

Research

The Susceptibility of Malaysian Rice Brands To *Sitophilus oryzae* and The Potential Application of Zinc Oxide Nanoparticles For Control Purposes

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ABSTRACT

The rice weevil, *Sitophilus oryzae*, is an insect pest that can pose severe harm to stored grains. Rice weevils, both adults and larvae, cause significant economic losses because they can infest a wide range of plants and stored goods. Nanoparticle insecticides are created and studied to replace the overly-used synthetic chemical insecticides. The purpose of this study is to investigate the susceptibility of several commercialized rice brands towards *S. oryzae* infestation and to assess the toxicity of different types of zinc oxide (ZnO) against *S. oryzae*. The study involved twelve rice brands, and *S. oryzae* infestation rates were observed in the laboratory for 17 weeks. The infestation rates were obtained at the end of the observation period. To obtain the required dosages of 0.1, 0.2, 0.4, and 0.8% (w/w), four different concentrations of each type of ZnO nanoparticle that had undergone various reactivity modifications were mixed with rice for bioassays test. *S. oryzae* has been proven to be susceptible to almost all commercialized rice brands studied in this research. Based on the data, it can be concluded that the ZnO nanoparticle modified with gamma-ray is the most effective at controlling *S. oryzae*, as 100% mortality was seen after three days of treatment at concentrations between 0.4 to 0.08% (w/w). The slow emergence of pests in monitored rice brands is attributed to the protectants and their residue, which work by eradicating eggs and adults that have consumed the grain. Zinc oxide type 7A is the most toxic towards *S. oryzae* because of its exposure to gamma-ray which has the highest energy compared to neutron and beta rays. This results in the release of more active molecules to act on the pest.

Key words: Modified zinc oxide, nanotechnology, rice weevils, sustainable control management, stored grains pest

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INTRODUCTION

Grains that are stored may sustain serious harm from insect infestations. These pests cause both quality and quantitative losses when they feed on grains that have been stored. Tiny beetles crawling on worktops, moths fluttering within rooms, or caterpillars moving up walls or on ceilings are the first signs of a pest infestation (Crawley & Bertone, 2021). Certain pests in products that are stored consume the entire kernel. Among them are *Sitophilus oryzae*, commonly known as rice weevils. The rice weevils are 1/8 to 1/4 inch long snout beetles that range from reddish brown to black (Mwenda *et al.*, 2019). Adults can be discovered up to a mile distant from affected commodities and can survive for up to eight months (Mwenda *et al.*, 2019). In the larval stage, a legless grub develops inside the kernels of wheat, maize, and other whole grains, including rice, which will eventually spoil the rice where the insects develop. *S. oryzae* which causes qualitative and quantitative losses in crops during storage (Ahmad *et al.*, 2021). It is important to understand the susceptibility of rice varieties to insect pest infestation. Susceptibility studies keep farmers, managers, and household users informed while also improving breeding program testing for infestation and damage caused by storage insect pests (Okpile *et al.*, 2021).

This will assist the appropriate authorities in taking responsibility by applying suitable precautions for rice in storage (Khan & Halder, 2012).

Several approaches to managing rice weevil infestations have been developed and implemented, using both traditional and modern technology. Chemical control is the most important and often used control measure. This means using synthetic insecticides like permethrin, pirimiphos-methyl, and others that are efficient in eliminating *S. oryzae* (Sharma et al., 2019). The frequent and ongoing application of different traditional insecticides by farmers and marketers has led to the development of resistance and the resurgence of insect pests, even though synthetic insecticides are effective against insect pests. The inability of chemicals to control pests has further led to insecticide resistance, resulting in food losses, and causing annual economic harm to the tune of several billion dollars worldwide (Odeyemi et al., 2010). Farmers may increase the rate or frequency of applications to cope with the resistance factor which causes additional selection pressure on the pests and eventually leads to a situation in which pests turn completely immune to the chemical insecticides (Khambay & Jewess, 2005).

Research and development are progressing to replace chemical synthetic insecticides with insecticides based on nanotechnology. Numerous studies demonstrate that nano-based pesticides, while beneficial for the environment and human health, also show potential in pest management. In the field of pesticides, ongoing systematic research is also needed to develop targeted pesticide formulations that are environmentally safe with sustained release. This is because nanoparticles have a system that can control and release active compounds when exposed to certain stimuli such as radiation. Thus, nanotechnology can be an alternative to reduce the damage caused by chemical pesticides on humans and the environment, increasing the efficacy of controlling pests while maintaining the safety of the products (Camara et al., 2019).

