

Characterization and evaluation of two amorphous silica powders against stored product insect
species on concrete surfaces and wheat

by

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B.Sc., Loyola College, 2005
M.Sc., Madras Christian College, 2007

AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

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Abstract

Silica based powders are gaining popularity in stored product insect pest management due to their low mammalian toxicity and ease in application. Amorphous silica powders 1 and 2 are two diatomaceous earth formulations provided by Imerys, Lompoc, California, USA. The particle diameter and the shape characteristics of the silica powders 1 and 2 were determined using Morphology G3 instrument. The particle diameter (D_{10} , D_{50} and D_{90}) of silica powder 1 was significantly smaller than silica powder 2. In terms of shape characteristics, the circularity, convexity, and elongation values of silica powder 1 were significantly higher compared to silica powder 2. The insecticidal efficacy of silica powder 1 and 2 against the red flour beetle, *Tribolium castaneum* (Herbst); confused flour beetle, *Tribolium confusum* Jacquelin du Val, saw-toothed grain beetle; *Oryzaephilus surinamensis* (Linnaeus); rice weevil, *Sitophilus oryzae* (Linnaeus); and lesser grain borer, *Rhyzopertha dominica* (Fabricius), was determined by exposing adults to different application rates and exposure times. On concrete surfaces, at application rates of 4.32 to 1.34 g/m², 99% mortality of *R. dominica* adults was observed when they were exposed to silica powder 1 for 24 to 48 h. In contrast, silica powder 2 at application rates of 0.63 to 0.19 g/m² produced 99% mortality of *R. dominica* adults when they were exposed for 4 to 12 h. At 15.81 to 5.35 g/m² of silica powder 1, and at 13.31 to 4.65 g/m² of silica powder 2, 99% mortality of *S. oryzae* adults was observed after exposure for 12 to 48 h. Lower application rates of powders and exposure times were required for both insect species to achieve complete suppression of progeny production, percentage of insect-damaged kernels, and percentage of grain weight loss compared to those required for 99% mortality. Complete mortality of *T. castaneum* adults was observed when they were exposed to 5 g/m² of silica powder 1 after 24 h, whereas a 48 h exposure to ≥ 0.75 g/m² of silica powder 2 was required to

achieve complete mortality. Complete mortality of *T. confusum* adults was achieved at ≥ 4.50 and ≥ 2.50 g/m² of silica powder 1 and silica powder 2, respectively. At 3.5 g/m² of silica powder 1, complete mortality of *O. surinamensis* adults was observed after 36 h, while only a 12 h exposure was required when adults were exposed to 4.0 g/m² of silica powder 2.

On grains, silica powder 1 applied to wheat at 0.5 g/kg rate produced more than 99% mortality of *O. surinamensis* and *T. castaneum* adults. However, ≥ 0.35 g/kg of silica powder 2 on wheat gave more than 99% mortality of *O. surinamensis* and *T. castaneum* adults. Complete suppression of *O. surinamensis* and *T. castaneum* adult progeny was achieved at ≥ 0.35 g/kg of silica powder 1 and 0.25 g/kg of silica powder 2. Complete mortality, adult progeny suppression of *R. dominica*, percentage of insect-damaged kernels, and percentage of grain weight loss was achieved only for silica powder 2 at 0.20 g/kg. Silica powder 2 at ≥ 0.25 g/kg on wheat produced 99% mortality of *S. oryzae* adults. Complete suppression of *S. oryzae* progeny production, percentage of insect-damaged kernels, and percentage of grain weight loss was achieved for both powders at 0.30 g/kg. Wheat mixed with 0.35 g/kg of silica powders 1 and 2 resulted in a decrease in bulk density of wheat and an increase in the angle of repose and tapped density. Treating wheat with the 0.35 g/kg of silica powders 1 and 2 caused wheat to change from good flowability to an acceptable flowability, based on the angle of repose, Hausner ratio, and Carr index values.

Both silica powders exhibited better insecticidal activity against *S. oryzae* at lower humidities of $30 \pm 2\%$ and $50 \pm 5\%$ compared to activity at a humidity of $75 \pm 4\%$. The combination of silica powders with a commercial insecticide powder Gravista-D at 0.09 g/kg containing two active ingredients (deltamethrin and S-methoprene) and an insecticide synergist piperonyl butoxide, resulted in enhanced efficacy in terms of adult mortality, suppression of

adult progeny production, inhibition of insect-damaged kernels and grain weight loss, compared to individual application of the powders. Overall, our findings demonstrate that silica powder 2 exhibited greater efficacy compared to silica powder 1 against all insect species on concrete surfaces and wheat, except for *T. castaneum* on concrete. Both the amorphous silica powders 1 and 2, hold significant promise for safeguarding grains from stored product insect pests in an environmentally friendly manner. Nevertheless, field-oriented studies are warranted to determine the efficacy of these powders applied as grain protectants across various agro-climatic conditions and their effects on grain quality to understand their commercial feasibility for stored product protection.

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Chapter 1 - Background and Review of Literature

1.1 Post-harvest losses and their mitigation

The world population is projected to reach approximately 9.1 billion by 2050, necessitating a 70% increase in food production to meet the escalating demand. This increase in food production requires a corresponding 60% rise in food supply (FAO, 2009; Hengsdijk et al., 2017). The Food and Agriculture Organization State of Food and Agriculture (2019) estimates that about 14% of the total food produced is lost during post-harvest operations. About 17 percent of the total food produced is wasted at the retail and consumer level. These collective post-harvest losses could otherwise provide sustenance for over 1.26 billion hungry individuals worldwide (FAO, 2019). It is estimated that the post-harvest losses caused by stored product insects are estimated to be around 9% in the developed countries and 20% in the developing countries (Pimentel, 1991; Phillips and Throne, 2010). Stored-product pests present substantial risks to dried, preserved, resilient agricultural goods, along with assorted value-enhanced food items and non-food derivatives of agricultural produce worldwide (Phillips and Throne, 2010). Moreover, they contaminate foodstuffs through the introduction of live insects, insect-derived substances like chemical secretions or silk, deceased insects and their fragments, infestation of edifices and storage facilities, and the buildup of chemical insecticide residues in food.

The quality of food grains may degrade if measures are not taken to prevent insect infestation, particularly in conditions of high relative humidity and temperature (Le Patourel, 1986). Management of insect pests in food grains predominantly involves fumigation (Rajendran and Sriranjini, 2008; Manivannan et al., 2016), use of grain protectants (Subramanyam et al., 2014; Hamel et al., 2020), sanitation of storage facilities, use of aeration and other Integrated Pest Management (IPM) approaches (Subramanyam and Roesli, 2000). However, pest control

efforts against stored product insects can expose humans to hazardous chemicals. In addition, the emergence of resistance to fumigants including phosphine among stored product insects (Benhalima et al., 2004; Daglish et al., 2014; Nguyen et al., 2015; Aulicky et al., 2019) has heightened the reliance on efficient grain protectants which are an integral part of the Integrated Pest Management system.

