

COMPARATIVE ANALYSIS AND IMPACT OF VARIOUS ENTOMOTOXIC NANOPARTICLES AGAINST *Sitophilus oryzae*

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ABSTRACT

Rice, an important cereal crop of Pakistan suffers 10 to 18% loss each year during harvesting, threshing, transportation, and storage. Several insect pests attack the grains during storage, but *Sitophilus oryzae* is one of the most destructive insect pests of rice grains, which causes substantial losses under warm climatic conditions. Conventional control practices such as use of chemical insecticides and fumigants have led to the resistance development in storage insect pests. Moreover, they also exert hazardous effects on human health and environment. This study evaluated the entomotoxic potential of zinc oxide (ZnO), titanium dioxide (TiO₂), and aluminum oxide (Al₂O₃) nanoparticles (NPs) against adult *Sitophilus oryzae* under controlled laboratory conditions. Custom-synthesized NPs with distinct surface properties viz., hydrophilic, hydrophobic, and lipophilic were tested at three concentrations (0.5, 1.0, and 2.0 g kg⁻¹ of rice grains), alongside untreated controls. The experiment was laid out in a completely randomized design (CRD) following a factorial arrangement, with each treatment replicated five times. After four days of aluminum oxide (1 g kg⁻¹) application, 90% of mortality of *S. oryzae* was recorded, while titanium oxide and zinc oxide showed similar results (85-90% mortality) with 2 g kg⁻¹ dose rate after 14 days of application. In short, it is preferred to use titanium oxide and zinc oxide than aluminum oxide which exhibits hazardous impacts on stored grains. Furthermore, titanium oxide and zinc oxide also have antimicrobial properties and can protect the stored grains from both insect pests as well as infection caused by microbes.

Key words: Nano particles, *Sitophilus oryzae*, Biosafety, Residual toxicity, Rice

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INTRODUCTION

Rice (*Oryza sativa* L.), an important cereal crop of Pakistan has a central position in the agricultural policies of Pakistan. It accounts for 76% of food grain production with 13.8% share to the value-added in agriculture and is spread on about 37% of the cropped area (Chandio *et al.*, 2020; World Bank, 2022). Rice suffers 10-18% loss every year during harvesting, threshing, transportation, and storage (Ahmed *et al.*, 2020). Several insect pests viz., *Sitophilus oryzae*, *Rhizopertha dominica* and *Trogoderma granarium* etc.

attack the grains during the storage (Rosentrator, 2022). Mostly insects belonging to the order Coleoptera and Lepidoptera attack on stored grains (Jamwal *et al.*, 2022). Weevils are major stored grain pest that cause huge damage to stored grains (Attia *et al.*, 2020). The *S. oryzae* (Order: Coleoptera; Family: Curculionidae) is most destructive pest of rice grains during storage. It is a primary storage pest that causes both qualitative and quantitative losses of rice in tropical areas of the world (Tadesse, 2020). Many chemical insecticides and fumigants are being used to protect the rice from storage pests (Qurban *et al.*, 2020). As these chemicals have

health risks, residual toxicity for mammals, and resistance development by stored grain pests, these conventional insecticides lead researchers to find alternative strategies (Aatif *et al.*, 2020). In the recent past, inert dusts, such as diatomaceous earths (DEs), silica nanoparticles, and silica aerogel have increasingly been used for commercial storage in developed countries, replacing traditional chemicals (Hanif *et al.*, 2021, 2022). But DEs also have some limitations, e.g., it led to lower bulk density, and its efficiency varies with geographic origin and moisture content in commodity (Jones *et al.*, 2022). Nanotechnology is innovative in the broad fields such as pesticides, agriculture, and pharmaceuticals (Acharya and Pal, 2020). Without harming nature, nanotechnology is an efficient alternative for the management of insect pests (Shahid *et al.*, 2021). Nanoparticles help to produce new pesticides, insecticides and insect repellants (Dangi and Verma, 2021). This study extends existing research by comparatively assessing the effectiveness of various entomotoxic nanoparticles against *S. oryzae* in stored rice. In contrast to earlier studies limited to individual nanoparticle types, this work provides an integrated comparative evaluation, elucidating differences in toxicity and potential as eco-friendly alternatives to conventional insecticides.