Therefore, the objectives of the study are to determine the susceptibility of commercialized rice brand varieties to the infestation of *Sitophilus oryzae* and to evaluate the toxicity of different types of Zinc oxide towards *Sitophilus oryzae* adults.

MATERIALS AND METHODS

Experimental procedures for susceptibility of rice brand varieties to the infestation of *S.*

Oryzae

There were 12 brands of rice used in this study; namely Lisa (L), Dari Pulau Jauh (DPJ), Sayang (S), Beras Wangi Nan Murah (BWNM), Bird of Pacific (BOP), Jasmin Suria (JS), Bird Jewel (BTJ), Fauna (F), Ros Utara (RU), Ras (R), Jejak Super Special (JSS) and Kami (K). These rice brands were purchased at stores around Perlis and Pulau Pinang. All rice brands were each poured into a 1L plastic container, covered with the lid, and monitored in the laboratory. A census of *Sitophilus oryzae* was done once in 7-day intervals. After each count, the dead weevils were discarded. The recording of the number of weevils was done from January to May 2022. This method was adapted from Okpile et al. (2021).

Experimental procedures for toxicity of ZnO towards *S. oryzae* according to Attia et al. (2020)

Sitophilus oryzae lab strain (LS)

Rice weevils were collected from infested rice packets from home, located at Kampung Sentua, Utan Aji, Perlis. Rice weevils collected were reared in the laboratory at Universiti Sains Malaysia to be used as the lab strain also called as control strain. At $28 \pm 2^\circ\text{C}$, 70 to 75% relative humidity, and 14h:10h (L:D) photoperiod, the rice weevils used in this study were reared in the laboratory.

Synthesis of zinc oxide nanoparticles (ZnO)

The ZnO nanoparticles were supplied by Solid State Lab, School of Physics, Universiti Sains Malaysia. All of the particles were synthesized to obtain less than 100 nm in size. The ZnO nanoparticles provided were in 4 different varieties and each had undergone different types of modification of reactivity. The ZnO used were 1A (exposed to neutron source), 3A (exposed to beta & gamma-ray), 7A (exposed only to gamma-ray), and B (non-modified).

Preparation of medium

Twenty grams of rice with chemical protectants in three replicates for each treatment were weighed in a 250 mL jar, for treatments and untreated (control) using a digital balance scale. Four different concentrations of each type of ZnO nanoparticles were prepared and admixed with rice to give the required doses which are 0.1, 0.2, 0.4, and 0.8% (w/w). Preparation of ZnO NPs doses was done by

using the formula: $[(\% \text{ conc.}) \times (20 \text{ g})]/100$. From the formula, the weight of ZnO NPs in grams was obtained, the weight of ZnO NPs obtained mixed with rice, and the total mixture must weigh 20 g. (Attia *et al.*, 2020).

Bioassay procedures

The rice and ZnO nanoparticles were admixed to form the feeding medium with different concentrations for the bioassay procedure. The feeding mediums were placed in a petri dish and shaken mechanically at a fixed time to ensure a complete mixing process. Ten adult *Sitophilus oryzae* were released in each petri dish that was filled with a ZnO-treated feeding medium. Rice weevil mortalities were calculated and recorded every 1, 2, 3, 7, and 15 days after exposure. When weevils did not move at all after having their abdomens pricked with a fine hair brush, they were considered dead (Attia *et al.*, 2020).

RESULTS AND DISCUSSION

Susceptibility of rice towards infestation of pest and its relation with protectant presence

According to the susceptibility statistics for commercialized rice brands in Table 1, 10 out of 12 brands were infested at least by week 16. Four brands show significantly high levels of infestation up to 12 weeks of observation i.e. brands BWNM, JS, R, and JSS with 36, 49, 65, and 124 individuals of *S. oryzae*, respectively. In addition, 3 brands which were DPJ, RU, and K were found infested with *Oryzaephilus surinamensis* as secondary infestation. Two brands showed no sign of infestation up to the end of the observation period i.e. brands L and S.