1.2 Grain protectants: Balancing efficacy and challenges

The application of chemical grain protectants represents a proactive strategy for treating uninfested grain during the loading process into bins. While these protectants may offer protection against stored product insects that develop externally, they may not adequately target all stages of insect life, particularly the development of larvae within kernels. Hence, it is essential to control adult stored-grain insects before they have the chance to reproduce by mating and laying eggs either on the surface (for internal insects) or within the kernels (for external insects) (Hagstrum and Subramanyam, 2006). To ensure the effective delivery of high-quality chemical insecticides, these substances are applied to grains during storage as they are loaded into bins or silos. However, the efficacy of synthetic pesticides in changing climatic conditions has attracted attention, with outcomes varying depending on the properties of the pesticide (Tsaganou et al., 2021). For instance, the effectiveness of fipronil as a grain protectant against lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) was compromised when applied at 30°C compared to lower temperatures of 20°C and 25°C (Kavallieratos et al., 2010). Temperature and humidity exerted significant yet intricate influences on the efficacy of spinosad as a grain protectant against various stored product insects. Notably, its effectiveness was observed to notably enhance at higher temperatures (e.g., 30°C compared to 20°C), particularly against rice weevil, *Sitophilus oryzae* (L.) (Athanassiou et al., 2008). Additionally, concerns have

arisen regarding control failures and reduced efficacy due to insect resistance to conventional grain protectants (Daglish and Nayak, 2018). Consequently, there is a substantial demand for safer, cost-effective alternatives that can effectively manage stored product insects.

1.3 Inert dusts as a sustainable approach to pest management

Stored product insects have been reported from residual grains and grain debris in empty bins, which serve as their food source until newly harvested grains are stored (Reed et al., 2003; Arthur et al., 2006). While sanitation of empty bins prior to introducing newly harvested grains can reduce insect numbers within stored grains (Reed et al., 2003; Arthur et al., 2006), this approach does not ensure complete control, particularly for long-lived insect species. Treatment of storage facilities and handling equipment with inert powders offers a cost-effective alternative to chemical treatments, providing durable protection (Desmarchelier and Dines, 1987). In addition, inert dust applications have demonstrated the ability to enhance the effectiveness of other pest management strategies, including aeration, fumigation, and heat treatments (Winks and Russel, 1994; Fields et al., 1997). Moreover, they have been recommended for use in resistance management strategies (Balliota and Athanassiou, 2023). Inert dusts powders are widely utilized for industrial and agricultural applications (Ebeling, 1971).

Diatomaceous earth formulations derived from the fossils of phytoplanktons or diatoms (Korunić, 1998; Losic and Korunić, 2018) are emerging as promising alternatives to synthetic insecticides worldwide (Subramanyam and Roseli, 2000). These formulations are mixed with food grains (Zeni et al., 2021) or used as structural treatments on empty grain bins and floors. The suggested mechanisms of action of diatomaceous earths on insect pests encompass diverse modes of action including, dehydration-induced surface enlargement of the insect integument (Zacher and Kunicke, 1931), damage to the digestive tract (Smith, 1969), and the blocking of

spiracles and trachea (Webb, 1945), and adsorption of epicuticular waxes, leading to death by desiccation (Ebeling, 1971; Subramanyam and Roesli, 2000). Many researchers have reported diatomaceous earth formulations to be effective on stored insect insects when admixed with grains. Additionally, the efficacy of diatomaceous earth products for insect pest management may vary depending on factors such as geographical origin, pH, diatom species, density, particle size distribution, and lipid adsorption capability (Korunić, 1997; Fields and Korunić, 2000).

Amorphous silica powders possess a unique capability to absorb water reversibly and absorb molecules of appropriate size, exchanging their inorganic cations without significant structural changes (Dakovic et al., 2007). Amorphous silica powders offer significant advantages as grain protectants in stored product protection, particularly because they are already used in food applications as anti-caking agents at concentrations below 1.5% w/w (Shawir et al., 1988). Moreover, they are considered safe for unlimited daily intake (Anonymous, 1974). These powders demonstrate effectiveness against stored product insects even at lower application rates (Manivannan and Subramanyam, 2023a,b; Manivannan et al., 2023). Diatomaceous earth formulations act on insects by physical means, resistance development is more unlikely to occur if proper application rate is administered.

1.4 Challenges with the usage of diatomaceous earths as grain protectants

Ideally, the application of diatomaceous earth formulations in storage environments includes, admixing with grains, structural treatment of walls and bin floors and surface treatment of grain bulks (Korunić, 2013). However, the widespread adoption of these formulations as grain protectants is restricted due to many limitations. Diatomaceous earth treatment with grains can impact the grain flowability, reduction in bulk density and grading value, impart dust residues, variations in insect species' susceptibility to diatomaceous earths, notable influences of grain

moisture and temperature on diatomaceous earths efficacy, and the substantial variation in diatomaceous earth effectiveness against insects across different commodities (Jackson and Webley, 1994; Subramanyam et al., 1994; Golob, 1997; Korunic, 2016). Grain bulk density plays a pivotal role in grading and assessing the economic value of wheat. Therefore, it is crucial for diatomaceous earth formulations intended for use as grain protectants to have minimal impact on the grain quality including bulk density and flowability.

Several studies have demonstrated that various diatomaceous earth formulations, applied at different rates, can significantly reduce the bulk densities of treated wheat. Bodroža-Solarov et al. (2012) reported a decrease in bulk density when wheat was treated with zeolite and diatomaceous earth formulations. Even very low concentrations (0.01 and 0.025 g/kg) of Celite 209 and DiaFil 610 were found to significantly reduce wheat bulk density (Korunic et al., 1996). Treatment with 0.05 g/kg of Dryacide® decreased bulk density and increased the angle of repose for wheat (Korunic et al., 1996). Since diatomaceous earth formulations treated to grains get adhered to kernel surfaces, this can increase friction between grains, resulting in elevated angle of repose and decreased bulk densities (Korunic et al., 1998). In addition, the milling industry is hesitant to accept diatomaceous earth-treated grains due to concerns about potential damage to machinery (Korunić, 1997, 1998; Subramanyam et al., 1994).

Wheat kernels subjected to inert dusts develop a granular system, which can transition among three distinct states: static, quasi-static, or dynamic (Lumay et al., 2012). A primary concern when applying any form of amorphous dust to grain for quality preservation is altering the treated grain's flow properties. A plethora of literature emphasizes the correlation between flowability and grain physical attributes such as particle size, particle shape, moisture content, and environmental factors like temperature and relative humidity (Ganesan, 2008; Emery et al.,

2009). Minor changes in wheat flowability could result in dramatic changes in basic flowability energy, minimum fluidization energy, and the economic implications, for the entire supply chain, are higher energy consumption and decreased profitability. The addition of inert dusts is not always detrimental to wheat flowability, though. Inert dusts may behave as flow additives and be desirable to improve grain processing by reducing the frictional resistance of particle-to-particle movement (Cooke and Freeman, 2006).

1.5 Justification for research

Many diatomaceous earth formulations are lethal to insects only at application rates of 1 g/kg or more (Fields and Korunić, 2000), and the recommended application rates of diatomaceous earth formulations lies between 0.5 g/kg to 3.5 g/kg (Korunić, 1997). However, researchers have evaluated an array of diatomaceous earth formulations from different geographical locations on stored product insects, very few products have shown efficacy at lower application rates. The effectiveness of diatomaceous earth formulations against stored product insects is influenced by several factors including application rate, duration of exposure, treatment conditions, type of commodity, insect species, and life stage (Korunić, 1998; Zeni et al., 2021; Agrafioti et al., 2023). Hence evaluation of newer formulations of diatomaceous earth should include testing their efficacy as grain protectants and as surface treatments. Previous research on diatomaceous earth has reported species specific insecticidal activity of these formulations on stored product insect pests. In addition, studies have shown that flat bodied insects such as the *Cryptolestes* spp. are highly susceptible to diatomaceous earth formulations (Eroglu et al., 2017), while the flour beetles, *Tribolium* spp. and the lesser grain borer, *Rhyzopertha dominica* (F.) are the most tolerant insect species (Golob, 1997; Korunić, 1998). In addition, the efficacy of diatomaceous earth powders against insect pests is known to be influenced by their physical

properties (pH, particle size distribution, lipid adsorption capability, moisture content of the powders, silicon dioxide content, and the diameter of inner pores of particles) of the formulations (Korunić, 1997; Fields and Korunić, 2000). However, very few studies have characterized the particle size and shape of the diatomaceous earth formulations on their insecticidal efficacy against stored product insects. Hence, diatomaceous earth formulations effective on internally and externally developing at lower application rates and shorter exposure time can be beneficial in grain processing environments.

