represented by blue color records, and alpha aluminum nanoparticles (B) represented by orange color records. γ -Al₂O₃ possesses a higher antifungal potency than α -Al₂O₃ as reflected on maximum inhibitory concentration value and the growth inhibition percentage estimated. In case of *A. flavus*, Table 1 and Figure 13 exhibited the maximum inhibitory concentration recorded in 1mg/dl for γ -Al₂O₃, the maximum inhibitory concentration for α -Al₂O₃ was at 3mg/dl with inhibition percentage (94.8 and 94.2) respectively.

Table 2 and Figure 14 show the fungal growth inhibition recorded at different concentrations of both types of aluminum nanoparticles against *F. oxysporum*. Data illustrated that the maximum inhibitory concentration detected for gamma and alpha aluminum nanoparticles was similar for both types at ratio 6mg/100ml with inhibition percentage (98.6 and 95%) respectively.

Table 1. *Aspergillus flavus* growth in response to different concentrations α and γ -Al₂O₃NPs (Dry weight g/100ml).

Concentration (mg/dl)	γ -Al ₂ O ₃ (A)		α -Al ₂ O ₃ (B)	
	Dry weight	Inhibition %	Dry weight	Inhibition %
0	1.55		1.70	
0.5	0.17	89.00	0.15	90.30
1	0.08	94.80	0.13	91.60
2	0.08	94.80	0.11	92.90
3	0.08	94.80	0.09	94.20

**Figure 13.** Mean growth Inhibition (A) and corresponding percentage (B) of *Aspergillus flavus* in response to different concentrations of α - and γ -AL₂O₃ NPs after five days of growth at 30 °C and 180 rpm in PDB growth medium. (Where R₂: the relation coefficient and y: the predicted fungal inhibition value at “X” nanoparticles concentration).**Table 2.** *Fusarium Oxysporum* growth in response to different concentrations α and γ -Al₂O₃NPs (Dry weight g/100ml).

Concentration (mg/dl)	γ -Al ₂ O ₃ (A)		α -Al ₂ O ₃ (B)	
	Dry weight	Inhibition %	Dry weight	Inhibition %
Control	0.70		0.80	
0.5	0.09	87.14	0.20	75.00
2	0.08	88.57	0.18	77.50
4	0.06	91.43	0.13	83.75
5	0.05	92.86	0.11	86.25
6	0.01	98.57	0.04	95.00

As for *Alternaria* sp. Table 3 and Figure 15 demonstrated that, the highest growth inhibitory concentration for both α - and γ -Al₂O₃ NPs was detected at 50 mg/100 ml with inhibition percentage of 98.75 and 93.3%, respectively.

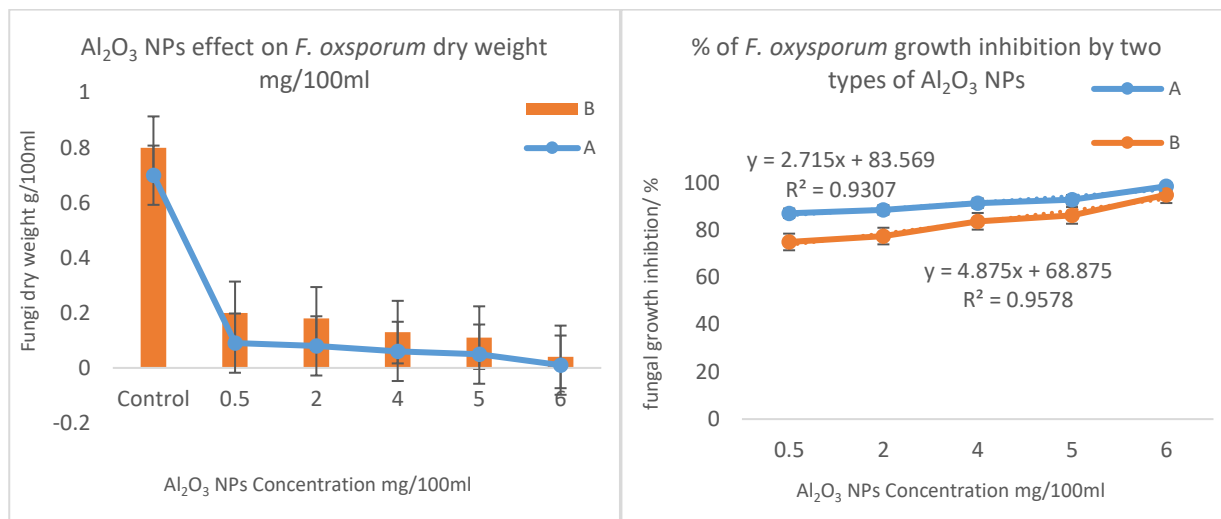


Figure 14. Mean growth Inhibition (A) and corresponding percentage (B), of *F. oxysporum* in response to different concentrations of α and γ -Al₂O₃ NPs after 5 days of growth at 30 °C and 180 rpm in PDB growth medium (Where R2: the relation coefficient and y: the predicted fungal inhibition value at "X" nanoparticles concentration).