MATERIALS AND METHODS

Nano formulation Development: Research trial was conducted, during May-July 2021, at the Entomology Laboratory, Bahauddin Zakariya University Bahadur Sub Campus Layyah, Punjab, Pakistan. Custom synthesized oxide nanoparticles (NPs) including hydrophilic ANP- α and ANP- γ , hydrophilic TNP (Anatase), hydrophilic and hydrophobic TNP (Rutile), hydrophilic, lipophilic and hydrophobic ZNP were procured from M K Implex, Canada. Titanium dioxide (TiO₂), aluminum oxide (Al₂O₃) and zinc oxide (ZnO) of micron size were procured from Pakistan Companies (PQ Chemicals, Sheikhpura and Naseem Enterprises, Lahore, Pakistan) to measure their insecticidal ability with nano-sized counterpart. Treatments detail given below (Table 1).

Insect culture: Insects were collected from traditional grain storage shops and milling units located in the District Layyah region of Punjab, Pakistan. *Sitophilus oryzae* was reared on rice grains in permanent darkness at temperature and relative humidity (R.H.) ranges of 30 ± 2 °C, 75±5%, respectively. The saturated solution of sodium chloride was used to maintain R.H. Adult insects (two weeks old) were used in the experimentation (Trematerra *et al.*, 2013).

Table 1: Description of nanoparticles evaluated in the bioassays

Sr #	Code	Nanoparticles
1	NP1	Al ₂ O ₃ (α) – hydrophilic
2	NP2	Al ₂ O ₃ (γ) – hydrophilic
3	NP3	Al ₂ O ₃ (bulk)
4	NP4	TiO ₂ (Anatase) – hydrophilic
5	NP5	TiO ₂ (Rutile) – hydrophilic
6	NP6	TiO ₂ (Rutile) – hydrophobic
7	NP7	TiO ₂ (Bulk)
8	NP8	ZnO – hydrophilic
9	NP9	ZnO – lipophilic
10	NP10	ZnO – hydrophobic
11	NP11	ZnO (bulk)

Evaluation of entomotoxic effectiveness of surface functionalized: Contact toxicity bioassays were performed in plastic boxes (99) having screw and perforated caps (diameter: 8 cm, height: 8.5 cm). Twenty gram of rice was taken in each plastic boxes and treated with nanoparticles at three dose rates 0.5, 1.0, and 2.0 g, while control was maintained without nanoparticles treatment. The experimental plastic boxes were shaken manually approximately for one minute to achieve equal dispersal of materials in rice (Subramanyam and Hangstrum, 2000). Twenty *S. oryzae* adults were released in each box. All bioassays were conducted at similar laboratory conditions as mentioned earlier. There were five replications for each dose. Insect mortality was noted after 1, 2, 4, 7, and 14 days of application. The corrected mortality was counted with Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality (\%)} = \frac{Mo - Mc}{100 - Mc} \times 100$$

Mo= Mortality observed

Mc= Mortality in control

Statistical analysis: The data collected were checked for normality using Shapiro-Wilk normality test, which confirmed the normal distribution. After that, the data were analyzed following two-way ANOVA under CRD with factorial arrangement using Statistix 8.1 software. However, the treatment means were compared using Tukey's HSD test at ($p \leq 0.05$) (Steel *et al.*, 1997).

RESULTS

All NPs with their respective doses significantly ($p \leq 0.05$) impacted the *S. oryzae* adults' mortality at various exposure intervals. In case of NP-2 (Al₂O₃ (γ) – hydrophilic) applied on rice, maximum mortality was assessed at all exposure intervals. Interaction between NP and dose rate, after 1 day of treatment, NP2 (Al₂O₃ (γ) – hydrophilic) showed maximum mortality (88%) when used at 2 g kg⁻¹ rate as compared to other NPs and their

used doses (Fig. 1). More than 90% mortality was attained, on day 2, with ANP along with its higher dosage rate (2 g kg^{-1}). On 2nd day, 100% mortality was recorded, in case of NP2 hydrophilic applied at 2 g kg^{-1} dose rate, while, NP1 (Al_2O_3 (α) – hydrophilic) showed 91% mortality with its higher dose application (2 g kg^{-1}). Around 40% insects were dead when NP-4 (TiO_2 (Anatase) – hydrophilic), NP6 (TiO_2 (Rutile) – hydrophobic) and NP9 (ZnO – lipophilic) were used with