Amorphous silica powders 1 and 2 are two diatomaceous earth formulations sourced from Imerys, Lompoc, California, USA. The elemental composition and the physical properties of these two silica powders 1 and 2 were characterized since they are new products and not registered for usage with the Environmental Protection Agency for usage in grain processing facilities. The efficacy of silica powder 1 and 2 against the *R. dominica*; red flour beetle, *Tribolium castaneum* (Herbst); saw-toothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus); and confused flour beetle, *T. confusum* Jacquelin du Val on concrete surfaces, and wheat was determined because there is no information on the insecticidal efficacy of these two formulations. In addition, studies have shown that even a very low concentration of diatomaceous earths (0.01 and 0.025 g/kg) can affect the bulk density of grains and flowability bringing down their grading and economic value. Hence, understanding the influence of silica powders on grain bulk density and flow rate is essential for effective flow control during grain transfer. Measurements such as bulk, tapped, and true densities, along with compressibility index and Hausner ratio, were crucial for assessing interparticle interactions and flowability. Additionally, dynamic flow properties were evaluated using instruments like the FT4 powder rheometer to provide insights into wheat treated with silica powder behavior during processing,

including energy required for flow, stability, flow rate, and aeration effects. Moreover, the efficacy of these silica powders is attributed to adsorption of the epicuticular waxes and at elevated humidity the absorbed water by the powders can influence their insecticidal efficacy (Ebeling 1971; Fields and Korunic 2000). Hence studies were also conducted to evaluate their efficacy at different humidity conditions to understand how these powders behave at different relative humidity conditions.

The objectives of the present study were:

- ❖ To determine the efficacy of two amorphous silica powders against the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on concrete surfaces,
- ❖ To determine the insecticidal activity of two silica powders applied to concrete arenas against the rice weevil, *Sitophilus oryzae* (L.), (Coleoptera: Curculionidae),
- ❖ To evaluate the insecticidal toxicity of silica powders against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae),
- ❖ To determine the insecticidal activity of two silica powders against the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae),
- ❖ To evaluate the efficacy of two silica powders against the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L), (Coleoptera: Silvanidae),
- ❖ To determine the efficacy of two amorphous silica powders applied to soft red winter wheat against the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae),
- ❖ To determine the insecticidal activity of two amorphous silica powders applied to hard red spring wheat against the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae),

- ❖ To evaluate the efficacy of two amorphous silica powders applied to soft red winter wheat against two externally developing stored product insects,
- ❖ To study the influence of two amorphous silica powders on the physical and flow properties of hard red spring wheat,
- ❖ To determine the efficacy of two amorphous silica powders at different relative humidity and evaluate Gravista-D plus silica powder combination against the rice weevil, *Sitophilus oryzae* (L).

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Chapter 2 - Insecticidal efficacy of two candidate diatomaceous earth powders against the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

2.1 Abstract

The insecticidal efficacy of amorphous silica powders 1 and 2 with different physical and elemental compositions were evaluated against the lesser grain borer, *Rhyzopertha dominica* (F.) using concrete arenas. The particle diameter and shape characterization (circularity, elongation, aspect ratio, and convexity) of the two silica powders were performed using Morphology G3 instrument. The particle diameter and shape characteristics (circularity and elongation) of silica powder 1 was significantly higher compared to silica powder 2. However, these factors did not influence the efficacy of silica powders against *R. dominica*. Silica powder 2 showed higher mortality at lower application rates and shorter exposure times compared to silica powder 1. The efficacy of silica powder 2 was due to its high oil adsorption capability, low pH, and silicon dioxide content compared to silica powder 1. Adult progeny production was inversely related to the powder application rate. Silica powder 2 was more effective on *R. dominica* in inhibiting adult progeny production at 0.25 g/m². Lower percentages of insect-damaged kernels and grain weight loss were observed at lower application rates (0.10 – 0.20 g/m²) following exposure to silica powder 2 compared to silica powder 1, which required higher application rates (2.0 – 3.5 g/m²). Overall, silica powder 2 demonstrated superior efficacy against *R. dominica* in terms of mortality, progeny production, insect-damaged kernels, and grain weight loss.

Keywords: Amorphous silica powders, *Rhyzopertha dominica*, Oil adsorption capability, Insect-damaged kernels, Grain weight loss.

2.2 Introduction

Stored product insects have been reported from residual grains and grain debris in empty bins, which serve as their food source until newly harvested grains are stored (Reed et al., 2003; Arthur et al., 2006). While sanitation of empty bins prior to introducing newly harvested grains can reduce insect numbers within stored grains (Reed et al., 2003; Arthur et al., 2006), this approach does not ensure complete control, particularly for long-lived insect species. Treatment of empty bins with approved insecticides after sanitation is recommended to achieve effective control of stored product insects (Bridgeman, 1994; Arthur and Subramanyam, 2012). The prolonged use of chemical insecticides has increased health risks to applicators and to the environment (Fields and Korunić, 2000). Inert dusts, especially silica-based diatomaceous earth and zeolites are potential alternatives to chemical insecticides (Subramanyam and Roseli, 2000; Baliota and Athanassiou, 2023). Non-diatomaceous earth inert dusts from Ethiopia such as filter cake and Triplex powders, which contain silica, were found to exhibit contact efficacy against the maize weevil, *Sitophilus zeamais* Motschulsky) and the rice weevil, *Sitophilus oryzae* (L.) (Tadesse and Subramanyam, 2018 a,b; Tadesse et al., 2019 a,b). Complete control of *S. zeamais* was achieved when adults were exposed to 7.5 g/m² of filter cake and 10 g/m² of Triplex powders in concrete arenas for 24 h (Tadesse and Subramanyam, 2018a). Tadesse et al. (2019a) reported 8.49 and 21.38 g/m² for effective control of *S. oryzae* following a 12 h exposure to filter cake and Triplex powders, respectively. Bohinc et al. (2020) reported 100% mortality of *S. zeamais* when adults were exposed for 7 days to Zeolite SI treated concrete arenas at 10 g/m² at 25 °C and 55% r.h. Complete mortality of the lesser grain borer, *Rhyzopertha dominica* (F.), *S. oryzae*, *S. zeamais*, the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), and red flour beetle, *Tribolium castaneum* (Herbst) adults was achieved after 24 h when exposed to 5 g/m² of

fine and coarse zeolites, except for *T. castaneum* which showed 96% mortality when exposed to coarse zeolite (Yao, 2014).