In this study, rice susceptibility is determined by how vulnerable it is to being damaged by pests found in stored goods. After observation, it was discovered that the main pest in the rice brands monitored was *S. oryzae* (rice weevil), with *O. surinamensis* (sawtoothed grain beetle) acting as a secondary pest. Even though all rice brands were processed in factories, there are still factors that can lead to pest outbreaks, such as the frequent or excessive application of insecticides, compost, or irrigation (Carvalho *et al.*, 2011). One of the main factors influencing insect pest outbreaks is the evolution of insecticide-resistant insects. Insecticides are used widely and repeatedly, creating a condition where resistant individuals are under strong selection pressure. Pests can still infest processed, stored goods like rice grains because these individuals can go across the area treated with insecticide and cause more outbreaks (Carvalho *et al.*, 2011; Hallam, 2022). As Malaysia is located in Southeast Asia, where rice is a staple food, it is critical to monitor the susceptibility status of the rice after processing and packing. With that data, various efficient control strategies can be developed to guarantee the quality of the rice sold and the health of the population (Hallam, 2022; Afandi & Lee, 2023).

From the data obtained, there were no patterns of emergence as every brand had different numbers of individuals that emerged at different times. This situation probably occurs due to the presence of grain protectants (Doherty *et al.*, 2023). Insecticides that are permitted to be used on grains to stop insect infestations during storage are known as grain protectants. Malathion and chlorpyrifos-methyl are two protectants that are used globally to prevent pests from invading stored grains (Kljajic & Peric, 2009). It was found that each of these pesticides poses a serious risk to the environment as well as to humans. The protectants and their residue, which kill eggs and adults that eat the grain, are responsible for the pests' gradual reappearance. They reappear as a result of the protectants' deteriorating effectiveness over time. Since chemical insecticides are proven to be hazardous to both human health and the environment (Ahmad *et al.*, 2024), modified zinc oxide (ZnO) is used to create safe nanopesticides that preserve both the environment and human health (Mason & Obermeyer, 2010).

Based on the results, the reason the two rice brands were not infested by insect pests until the end of observation was due to the high efficacy of the protectants in both brands. According to Mason & Obermeyer (2010), protectants offer protection for 3 to 6 months. All of the rice brands were manufactured in early November and were monitored until May, assuming the efficacy of protectants in other rice brands had decreased thus resulting in the emergence of pests. There was still residue in the two rice brands resulting in no emergence of pests.

Efficacy of zinc oxide nanoparticles based on the reactivity modification

The toxicity effect of different types of ZnO treatments at 4 different concentration percentages (w/w) towards *S. oryzae* is shown in Table 2. For the gamma-ray modified-ZnO (7A) treatment, 100% mortality was found after 3 days of treatment with a concentration range of 0.4 to 0.8% (w/w) concentration. For beta-gamma ray modified-ZnO (3A) and neutron modified-ZnO (1A) treatments, 100% mortality was only obtained after 7 days period of exposure with the concentrations range of 0.4 to 0.8% (w/w). However,

for non-modified ZnO (B) treatment, 100% mortality was only obtained for 0.8% (w/w) concentration after 7 days of the ZnO exposure. The data shows that the ZnO nanoparticle modified with the gamma-ray is the most efficient to control *S. oryzae*.

Table 1. Susceptibility of rice brands towards *Sitophilus oryzae*

Rice brands	Period of observation of <i>Sitophilus oryzae</i> (primary infestation) (weeks)																	Occurrence of secondary infestation		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		Total	
L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	ND	
DPJ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	<i>Oryzaephilus surinamensis</i>	
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	ND	
BWNM	0	0	0	0	0	0	0	0	0	0	0	1	2	5	9	17	36	36	ND	
BOP	0	0	1	1	1	1	1	1	1	1	1	1	1	2	3	4	4	4	ND	
JS	0	0	0	0	0	0	0	0	0	0	0	6	6	7	15	31	49	49	ND	
BTM	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	5	5	5	ND	
F	1	1	1	1	1	1	1	1	1	4	5	5	6	6	7	10	14	19	19	ND
RU	0	0	0	0	0	0	0	0	0	0	0	2	2	3	5	12	19	19	<i>Oryzaephilus surinamensis</i>	
R	1	1	1	1	1	3	3	3	4	5	6	18	20	20	25	39	65	65	ND	
JSS	1	1	1	1	2	6	6	6	6	10	12	38	49	64	72	98	124	124	ND	
K	0	0	0	0	0	0	0	0	0	0	0	0	0	5	7	10	14	14	<i>Oryzaephilus surinamensis</i>	

L: Lisa ; DPJ: Dari Pulau Jauh ; S: Sayang ; BWNM: Beras Wangi Nan Murah ; BOP: Bird of Pacific ; JS: Jasmin Suria; BTJ: Bird Jewel ; F: Fauna ; RU: Ros Utara ; R: Ras ; JSS: Jejak Super Special; K: Kami; ND: Non determined.