2 g kg^{-1} (Fig. 2). Mortality was more than 90%, after day 4, in case of ANP applied at 1 g kg^{-1} and 0.5 g kg^{-1} dose rate, whereas, 100% mortality was observed when ANP was applied with higher dose rate (2 g kg^{-1}). Application of ZNP with 2 g kg^{-1} dose rate exhibited more than 45% mortality of insects. Approximately 60% insect mortality was assessed where TNP- (Anatase) and TNP- (Rutile) were used along with their respective higher dose (Fig. 3).

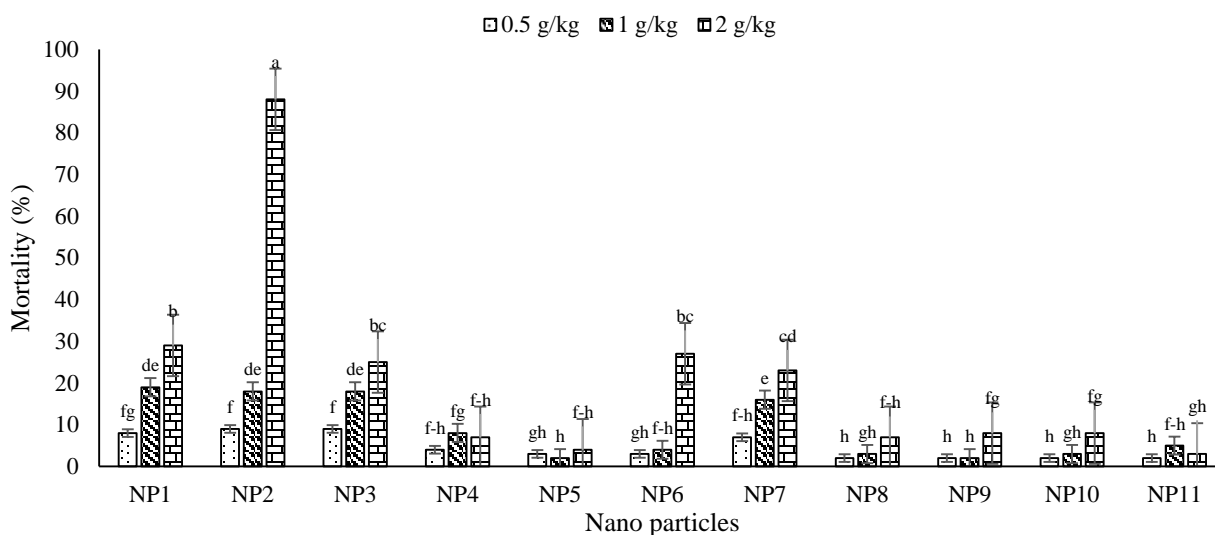


Fig. 1. Interactive effect of NP and D on mortality of *Sitophilus oryzae* at day 1. Similar letters on the bars represent non-significant difference among treatments ($p = 0.05$). (ANPs) NP1= Al_2O_3 (α) - hydrophilic, NP2= Al_2O_3 (γ) - hydrophilic, NP3= Al_2O_3 (bulk), (TNPs) NP4= TiO_2 (Anatase) – hydrophilic, NP5= TiO_2 (Rutile) – hydrophilic, NP6= TiO_2 (Rutile) – hydrophobic, NP7= TiO_2 (Bulk), (ZNPs) NP8= ZnO – hydrophilic, NP9= ZnO – lipophilic, NP10= ZnO – hydrophobic, NP11= ZnO (bulk).