Diatomaceous earth dusts can offer long term protection of stored food grains (Korunić, 1999; Subramanyam and Roesli, 2000) compared to chemical insecticides, which can degrade over time. Moreover, diatomaceous earth dusts have low mammalian toxicity but possess strong insecticidal activity. They kill insects by adsorbing the epicuticular lipids by direct contact leading to death by desiccation (Ebeling, 1971; Shawir et al., 1988; Subramanyam and Roesli, 2000; Mewis and Ulrichs, 2001). Diatomaceous earth has become one of the integral part of integrated pest management of stored product insects (Korunić, 1999). A majority of studies on diatomaceous earths focused on their usage as grain protectants (Subramanyam and Roseli, 2000; Chanbag et al., 2007; Vardeman et al., 2007a; Kavallieratos et al., 2010; Korunić, et al., 2017; Paponja et al., 2020). There are limited published studies reporting the efficacy of diatomaceous earth for treatment of surfaces such as empty bins and floors. At an application rate of 10 g/m², Silico-sec[®] dry dust demonstrated remarkable efficacy, leading to over 90% mortality of the granary weevil, *Sitophilus granarius* (L), Mediterranean flour moth, *Ephestia kuehniella* (Zeller), and groceries mite, *Lepidoglyphus destructor* (Schrank), when exposed to treated wood surfaces for 7 days (Collins and Cook, 2006). In contact bioassays conducted in concrete arenas (Frederick and Subramanyam, 2016), DiaFil[®] 610 demonstrated 100% control of *T. castaneum* at application rates of 2.5 and 5.0 g/m² on concrete arenas for a period of 24 h. Complete mortality was achieved when adults of *R. dominica* were exposed to SilicoSec[®] and Protect-It[®] treated concrete surfaces at 2 g/m² application rate for 3 days (Delgarm and Ziaee, 2021). A recent study from Greece (Baliota and Athanassiou, 2023) demonstrated that *R. dominica* adults can be controlled by exposing them to steel or concrete surfaces treated with

natural inert dusts such as DE 5, DE 6, and zeolite at an application rate of 0.5 g/m² when exposed for 3 days or more. The insecticidal activity of inert dusts can vary based on the geographical location, diatom species, pH, particle size distribution, internal surface areas, and oil adsorption capability (Korunić, 1997; Korunić, 1999; Fields and Korunić, 2000). In this study, two candidate amorphous diatomaceous earths, supplied by Imery's chemicals, Lompoc, California, USA, referred to here as silica powders 1 and 2, were evaluated for their efficacy against *R. dominica* adults using concrete arenas to simulate empty bin floors. The objective of this study was to determine the effective concentrations and times required for 99% mortality of adults and 99% reduction in adult progeny production of *R. dominica* exposed to the two amorphous silica powders. In addition, our study determined the percentage of insect-damaged kernels and percentage of grain weight losses to illustrate which amorphous silica powder was more effective for *R. dominica* control.

2.3 Materials and Methods

2.3.1 Particle size analysis and shape characterization of amorphous silica dusts

Two amorphous silica powders 1 and 2, with different elemental compositions (Table 2.1) were supplied by the manufacturer, Imery's Chemicals, Lompoc, California, USA. The particle size and particle size distribution analysis (D₁₀, D₅₀ and D₉₀ – diameter fractions where 10, 50, and 90% of particles were within a certain size range) of the two amorphous silica powders were performed using Morphologi G3 SE instrument (Malvern Instruments, Malvern, UK). A silica dust sample of 3 mm³ was dispersed on a glass slide using a dispersion unit with pressurized air. The dust particles were imaged at 10× magnification, and the system was programmed to select 20,000 particles at random (Borretto et al., 2022). The shape characteristics such as circularity, convexity, elongation, and aspect ratio were also determined

using Malvern Morphologi G3 SE instrument. Circularity defines how close the particle resembles a perfect circle; a value close to 1 indicates a perfect circle shape of the dust particle. Convexity quantifies the surface roughness of the particle; smooth particles have convexity values close to 1, while rough particles have values closer to 0 (Malvern Instruments Ltd., 2015; Borretto et al., 2022). Aspect ratio is used to classify the dust particles as fibrous, acicular, and equant (Olson, 2011) and it is the ratio of Feret's minimum length to its maximum length. Elongation defines how elongated the particles are; values closer to 1 indicate very elongated particles. All these characteristics on each silica powder were performed in triplicates.

2.3.2 Wheat grains

Organic soft red winter wheat from Great River Organic Milling Company, Fountain City, Wisconsin, USA, was used for culturing insects. The wheat samples were kept in a deep freezer at -13°C for a week to kill any live insects present. The moisture content of the grain sample was determined using a Moisture Analyzer Model 930 (Shore Sales Co., Pantoul, Illinois, USA). To ensure that the development of insects was uniform in wheat grains, a moisture content of 12% (wet basis) was maintained for wheat samples throughout the study. When the moisture content of the wheat grains was not within the desired level (12%), the grains were either tempered with distilled water when too dry or were allowed to equilibrate inside a temperature humidity-controlled chamber (Pervical Scientific, Inc., Model I-36 VL, Perry, Iowa, USA) if moisture was > 12%. The tempering of the grains was performed using the following formula:

$$\text{Amount of water in ml required for tempering} = \frac{100 - \text{current moisture}}{100 - \text{desired moisture}} \times \text{grain weight}.$$

2.3.3 Test insects

Cultures of *R. dominica* used in experiments originated from insect colonies maintained since 1999 in the Department of Grain Science and Industry, Kansas State University,

Manhattan, Kansas, USA. Insect cultures from a laboratory strain were reared in 0.94-L glass mason jars containing preconditioned organic soft winter wheat. The jars were covered with wire mesh screen (250 μm) and filter paper lids (9 cm diameter). Jars were placed inside a temperature/humidity chamber at 28°C and 65% r.h. with a light:dark photoperiod of 14:10.

2.3.4 Concrete poured Petri dishes

Ready mix concrete (Rockite, Hartline Products Company, Inc., Cleveland, Ohio, USA) slurry was prepared by mixing concrete mix with tap water in 2:1 ratio. Concrete dishes were made by pouring concrete slurry into 9 cm diameter and 1.5 cm high plastic Petri dishes (Midwest Scientific Co., Fenton, Missouri, USA). After pouring, the dishes were allowed to dry on a laboratory bench for 24 h. The inside walls of Petri dishes were coated with polytetrafluoroethylene (Insect-a-slip, Bio Quip Products, Inc, Rancho Dominguez, California, USA) to prevent insects crawling on the sides thereby avoiding contact with treated surface (Tadesse and Subramanyam, 2018 a,b; Tadesse et al., 2019a). The concrete arenas were used to simulate empty bin floors.

2.3.5 Bioassays

Three different experiments were performed to determine the effect of the two amorphous silica powders on *R. dominica*. In the first experiment, concrete poured Petri dishes were treated with each silica powder at twenty different application rates: 0 (control), 0.1, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5 g/m². Once the silica powder was transferred to concrete arenas, a dissecting needle was used to distribute the powder on concrete arenas. Ten unsexed adults (1-4-week-old) were exposed to each powder application rate for 4, 8, 12, 24, 36, and 48 h. Five replications were maintained for each powder

application rate and exposure time combination. Concrete arenas after insect introduction were secured with Petri dish lids and held in a growth chamber at 28°C and 65% r. h.

In the second experiment, 10 unsexed adults of *R. dominica* were exposed to 1.5 g/m² of silica powder 1 and 0.25 g/m² of silica powder 2, for 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h. These rates were selected based on the outcomes of the initial experiments, which resulted in complete mortality of *R. dominica* adults at these rates after 24 h for silica powder 1 and 2, respectively. Each of the silica powder application rate and exposure time combination had five replications. All the concrete arenas were held in a growth chamber at 28°C and 65% r.h. After the exposure period, mortality assessments were carried out for each treatment combination. The adults that did not move when prodded with camel's hair brush were regarded as dead.