Table 2. Toxicity of Zinc oxide with different modifications on *S. oryzae* (% of mortality)

Treatments (ZnO types)	Concentration % (w/w)	Periods of exposure (day) with percentage mortality (%)				
		1	2	3	7	15
7A	0.1	0	0	3.0	46.7	83.3
	0.2	10.0	16.7	70.0	100	-
	0.4	0	33.0	100	-	-
	0.8	0	56.7	100	-	-
	0.1	0	13.0	40.0	63.0	96.7
3A	0.2	0	40.0	73.0	83.0	100
	0.4	3.0	13.0	60.0	100	-
	0.8	6.0	83.0	86.7	100	-
	0.1	0	6.6	56.7	93.0	100
1A	0.2	6.6	16.7	83.0	90.0	100
	0.4	0	53.0	86.7	100	-
	0.8	3.0	76.7	83.0	100	-
B	0.1	0	0	6.6	10.0	26.7
	0.2	0	0	10.0	13.0	16.7
	0.4	0	3.0	13.0	40.0	73.3
	0.8	3.0	33.3	53.3	100	-

7A: gamma ray modified-ZnO; 3A: beta-gamma ray modified-ZnO; 1A: neutron modified-ZnO; B: Non modified-ZnO; w/w: weight per weight; ZnO: Zinc oxide.

Zinc oxide nanoparticles are photo-responsive nanoparticles. Photo-responsive nanoparticles enable the control of active molecule release via exposure to light radiation. These nanoparticles can absorb light of various wavelengths in the UV, visible, and infrared ranges (Camara *et al.*, 2010). In this study, modified ZnO nanoparticles were exposed to different types of radiation, therefore, ZnO releases active molecules based on the types of light absorbed (Sirelkhatim *et al.*, 2015). This explains the reason behind type B being the least effective to control pests because type B was not exposed to any radiation thus the release of active molecules was probably too slow or too little for it to be toxic enough to kill *S. oryzae*. Zinc oxide type 7A is the most toxic towards *S. oryzae* because it was exposed to gamma-ray which has the highest energies compared to neutron and beta rays, so more active molecules were released to act on the pest.

Several studies have found that the various morphologies of ZnO nanoparticles have a significant

impact on toxicity (Peng *et al.*, 2011; Sirelkhatim *et al.*, 2015; Pereira *et al.*, 2020). The modification of reactivity also affects the morphologies which in turn influence the level of toxicity.

Insect mortality could be caused by injury to the digestive tract or enlargement at the surface of the integument as a result of dehydration or spiracle and trachea blockage. Absorption and abrasion cause damage to the insect's protective wax coat on the cuticle. As the water barrier deteriorates, the insects will start to lose water and moisture inside their body and die from desiccation (Alexander *et al.*, 2008; Attia *et al.*, 2020). This physical mode of action hypothesis strengthens the case for the use of nanoparticles as an insecticide. Insects are unlikely to develop genetic or physiological resistance to such a mode of action. However, insects may develop a specific pattern which is a behavioral response to the particles used and avoid contact (Attia *et al.*, 2020).

CONCLUSION

According to the findings of this study, ten out of twelve rice brands were infested by *S. oryzae* at least by week 16. Brands Lisa (L) and Sayang (S) showed no signs of infestation until the end of the observation period. Brands namely Beras Wangi Nan Murah (BWNM), Jasmin Suria (JS), Ras (R), and Jejak Super Special (JSS) exhibited significantly high infestation up to 12 weeks of observation, with 36, 49, 65, and 124 individuals of *Sitophilus oryzae*, respectively. Furthermore, three brands, DPJ, RU, and K, were found to be infested with *Oryzaephilus surinamensis* as a secondary infestation. This is because protectants only protect stored grain for 3 to 6 months and protectants in commercialized rice used in the study had reduced efficiency. According to the data, the 7A treatment is the most effective at controlling *S. oryzae*. Zinc oxide type 7A is the most toxic to *S. oryzae*. After all, it is exposed to gamma rays, which have the highest energies compared to neutron and beta rays, resulting in the release of more active molecules to act on the pest because ZnO nanoparticles inhibit the photoresponsive mechanism. For future studies, ZnO nanoparticle usage can be widely explored to be used as a protectant in stored products such as commercialized rice brands in our country by testing the ZnO NPs to all the twelve brands used in this study. This could also ensure that researchers implement goal number 12 in sustainable development goals (SDG) which is to ensure sustainable development and production patterns by securing and improving food safety and security.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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