Table 3. *Alternaria* sp. growth in response to different concentrations α and γ -AL₂O₃ NPs (Dry weight g/100ml).

Concentration (mg/dl)	γ -Al ₂ O ₃ (A)		α -Al ₂ O ₃ (B)	
	Dry weight	Inhibition %	Dry weight	Inhibition %
Control	0.80		0.90	
5	0.20	75.00	0.20	77.78
10	0.13	83.75	0.13	85.56
20	0.07	91.25	0.07	92.20
30	0.01	98.75	0.07	92.20
50	0.01	98.75	0.06	93.30

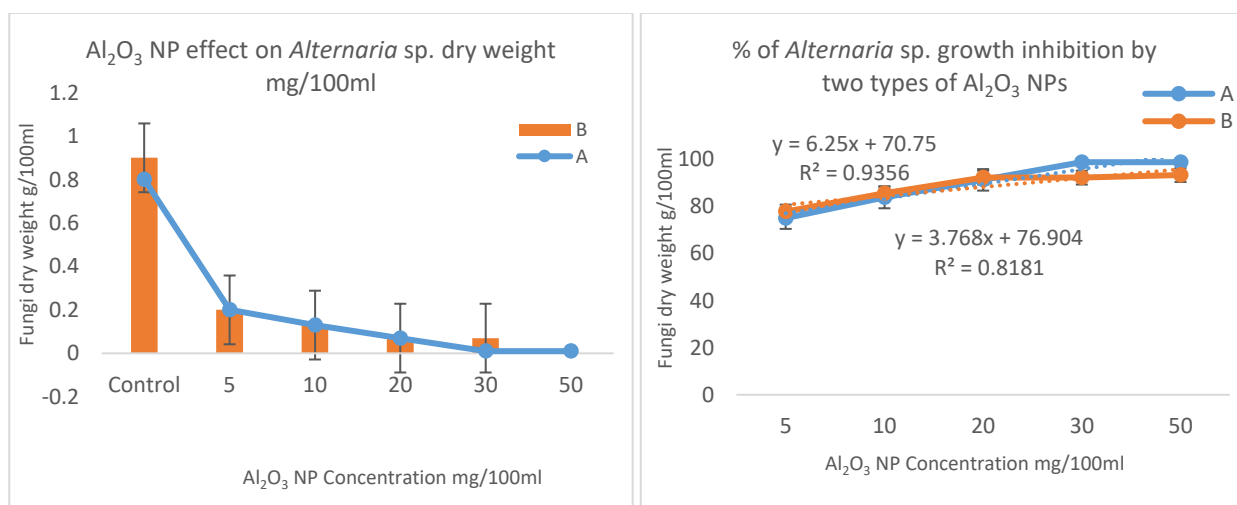


Figure 15. Mean growth inhibition (A) and corresponding percentage (B) of *Alternaria* sp. in response to different concentrations of α - and γ -Al₂O₃NPs after five days of growth at 30 °C and 180 rpm in PDB growth medium (Where R2: the relation coefficient and y: the predicted fungal inhibition value at "X" nanoparticles concentration).

DISCUSSION

In recent years, there has been a lot of interest in the synthesis of nanoparticles from waste materials as well as in investigating the potential use of metal oxide nanoparticles as innovative fungicides and insecticides (Elbehery *et al.* 2022; Ibrahim *et al.* 2022; Samy *et al.* 2023). In this work, α - and γ -Al₂O₃ NPs were synthesized from aluminum cans in the waste. The morphological, optical and thermal results of Al₂O₃ NPs were in agreement with the previous studies (Mohamad *et al.* 2019; Ziva *et al.* 2021; Thanigachalam *et al.* 2022; Hoque *et al.* 2023).