In the third test, the effect of two amorphous silica powders on the progeny production of *R. dominica* was evaluated at 42 d by exposing the adults to the application rates and exposure periods similar to first experiment. Five replications were maintained for each powder application rate and exposure time combination. After the intended exposure, insects from each concrete arena were carefully transferred to individual 150 ml round bottom plastic containers containing 30 g of organic soft winter wheat. After 42 d, the wheat was sieved using a 2.38 mm circular holed aluminum sieve to separate the adults. The adult progeny production was calculated by subtracting the 10 parental adults from the total number of adults obtained from each powder application rate and exposure time combination replicate. The wheat grains from each treatment combination were passed through a Boerner divider (Seedburo Equipment Company, Des Plaines, Illinois, USA) to obtain a working sample of ≈ 3.5 g to determine the number of insect damage kernels and undamaged kernels (Tadesse et al., 2019 a,b). The percentage of insect damage kernels was estimated by counting the number of damaged kernels

and undamaged kernels and weighing damaged and undamaged kernels from the working samples. The weight loss was calculated by using the equation:

$$\text{Weight loss (\%)} = 100 \times [(UN_d - DN_u) \div (U \times (N_d + N_u))]$$

where, U is the weight of undamaged kernels, N_d is the number of damaged kernels, D is the weight of damaged kernels, and N_u is the number of undamaged kernels (Adams and Schulten, 1978; Boxall, 1986).

2.3.6 Data analysis

The particle diameter and shape characteristics of the silica powders 1 and 2 were subjected to two sample *t*-test to determine the differences between the powders (SAS Institute, 2012). The mortality of *R. dominica* exposed to silica powders 1 and 2 were corrected for control mortality (Abbott, 1925). The lethal concentrations (LCs) required to achieve 50 and 99% mortality, and effective concentrations (ECs) required to achieve 50 and 99% reduction in adult progeny production for both the silica powders were estimated using probit analysis (SAS Institute, 2012). The LC_{99} , LT_{99} , EC_{99} and ET_{99} values for silica 1 powder were compared with silica 2 powder using ratio tests (Robertson et al., 2007). The differences between any two $LC_{99}/LT_{99}/EC_{99}$ or ET_{99} values were considered significant ($P \leq 0.05$) if the 95% confidence interval for the ratio tests did not include 1 (Robertson et al., 2007).

The non-linear model, $y = (a + cx^{0.5} + ex)/(1 + bx^{0.5} + dx)$, was fit to the percentage of insect-damaged kernels as a function of application rate of silica powder 1 and silica powder 2, using TableCurve 2D software, Version, 5.01.01 (Systat Software, Inc., San Jose, California, USA). Similarly, non-linear model, $\ln y = a + bx$, was fit to the percentage of grain weight loss as a function of application rate of silica powders 1 and 2, respectively, using TableCurve 2D software, Version, 5.01.01. The non-linear model, $\ln y = a + bx^{0.5}$, was fit to the percentage of

insect-damaged kernels and percentage of grain weight loss as a function of exposure time for both the powders. The parameters a and b were estimated by fitting equations to the percentage insect-damaged kernels and percentage of grain weight loss as a function of application rate and exposure time. Each possible pairwise comparison between powders was performed by comparing individual models with the pooled model (Draper and Smith, 1998). Any two models were significant ($P < 0.05$) from one another if the F -test showed individual models to deviate from the pooled model. All the graphs were plotted using SigmaPlot, version 12.5 software (Systat software, Inc, San Jose, California, USA).

2.4 Results

2.4.1 Particle size and shape characteristics of silica powders

The two- sample t -tests showed that silica powder 1 had significantly smaller in size particles compared to silica powder 2 at the D_{10} , D_{50} , and D_{90} diameter fractions ($P < 0.05$) (Table 2.2). Particles with diameters of at most 26.4 and 31.4 μm dominate the silica powder 1 and silica powder 2, respectively. In terms of shape characteristics, the circularity, convexity, and elongation values of silica powder 1 was significantly higher ($P < 0.05$) compared to silica powder 2. On the other hand, the aspect ratio was significantly higher ($P < 0.05$) for silica powder 2 compared to silica powder 1.

2.4.2 Responses of *R. dominica* adults to silica powders

The mean $\pm$ SE mortality of *R. dominica* adults in controls ranged from 0 to $6.0 \pm 4.0\%$. The mean $\pm$ SE percentage corrected mortality of *R. dominica* adults exposed to 0.1 to 5.0 g/m^2 of silica powder 1 for 4, 8 and 12 h produced a mortality of $16.0 \pm 4.0\%$. The corresponding mean $\pm$ SE percentage corrected mortality of *R. dominica* exposed to the same application rate of silica powder 1 for 24, 36 and 48 h ranged from 16.0 ± 4.0 to 100.0%. Therefore, probit

regression estimates were performed only for 24, 36, and 48 h mortality response data for silica powder 1. Adults of *R. dominica* were more susceptible to silica powder 2. Exposure of *R. dominica* to lowest rate of 0.1 g/m² of silica powder 2 gave adult mortalities more than 80% when exposed for 24, 36 and 48 h. Therefore, there were not enough data points to perform probit analysis. The Chi-square (χ^2) values for goodness-of-fit test of model to data were significant ($P < 0.05$) for both the silica powders, 1 and 2, indicating poor fit of the probit model to the data. Heterogeneity could be the possible factor responsible for the poor fit of model to the data. The lethal concentrations for 50 and 99% mortality of *R. dominica* of silica powder 1 were 0.19 and 4.32 for 24 h, 0.17 and 1.62 for 36 h, and 0.17 and 1.34 g/m² for 48 h exposures, respectively (Table 2.3). While the estimated lethal concentrations for 50 and 99 % mortality of *R. dominica* adults were comparatively lower for silica powder 2 compared to silica powder 1 with 0.19, and 0.63 for 4 h, 0.14 and 0.62 for 8 h, and 0.08 and 0.19 g/m² for 12 h exposure, respectively (Table 2.4). This indicated that silica powder 2 was more effective than silica powder 1. Comparing the LC₉₉ values for silica powder 1 at 24, 36 and 48 h exposures to LC₉₉ values of silica powder 2 even at 4, 8 and 12 h exposures, showed that silica powder 1 had significantly higher ($P > 0.05$) LC₉₉ values than silica powder 2 [Silica powder 1 vs silica powder 2, 24 h vs 48 h] (Table 2.5).

The mean $\pm$ SE mortality of *R. dominica* adults in controls ranged from 6.3 $\pm$ 1.3%. The mean $\pm$ SE percentage corrected mortality of *R. dominica* exposed to silica powder 1 at 1.5 g/m² for 1 to 24 h ranged from 11.0 $\pm$ 3.6 to 100%. The mean $\pm$ SE percentage corrected mortality of *R. dominica* exposed to silica powder 2 at 0.25 g/m² for 1 to 24 h ranged from 3.3 $\pm$ 1.2 to 96.7 $\pm$ 3.3 %. The Chi-square (χ^2) value for goodness-of-fit of model to data was not significant ($P > 0.05$) indicating good fit of model to the data. The lethal times required for 50 and 99% mortality

of *R. dominica* adults exposed to 1.5 g/m² of silica powder 1 was 15.49 and 60.25 h. On the other hand, the lethal times required for 50 and 99% mortality of *R. dominica* adults exposed to 0.25 g/m² of silica powder 2 was 3.82 and 12.13 h (Table 2.6). These results confirmed that silica powder 2 was more efficacious than silica powder 1. Silica powder 1 required a longer time to produce 99% mortality of adults than silica powder 2 [LT₉₉ ratio (95% CI) = 5.20 (CI could not be estimated)].