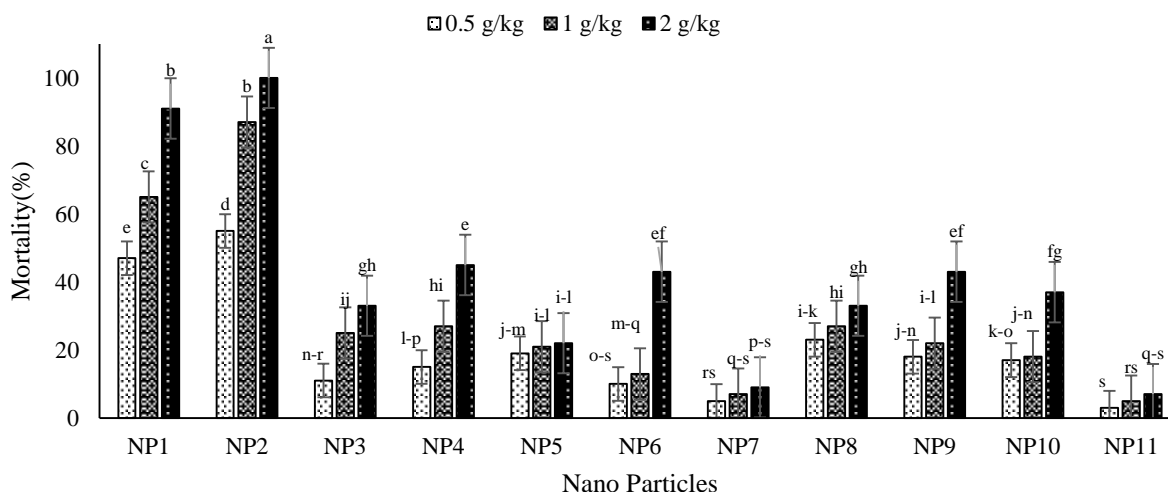


Fig. 2. Interactive effect of NP and D on mortality of *Sitophilus oryzae* at day 2. Similar letters on the bars represent non-significant difference among treatments ($p = 0.05$). (ANPs) NP1= Al_2O_3 (α) - hydrophilic, NP2= Al_2O_3 (γ) - hydrophilic, NP3= Al_2O_3 (bulk), (TNPs) NP4= TiO_2 (Anatase) – hydrophilic, NP5= TiO_2 (Rutile) – hydrophilic, NP6= TiO_2 (Rutile) – hydrophobic, NP7= TiO_2 (Bulk), (ZNPs) NP8= ZnO – hydrophilic, NP9= ZnO – lipophilic, NP10= ZnO – hydrophobic, NP11= ZnO (bulk).

ANPs, killed nearly all the insects at 1 g kg⁻¹ and 2g kg⁻¹ dose rate, while, Hydrophobic TNP - (Rutile) showed 94% insect mortality when applied with 2 g kg⁻¹ dose rate after 7 days of treatment. In contrast, ZNPs exhibited more than 60% mortality at similar higher dosage rate (Fig. 4). After 14 days, ANPs showed 100% mortality of insects when applied with 2g kg⁻¹ dose rate,

while, application of TNPs and ZNPs exhibited 90% and 80% mortality of insects at higher dose rate (2 g kg⁻¹). Mortality of insects was 60-75% when ZNPs and TNPs were applied at 1 g kg⁻¹ dose rate (Fig. 5). Nano particles impacted more efficiently and significantly as compared to their bulk sizes across all days. Progeny of *S. oryzae* was not observed in stock of rice even after 2 months.