Furthermore, α - and γ -Al₂O₃ NPs have been proposed as an effective control method against females, nymphs and larvae stages of *M. fungivorus* and *C. mycophagus* stored grain mites, as well as the mycotoxin producing fungi, *Aspergillus*, *Alternaria* and *Fusarium* sp. For mite toxicity, γ -Al₂O₃ NPs were more efficient than alpha phase on tested mites' post-initial exposures. The effect was significantly increased by increasing exposure time and concentrations. Also, the females' oviposition was significantly influenced and did not hatch at all. Moreover, the toxic efficacy remained strong after a period of time. Indeed, SEM images concluded that a strong adhesion of α and γ -Al₂O₃ NPs was visibly bonded to various areas of dead females. Similarly, there was a significant influence of Al₂O₃ NPs toxic activity on stored insects, *O. surinamensis* and *S. oryzae*, when the NPs bind to insect cuticle (Stadler *et al.* 2017; López-Muñoz *et al.* 2019; Elbehery *et al.* 2022).

The responses discrepancy in toxicity may be due to NPs characteristic differentiations, the mode of action, and the pest sensitivity. Based on NPs nature and mode of action, Al₂O₃ NPs toxicity may be attributed to adhesion to surfaces that lead to changes in membrane characteristics that impact permeability and cellular respiration (Park *et al.* 2016; Benelli 2018). Surface of Al₂O₃ NPs are electrically charged as by product from aluminum oxidations. These NPs can interact with the pests' protective cuticular wax coating more efficiently due to their expanded exposed surfaces. The pests die as their wax layer is worn down by abrasion and lipid adsorption; they begin to lose water from their bodies, and they perish from desiccation (Ibrahim *et al.* 2022). Similar to this, Lazarevic *et al.* (2018) showed the substantial toxicity of alumina powder on *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae) to the high specific surface area.

Based on the pest mite sensitivity, the present study concluded that *M. fungivorus* was more sensitive than *C. mycophagus* mite. The variances in the toxicity rates between the two mites could be due to variations in the metabolism, sizes and target body surfaces. Barghout and Samy (2023) concluded the same result on the same pest mites by zinc oxide nanoparticles. Since the acaricidal effect of Al₂O₃ NPs are mostly physical in nature, it is expected that pests will not quickly develop a resistance to this mechanism of action. Our findings suggest that Al₂O₃ NPs could be a useful tool for controlling any Acaridae mites infesting stored grains.

Additionally, determining the optimum fungicidal concentration of α and γ -Al₂O₃ NPs against the fungal isolates has been the subject of numerous research studies that have shown the role of pathogenic mycotoxin-producing fungi such as *Aspergillus*, *Fusarium*, and *Alternaria* in causing infections in grains and green plants that can be used as animal feedstock (USDA 2016; Elbehery *et al.* 2022). The obtained results exhibited that, aluminum nanoparticles possess a potent fungicidal activity in the two forms, however γ -Al₂O₃ was more effective as a fungicidal agent than α -Al₂O₃ NPs which reflected on lower fungicidal concentration specially against *A. flavus* (1 mg/100 ml) with approximately 95% of growth inhibition.

The common pathway for aluminum nanoparticles fungicidal activity is through the induction of multiple cellular site destruction: either due to its linkage to cellular proteins and enzymes or direct DNA binding that causes mutation and adversely affect cellular replication and proliferation. The antifungal activity may also attribute to linkage to the free hydrogen bond on the cellular membrane which alter the lipopolysaccharide layer configurations that eventually lead to cell membrane damage and cell lysis reported by Capeletti *et al.* (2014).

Similarly, Suryavanshi *et al.* (2017) evaluated the antifungal activity of aluminum nanoparticles against different food borne pathogenic fungi including *F. oxysporum* and *A. flavus*. The data showed that aluminum nanoparticles exhibited antifungal activity against *F. oxysporum* and *A. flavus* with MIC concentration of 250 and 150 µg/ml respectively. It is worthy to mention that each fungal strain responded differently to the different nanoparticle concentration gradients according to their susceptibility and the ability to proliferate under this stress condition; where *A. flavus* was the most susceptible to the presence of lower concentrations of nanoparticles (fungicidal dose at 1 mg/100 ml), and *Alternaria* sp. was the most resistant with fungicidal dose at 30 mg/100 ml. Another study demonstrated the efficacy of aluminum nanoparticles in controlling fungal growth. The antifungal activity of mesoporous aluminum nanoparticles against *Fusarium oxysporum* was found by Shenashen *et al.* (2017). It was noted that when compared to the control treatment, the highest antifungal activity was observed at the concentration of 400 mg/L and the maximum inhibition rate was 78.57% after growth on PDA sheets.