2.4.3 Adult progeny production

The mean $\pm$ SE number of *R. dominica* adults produced in controls ranged from 69.0 $\pm$ 5.6 to 85.6 $\pm$ 4.1%. The mean $\pm$ SE number of *R. dominica* adult progeny produced after exposure to 0 to 5 g/m² of silica powder 1 for 4 to 24 h exposure times ranged from 0 to 85.6 $\pm$ 4.1. The mean $\pm$ SE number of *R. dominica* adult progeny produced after exposure to 0 to 5 g/m² of silica powder 2 ranged from 86.2 $\pm$ 5.00. The adult progeny production was inversely proportional to the powder application rate. Adult progeny was 0% when adults were exposed to silica powder 1 at 4.5 and 5 g/m² application rates after 12 h exposure. However, a 100% reduction in progeny production was achieved after exposure for 8 h to 0.25 to 5 g/m² of silica powder 2. The χ^2 values for goodness-of-fit of model to data were significant ($P < 0.05$) for silica powder 1, indicating poor fit of the probit model to the adult mortality data. The χ^2 values for goodness-of-fit for silica powder 2 exposed for 4 and 8 h was not significant ($P > 0.05$), indicating good fit of the probit model to the percent reduction in adult progeny data. The probit model could not be fit for 48 h of silica powder 1 and 12, 24, 36 and 48 h for silica powder 2, since 100% reduction in adult progeny production was observed at all the tested application rates (0.1 to 5 g/m²).

The effective concentrations (ECs) of silica power 1 to reduce 50 and 99% of adult progeny production of *R. dominica* was 0.08, 0.05, 0.03, 0.02 and 0.02 g/m², and 5.88, 4.64, 3.83, 0.27 and 0.19 g/m² for 4, 8, 12, 24 and 36 h exposures, respectively (Table 2.7). The ECs to reduce 50 and 99% of progeny production were 0.05 and 0.02 g/m², and 0.26 and 0.14 g/m² when adults were exposed to 0 – 5 g/m² of silica powder 2 for 4 and 8 h, respectively (Table 2.8). Silica powder 1 had significantly higher EC₉₉ values than silica powder 2 after 4 h and 8 h exposures (Table 2.9), indicating silica powder 2 to be more efficacious than silica powder 1.

The mean $\pm$ SE number of *R. dominica* adult progeny produced at 42 d in controls ranged from 71 $\pm$ 3.1. The mean $\pm$ SE number of *R. dominica* adult progeny produced at 42 d after exposure to 1.5 g/m² of silica powder 1 for 1 to 24 h ranged from 0 to 66.3 $\pm$ 5.6. The mean $\pm$ SE number of *R. dominica* adult progeny produced at 42 d after exposure to 0.25 g/m² of silica powder 2 ranged from 0 to 121.0 $\pm$ 11.8. The χ^2 values of goodness-of-fit of the model data to the percentage adult progeny production data recorded at 42 d after exposure to 1.25 g/m² of silica powder 1 was significant ($P < 0.05$), but not when exposed to 0.25 g/m² of silica powder 2, indicating the probit model fit the data well for silica powder 2. The effective times (ETs) required for 50 and 99% reduction of adult progeny production were 0.8 and 68.4 h respectively, when adults were exposed to 1.5 g/m² of silica powder 1. The corresponding values were 0.06 and 19.9 h, respectively, when exposed to 0.25 g/m² of silica powder 2 (Table 2.10). Silica powder 1 required a significantly longer time to reduce 99% of adult progeny production than silica powder 2 [ET₉₉ ratio (95% CI) = 3.44 (1.62 – 7.29)].

2.4.4 Percentage of insect-damaged kernels

The mean $\pm$ SE of insect-damaged kernels in controls after 42 d ranged from 17.5 $\pm$ 1.4%. The mean $\pm$ SE of insect-damaged kernels after 42 d when adults of *R. dominica* were

exposed to 0.1 to 5 g/m² application rates ranged from 0 to 12.7 ± 0.2% of silica powder 1 for 4 to 48 h. No insect-damaged kernels were observed when adults were exposed to 0.15 to 5 g/m² of silica powder 1 for 36 and 48 h. The percentage of insect-damaged kernels decreased as the powder application rate increased. The mean ± SE percentage insect-damaged kernels caused by *R. dominica* ranged from 0 to 10.5 ± 0.5 after exposure to 0.1 to 5 g/m² of silica powder 2 for 4 and 8 h. Complete reduction of insect-damaged kernels was achieved when *R. dominica* adults were exposed to silica powder 2 treated concrete arenas at 0.1 to 5 g/m² for 12 to 48 h exposure. The decrease in the percent insect damaged kernels at increasing application rates of silica powder 1 and 2 were nonlinear. The fitted nonlinear models satisfactorily explained the relationship between the insect-damaged kernels and the application rates of silica powder 1 ($r^2_{\text{range}} = 0.75 - 0.99$) at 4 to 48 h exposures times (Fig. 2.1), and silica powder 2 ($r^2_{\text{range}} = 0.78 - 0.81$) at 4 and 8 h (Fig. 2.2). Comparison of nonlinear models fitted for insect-damaged kernels were significantly higher for silica powder 1 compared to silica powder 2 after 4 ($F = 159.80$; $df = 5, 30$; $P = 0.000$) and 8 h exposure ($F = 124.28$; $df = 6, 29$; $P = 0.000$).

No insect-damaged kernels were observed when *R. dominica* adults were exposed for 18 to 24 h at 1.5 g/m² of silica powder 1 and for 10 to 24 h at 0.25 g/m² of silica powder 2. The decrease in insect-damaged kernels as a function of exposure time was nonlinear. The fitted models satisfactorily explained the relationship between insect-damaged kernels and the exposure time data of silica powder 1 ($r^2 = 0.97$) and silica powder 2 ($r^2 = 0.98$) (Fig. 2.3). Comparison of nonlinear models fitted for insect-damaged kernels showed no significant difference between the silica powder 1 and 2 ($F = 3.289$; $df = 2, 24$; $P = 0.0546$).

2.4.5 Grain weight loss

The mean $\pm$ SE of grain weight loss in control ranged from 9.5 ± 1.3 to $16.1 \pm 2.0\%$. The mean $\pm$ SE of weight loss after 42 d when adults of *R. dominica* were exposed to 0.1 to 5 g/m² application rates ranged from 0 to $14.3 \pm 0.2\%$ of silica powder 1 for 4 to 48 h. The minimum application rate required to achieve complete reduction in grain weight loss were 5, 4, 3, 0.30, 0.15 and 0.1 g/m² when adults were exposed to silica powder 1 for 4 to 48 h. The mean $\pm$ SE of weight loss after 42 d when adults of *R. dominica* were exposed to 0.1 to 5 g/m² application rates ranged from 0 to $6.4 \pm 0.2\%$ of silica powder 1 for 4 to 48 h. Complete reduction in grain weight loss was achieved when *R. dominica* adults were exposed to 0.25 g/m² of silica powder 2 at all exposure times. The decrease in weight loss with increasing powder application rates of silica powder 1 and silica powder 2 was nonlinear. The fitted models satisfactorily explained the relationship between the grain weight loss and the application rates of silica powder 1 exposed for 4 to 48 h ($r^2_{\text{range}} = 0.81 - 0.97$) (Fig. 2.4) and silica powder 2 ($r^2 = 0.96$) exposed for 4 h (Fig. 2.5). Models fitted to grain weight loss data was significantly higher across all application rates when adults of *R. dominica* were treated to silica powder 1 for 4 h compared to silica powder 2 for 4 h ($F = 3.132$; $df = 2, 24$; $P = 0.0318$).