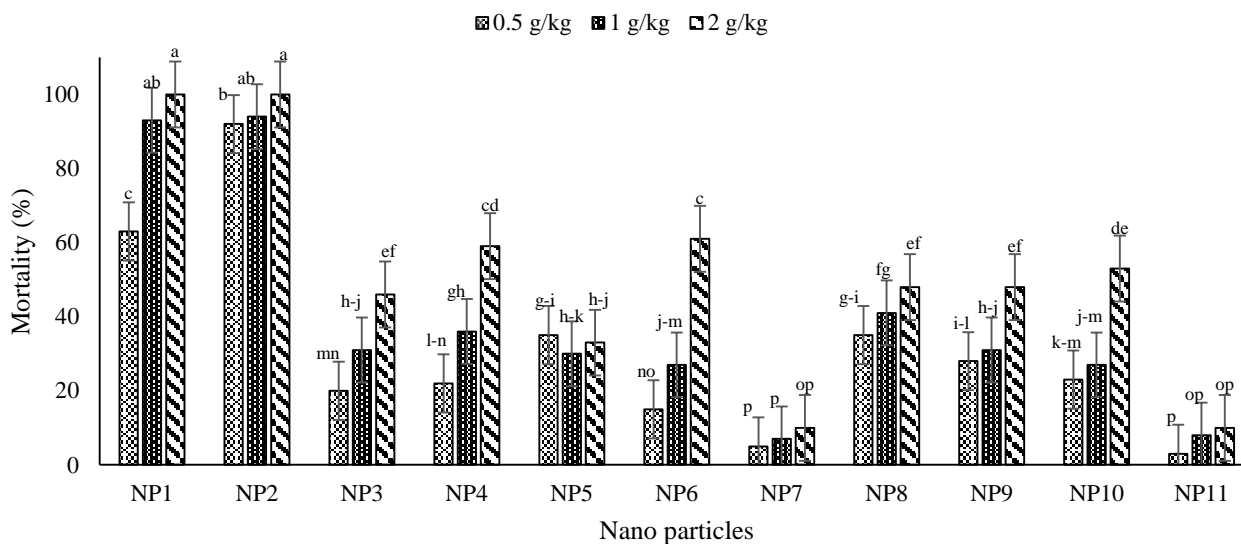


Fig. 3. Interactive effect of NP and D on mortality of *Sitophilus oryzae* at day 4. Similar letters on the bars represent non-significant difference among treatments ($p = 0.05$). (ANPs) NP1= Al₂O₃ (α) - hydrophilic, NP2= Al₂O₃ (γ) - hydrophilic, NP3= Al₂O₃ (bulk), (TNPs) NP4= TiO₂ (Anatase) - hydrophilic, NP5= TiO₂ (Rutile) - hydrophilic, NP6= TiO₂ (Rutile) - hydrophobic, NP7= TiO₂ (Bulk), (ZNPs) NP8= ZnO - hydrophilic, NP9= ZnO - lipophilic, NP10= ZnO - hydrophobic, NP11= ZnO (bulk).

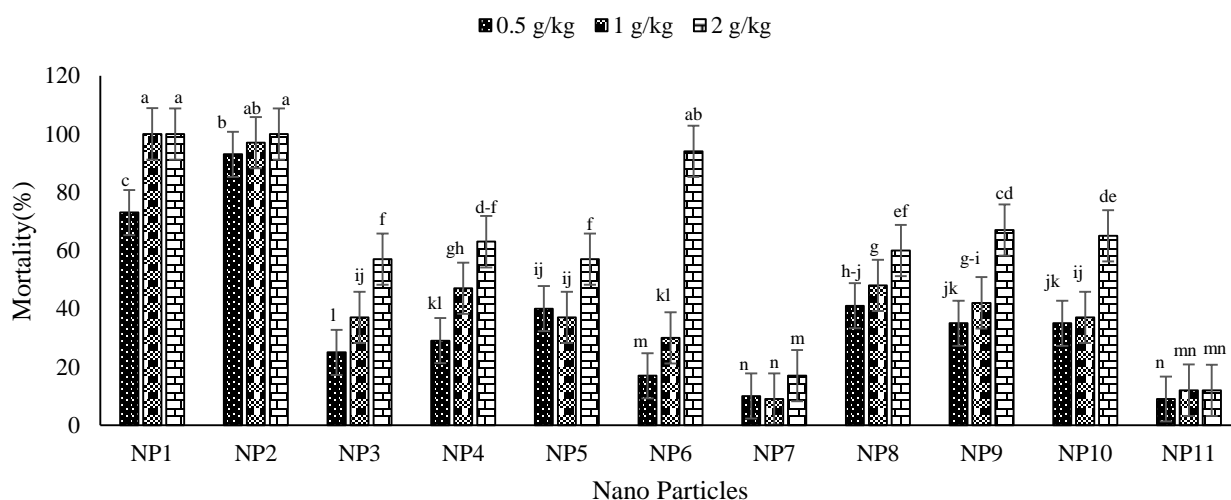


Fig. 4. Interactive effect of NP and D on mortality of *Sitophilus oryzae* at day 7. Similar letters on the bars represent non-significant difference among treatments ($p = 0.05$). (ANPs) NP1= Al₂O₃ (α) - hydrophilic, NP2= Al₂O₃ (γ) - hydrophilic, NP3= Al₂O₃ (bulk), (TNPs) NP4= TiO₂ (Anatase) - hydrophilic, NP5= TiO₂ (Rutile) - hydrophilic, NP6= TiO₂ (Rutile) - hydrophobic, NP7= TiO₂ (Bulk), (ZNPs) NP8= ZnO - hydrophilic, NP9= ZnO - lipophilic, NP10= ZnO - hydrophobic, NP11= ZnO (bulk).