CONCLUSION

Agriculture as we know it today has been replaced by contemporary agriculture thanks to nanotechnology. The synthesized α - and γ -Al₂O₃ NPs revealed significant toxic effects against females, nymphs and larvae of *M. fungivorus* and *C. mycophagus* mites. The effectiveness of α - and γ -Al₂O₃ NPs against the tested mites varied depending on concentrations. By extending the exposure duration and increasing the concentration, the mortality of both mite stages increased. Additionally, the female's oviposition was severely impacted and the eggs failed to develop. In addition, the efficacy persisted even after a while. It was discovered via Scanning Electron Microscopy (SEM) photos that the dead females' various body parts were visibly bound with a powerful adhesive made of α and γ -Al₂O₃ NPs. Moreover, the synthesized nanoparticles proved their efficacy in controlling and eliminating all tested fungal mediated plant infections related to the presence of *A. flavus*, *F. oxysporum* and *Alternaria* sp. fungal species, with considering these species varied susceptibility and resistance. Successful use of these metal oxide nanoparticles may give protection not only against stored grain pests but also prevent the agricultural produce from microbial infection. Further investigation is needed for evaluating the side effects on beneficial organisms.

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خواص ریخت‌شناختی، نوری و حرارتی نانوذرات آلفا و گاما-آلومینیوم: ارزیابی فعالیت‌های زیستی آنها در برابر کنه‌های انباری و قارچ‌های مولد مایکوتوکسین

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چکیده

به تازگی استفاده از روش‌های مهار سازگار با محیط زیست در برابر آفات مورد توجه زیادی قرار گرفته است. بنابراین، این مطالعه با هدف ارزیابی سنتز دو فاز مختلف نانو اکسید آلومینیوم آلفا و گاما (α - و γ -Al₂O₃ NPs) از پسماندها انجام شد. افزون بر این، آنها را برای کنترل کنه‌های محصولات انباری، *Mycetoglyphus fungivorus* و *Caloglyphus mycophagous*، و قارچ‌های *Aspergillus flavus*، *Fusarium oxysporum* و *Alternaria sp.* ارزیابی شدند. دو فاز نانوذرات Al₂O₃ در محیط اسیدی و بازی سنتز شدند و با استفاده از میکروسکوپ الکترونی روبشی نشر میدانی (FESEM)، طیف‌سنجی پرتو ایکس پراکنده انرژی (EDX)، فرابنفش مرئی (UV-Vis) و تجزیه و تحلیل گرما گرانشی (TGA) تشخیص داده شدند. درصد تلفات کنه‌ها در غلظت‌های ۱۰، ۳۰ و ۶۰ پی پی ام پس از ۱، ۲، ۳ و ۴ روز بررسی شد. رشد قارچ‌ها برای تعیین غلظت قارچ‌کشی در کشت‌های PDB با استفاده از روش وزن‌های خشک میسلیم ارزیابی شد. این مطالعه تشکیل و پایداری حرارتی نانوذرات α - و γ -Al₂O₃ را با استفاده از FESEM، UV-Vis، TGA تایید می‌کند. اندازه متوسط ذرات α -Al₂O₃ ۱۰-۴ نانومتر و برای γ -Al₂O₃ ۳۰-۱۴ نانومتر است. هر دو فاز برای مراحل آزمایش شده کهنه سمی بودند. افزایش غلظت و زمان در معرض قرار گرفتن باعث افزایش میزان مرگ و میر کنه‌ها می‌شود. نانوذرات γ -Al₂O₃ کارآمدتر از α -NP ها پس از در معرض قرار گرفتن اولیه بودند، سپس در طول زمان اثر یکسانی دارند. باروری ماده به مقدرا زیادی تحت تاثیر قرار گرفت. تخم‌های آزمایش شده هرگز از تخم خارج نشدند. فعالیت سمی پس از ۱، ۲ و ۳ ماه در معرض قرار گرفتن قوی باقی ماند. تصاویر SEM چسبندگی قوی از α - و γ -NPshas را نشان داد که به طور آشکار به نواحی مختلف بدن کنه‌های ماده مرده پیوند خورده است. γ -Al₂O₃ فعالیت ضد قارچی بیشتری نسبت به α -NPs نشان داد. غلظت قارچ‌کش‌ها به روشی وابسته به حساسیت سویه قارچی است. نتیجه‌گیری می‌شود که نانوذرات α - و γ -Al₂O₃ عوامل سازگار با محیط‌زیست در برابر تکثیر کنه‌های انباری و قارچ‌های مولد مایکوتوکسین هستند.

واژگان کلیدی: نانوذرات آلفا و گاما اکسید آلومینیوم، قوطی‌های آلومینیومی، محصولات سازگار با محیط زیست، زمان در معرض قرار گرفتن، کنه‌های انباری.

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