Grain weight loss was 0% when *R. dominica* adults were exposed for 18 – 24 h at 1.5 g/m² of silica powder 1 and for 10 – 24 h at 0.25 g/m² of silica powder 2. Grain weight loss decreased with increasing exposure times and the decrease in weight loss over increasing exposure times was nonlinear. The fitted models satisfactorily explained the relationship between grain weight loss and powder exposure times of silica powder 1 ($r^2 = 0.95$) and silica powder 2 ($r^2 = 0.84$) (Fig. 2.6). Comparison of nonlinear models fitted to grain weight loss data of the two

silica powders exposed for 1 to 24 h, showed no significant difference between the powders ($F = 3.132$; $df = 2, 24$; $P = 0.0618$).

2.5 Discussion

Numerous studies have investigated the effectiveness of inert dusts against *R. dominica* (Fields and Korunic, 2000; Athanassiou and Korunić, 2007; Vardeman et al., 2007 a,b; Saez and Mora, 2007; Kljajic et al., 2010; Sadeghi et al., 2012; Stadler et al., 2012; Derharm and Ziaee, 2021; Balliota and Athanaissou, 2023). The mechanism of action for inert dusts is adsorption of epicuticular waxes resulting in death of insects by desiccation (Ebeling, 1971; Subramanyam and Roseli, 2000; Mewis and Ulrichs, 2001; Prasantha, 2003). Additionally, inert dusts can act as a physical repellent (White et al., 1966), causing damage to the digestive tract (Smith, 1969) and suffocation caused by blockage of spiracles and tracheae in insects (Webb, 1945). Insect mortality occurs through desiccation when insects lose more than 60% of their water content (Ebeling, 1971). Our observations indicated that adults treated with silica powder 1 and silica powder 2 became brittle, suggesting significant water loss from the insect's body. Contact efficacy bioassays further revealed that silica powder 2 was more effective than silica powder 1, even at lower application rates and shorter exposure periods.

The efficacy of inert dusts against stored product insects can vary based on factors such as amorphous silica content, particle size, oil adsorption capability, pH values (< 8.5), clay quantity, and other impurities (Korunić, 1998; Korunić, 2013; Liška et al., 2017). Therefore, the elemental composition and the physical properties of the two silica powders were linked with their efficacy. Silica powder 2, with a desirable pH value < 8.5 , exhibited a better response on *R. dominica* adults compared to silica powder 1, which had a pH of 9.6. The silicon dioxide content in the inert dusts can also contribute to insect mortality by adsorbing the epicuticular wax layers

from the insect integument (Subramanyam and Roseli, 2000; Astuti et al., 2019). It was anticipated that both the amorphous silica powders would display higher mortality response on *R. dominica* as their silicon dioxide contents were above 94%, especially for silica powder 2 which had 97% silicon dioxide content in its total composition. Vaiyas and Athanassiou (2004) indicated that smaller particle sizes are crucial for better insecticidal efficacy of inert dusts against stored product beetles. The particle diameter (D10, D₅₀ and D₉₀) of silica powder 1 was significantly smaller compared to silica powder 2. The shape characteristics such as the circularity, convexity, and elongation values were significantly higher for silica powder 1 compared to silica powder 2. However, these factors did not appear to influence the efficacy of silica powders against *R. dominica*. The insecticidal activity of zeolites on stored product insects was not associated with the particle size (Rumbos et al., 2016). Similarly, Korunić (1997) reported that the origin of diatomaceous earth could have significant effect on insecticidal activity. In their study, they mentioned that the particles with a high size range (i.e., 0 –192 µm) can produce similar results, highlighting the importance of particle shape. Although there were notable differences, the low elongation values, which are closer to 0, suggest that the particles have a lesser degree of elongation. The convexity values ranged from 0.95 to 0.97 which was closer to 1, indicating both the silica powders had smooth surfaces.

The mortality response of the adults could be linked to the oil adsorption capability of the two silica powders, particularly silica powder 2, which had a higher adsorption capability (386%) compared to silica powder 1 (231%). Due to the variation in susceptibility of *R. dominica* adults with the two powders, it was necessary to increase the number of application rates to perform probit analysis. Additionally, adults exposed to the highest application rate of 5 g/m² of silica powder 1 achieved mortalities of less than 20% after 12 h of exposure. In contrast,

adults showed greater susceptibility to silica powder 2, with mortalities exceeding 90% at the lowest application rate of 0.1 g/m² after exposure for 24 h or more, making it impossible to perform probit analysis at these exposures times.

Among the two amorphous silica powders tested, *R. dominica* adults exhibited higher susceptibility to silica powder 2 at an application rate of 0.25 g/m² after exposure for 12 h, which was relatively lower than other studies reporting minimal effective application rate of 1 g/m², requiring longer exposures to control *R. dominica* populations. Balliota and Athanassiou (2023) reported that *R. dominica* was the most susceptible insect species compared to *S. oryzae* and *T. confusum* when exposed to steel and concrete surfaces treated with zeolites, DE 5 and DE 6 at 0.5 or 1 g/m² for 1, 3, 7 and 14 d. The efficacy of silica powder 2 against adults at lower doses (0.25 g/m² or above) confirms the previous report indicating *R. dominica* as a susceptible insect species to inert dusts.

The goodness-of-fit test (χ^2 values) for determining LC₅₀ and LC₉₉ values for silica powder 1 and silica powder 2 indicated a significant deviation from the probit model, suggesting a poor fit of the model to the data. These heterogeneous responses may be attributed to the use of unsexed 1- to 4-week-old adults in the experiment, due to variation in adult activity leading to differential pick up (Le Patourel et al., 1989; Malia et al., 2016). Similar heterogeneous variations have been reported in studies involving Ethiopian powders (Tadesse and Subramanyam, 2018a), Spinosad (Subramanyam et al., 2014), phosphine (Manivannan et al., 2016), and chlorine dioxide (E et al., 2017). If heterogeneity is present, the variances and covariances of the probit regression analysis were adjusted using the heterogeneity factor. The heterogeneity factor is determined by dividing the χ^2 values by the degrees of freedom (df) and a critical value from the *t*-distribution is then used to compute the 95% confidence interval

(Subramanyam et al., 2014, E et al., 2018).

The LC₉₉ values after exposure to silica powder 2 for 4, 8, and 12 h were significantly lower ($P < 0.05$) than the LC₉₉ values determined after exposure to silica powder 1 for 24, 36, and 48 h, respectively. Ratio tests confirmed significantly higher toxicity of silica powder 2 compared to silica powder 1 even at shorter exposures. Similarly, the LT₉₉ and ET₉₉ values were 5 and 3 times lower, respectively, for silica powder 2 after exposure to 1.5 g/m² of silica powder 1 and 0.25 g/m² of silica powder 2, confirming the greater efficacy of silica powder 2. Complete mortality of adults was achieved when exposed to 1.5 g/m² of silica powder 1 for 24 h and 0.25 g/m² of silica powder 2 for 12 h. Ertürk et al. (2020) demonstrated complete mortality of *S. oryzae* following a slurry application of Delech WP diatomaceous earth at an application rate of 2 g/m² after 288 h exposure. Baliota and Athanassiou (2023) observed more than 90% mortality of *R. dominica* adults exposed to 1 g/m² of zeolite for 1 day and 0.5 g/m² treatment after 72 h exposure.