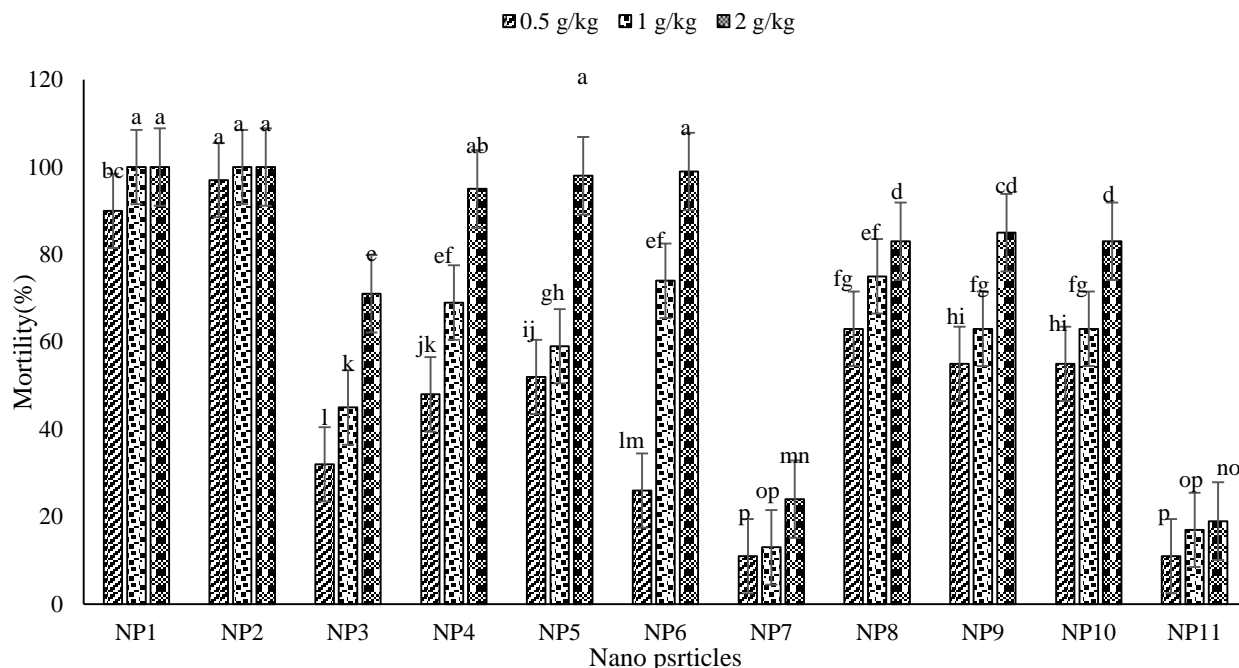


Fig. 5. Interactive effect of NP and D on mortality of *Sitophilus oryzae* at day 14. Similar letters on the bars represent non-significant difference among treatments ($p = 0.05$). (ANPs) NP1= Al_2O_3 (α) - hydrophilic, NP2= Al_2O_3 (γ) - hydrophilic, NP3= Al_2O_3 (bulk), (TNPs) NP4= TiO_2 (Anatase) - hydrophilic, NP5= TiO_2 (Rutile) - hydrophilic, NP6= TiO_2 (Rutile) - hydrophobic, NP7= TiO_2 (Bulk), (ZNPs) NP8= ZnO - hydrophilic, NP9= ZnO - lipophilic, NP10= ZnO - hydrophobic, NP11= ZnO (bulk).

DISCUSSION

Researchers are increasingly exploring the use of diatomaceous earth (DE) dusts as ecofriendly insecticides for managing a range of insect pests (Hanif *et al.*, 2021, 2022). However, the efficacy of diatomaceous earth (DE) is influenced by several factors, including temperature, relative humidity, and the geographical origin of the material (Hasan *et al.*, 2022). In modern agriculture there is significant increase in the use of nanotechnology (Das *et al.*, 2019). Under controlled laboratory conditions, nanoparticles (NPs) have gained attention for insect pest management owing to their high surface area, precision in target delivery, and ability to enable controlled release of active agents (Thabet *et al.*, 2021). Results of the current study showed that these NPs have insecticidal effects on *S. oryzae*. In the current experiment, oxide forms of NPs were used which are more effective due to their size. Owing to the micron size of NPs, these interact with protective waxy layer of insects. The NPs damage the wax layer due to abrasion and adsorption of lipids, and desiccation occurs leading to insect death (Fraceto *et al.*, 2020). The physical mode of action of NPs, including cuticle abrasion and disruption, has been documented through scanning electron microscopy. For instance, nano silica particles showed degradation of *S. oryzae* cuticle and mandible

damage leading to desiccation and death (Padmasri *et al.*, 2018), which may explain the persistent high mortality across our tested NPs, especially aluminum and zinc oxide types. Al_2O_3 (γ) was found more effective to control *S. oryzae* at 14th day at dose rate of 2 g kg^{-1} . According to previous studies, higher concentrations and longer exposure times enhance insect mortality (Srinivasan *et al.*, 2023). The results for the hydrophilic nanoparticle Al_2O_3 (γ) are particularly noteworthy since they indicate a better insecticidal mechanism than other materials studied (El-Barkey *et al.*, 2023). Hamza *et al.* (2012) reported that TNP (titanium dioxide nanoparticles) and ZNP (zinc oxide nanoparticles) showed 100% mortality of rice weevil at 7th day and 14th day at 2 g kg^{-1} . The dose-dependent nature of insect mortality observed in this study supports by earlier work (Ismail *et al.*, 2021), which showed that increasing aluminum nanoparticle concentration significantly raised *S. oryzae* mortality and decreased progeny emergence, supporting our findings where the highest dose caused 100% mortality on day 2 and suppressed progeny for two months. According to Saeed *et al.* (2025), the study demonstrates that the nanoparticles not only cause acute adult mortality but also interfere with population dynamics, limiting the establishment of succeeding generations, which is essential for long-term storage protection. Furthermore, TNP or ZNP are strong anti-

microbial agents and have benefit over conventional insecticides (Wang *et al.* 2016). These NPs not only provide protection from insects' pests but also minimize microbial infection of agricultural products. Titanium dioxide and Zinc oxide are used in cosmetics from several decades (Mallakpour and Mustansar, 2022) and it is safe for humans. Results of the current study support the broader evidence that TNP and zinc oxide nanoparticles can be developed as safer, residue-free alternatives to conventional insecticides for stored product protection. Further investigations into the mechanisms of toxicity, environmental safety, and field-scale evaluations are required to optimize formulations and application strategies for commercial use.

Conclusion: The study revealed that all tested nanoparticles significantly increased the mortality of *S. oryzae* in stored rice, with efficacy influenced by nanoparticle type, dose, and exposure time. Among them, titanium oxide and zinc oxide showed similar results (85-90% mortality) with 2 g kg⁻¹ dose rate after 14 days of application. Aluminum oxide nanoparticles were generally more effective than titanium dioxide and zinc oxide types. The absence of progeny even after two months indicates strong long-term effects. Overall, titanium oxide and zinc oxide nanoparticles demonstrate great potential as eco-friendly alternatives to conventional insecticides for protecting stored rice, offering both effectiveness and environmental safety.

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Conflicts of Interest: All of the authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contribution: Ch. Muhammad Shahid Hanif, Hafiz Muhammad Aatif and Muhammad Zeeshan Mansha conducted the experiments, Ibrahim Al-Ashkar and El-Sabagh Ayman analyzed the data. Kamran Ikram, Zahid Mahmood Sarwar and Qamar uz Zaman wrote the main manuscript text. Muhammad Zeeshan Mansha, Qamar uz Zaman, Ibrahim Al-Ashkar, Muhammad Kamran and El-Sabagh Ayman revised and checked the manuscript. All authors have read and agreed to publish this version of the manuscript.

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