The EC₉₉ values of silica powder 2 were 22 and 33 times lower than silica powder 1 after 4 and 8 hours, respectively. The corresponding ET₉₉ value for silica powder 2 was 3 times lower than silica powder 1 after exposure to 1.5 g/m² of silica powder 1 and 0.25 g/m² of silica powder 2. No adult progeny was produced 42 days after exposure to 4.5 g/m² of silica powder 1 for 12 h, while complete reduction of adult progeny was achieved with 0.25 g/m² of silica powder 2 after 8 h exposure. When adults were exposed for 18 h to 1.5 g/m² of silica powder 1, there were no insect-damaged kernels and no grain weight losses. Complete suppression of insect-damaged kernels and grain weight loss were achieved after 10 h of exposure to 0.25 g/m² of silica powder 2. Tadesse et al. (2019a) observed complete inhibition of insect-damaged kernels and grain weight loss after exposing *S. zeamais* and *S. oryzae* to a much higher application rate of 4 to 8

g/m² of filter cake and 9 g/m² of Triplex powder for 12 h. The difference in response between the study by Tadesse et al. (2019a) and the present study could be attributed to the insect species tested, the silicon dioxide content and oil adsorption capability of the two powders used. Tadesse et al. (2019a) did not study the influence of oil adsorption capability of the two powders. The higher susceptibility of *R. dominica* adults to the silica powders, especially with silica powder 2, are in line with the results reported by Baliota and Athanassiou (2023), who indicated *R. dominica* to be the most susceptible species compared to *S. oryzae* and *T. castaneum*, despite being the less mobile species than the other two species (Arthur et al., 2020; Agrafioti et al., 2023).

The present study provides a valid basis for the effective utilization of amorphous silica powders for controlling *R. dominica* in empty bins at lower application rates. There was compelling evidence to suggest that silica powder 2 exhibited greater efficacy in reducing progeny production and grain damage. Moreover, the oil adsorption capability, pH, and the silicon dioxide contents may have a significant impact on insect response to amorphous silica powders. Given the increasing concerns surrounding the use of residual insecticides in post-harvest management systems, the application of silica powders, particularly in amorphous forms, can offer benefits by reducing the need for higher application rates of inert dusts. However, additional investigation is warranted to assess the long-term efficacy of these powders in real world empty storage facilities against stored product insects.

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Table 2.1. Physical properties and elemental compositions of silica powders 1 and 2^a

Test	Silica powder 1	Silica powder 2
Oil Abs. %	231	386
Moisture %	4.1	6.8
Loss of ignition (LOI %)	2.9	2.1
Bulk density g/c ³	0.12	0.13
pH	9.6	7.2
Calcium Oxide (CaO %)	0.62	0.03
Magnesium oxide (MgO %)	0.33	0.02
Aluminum oxide (Al ₂ O ₃ %)	2.86	0.21
Silicon dioxide (SiO ₂ %)	94.5	97.3
Phosphorous penta oxide (P ₂ O ₅ %)	0.07	0.01
Potassium Oxide (K ₂ O %)	0.20	nd ^b
Titanium oxide (TiO ₂ %)	0.13	0.01
Iron (III) oxide (Fe ₂ O ₃ %)	0.93	0.03
Sulphur trioxide (SO ₃ %)	0.02	1.02

^aInformation provided by Imery's Chemicals, Lompoc, California, USA.

^bnd - not detected.

Table 2.2. Particle size and shape characteristics (mean $\pm$ SE) of silica powders 1 and 2.

Powders	Silica powder 1	Silica powder 2	<i>t</i> -value (df)	<i>P</i> - value*
Particle size distribution (μm) ^a				
D ₁₀	4.61 $\pm$ 0.04	6.58 $\pm$ 0.23	-8.33 (4) ^b	0.0011*
D ₅₀	12.71 $\pm$ 0.21	15.84 $\pm$ 0.28	-8.97 (4) ^b	0.0009*
D ₉₀	26.40 $\pm$ 0.50	31.43 $\pm$ 0.20	-8.97 (4) ^b	0.0009*
Shape characteristics				
Circularity	0.65 $\pm$ 0.00	0.61 $\pm$ 0.00	4.14 (4) ^b	0.0144*
Convexity	0.97 $\pm$ 0.00	0.95 $\pm$ 0.00	4.96 (4) ^b	0.0077*
Elongation	0.44 $\pm$ 0.01	0.37 $\pm$ 0.00	5.55 (2.0) ^c	0.0306*
Aspect ratio	0.59 $\pm$ 0.03	0.67 $\pm$ 0.01	-8.11 (4) ^b	0.0013*

^aD₁₀, D₅₀ and D₉₀ are the diameter fractions where 10, 50, and 90% of particles were within a certain size range.

^bVariances between silica powder 1 and 2 were equal ($F_{\text{range}} = 1.76 - 37.0$; $df = 2, 2$; $P_{\text{range}} = 0.0526 - 0.7234$).

^cVariances between silica powder 1 and 2 were unequal ($F = 360.75$; $df = 2, 2$; $P = 0.0055$).

*Significant ($P < 0.05$; two-sample *t*-test).

Table 2.3. Probit regression estimates and concentrations required for 50 and 99 % mortality for *R. dominica* exposed to silica powder 1 treated concrete arenas at various application rates for different time periods at 28 °C and 65% r.h.

Duration (h) ^a	N ^b	Mean $\pm$ SE		Lethal concentration (g/m ² , 95% CI)		χ^2 (df)	P ^c
		Intercept	Slope	LC ₅₀	LC ₉₉		
24	950	1.23 $\pm$ 0.12	1.72 $\pm$ 0.21	0.19 (0.13-0.30)	4.32 (2.61-9.63)	2837.31 (93)	<0.0001
36	560	2.06 $\pm$ 0.19	2.95 $\pm$ 0.31	0.19 (0.17-0.22)	1.23 (0.92-2.88)	557.08 (48)	<0.0001
48	450	1.80 $\pm$ 0.14	2.30 $\pm$ 0.21	0.17 (0.14-0.18)	1.69 (1.23-2.67)	227.38 (43)	<0.0001

^a Probit analysis could not be performed for 4, 8 and 12 h exposure data as the observed mortalities at the highest application rate of 5 g/m² was < 20 %.

^b N=total number of insects used in generating the probit regression estimates.

^c χ^2 values for goodness-of-fit of model to data were significant ($P < 0.05$), indicating poor fit of model to data.

Table 2.4. Probit regression estimates and concentrations required for 50 and 99 % mortality for *R. dominica* exposed to silica powder
2 treated concrete arenas at various application rates for different time periods at 28 °C and 65% r.h.

Duration (h) ^a	N ^b	Mean $\pm$ SE		Lethal concentration (95% CI) (g/m ²)		χ^2 (df)	P ^c
		Intercept	Slope	LC ₅₀	LC ₉₉		
4	350	3.21 $\pm$ 0.39	4.47 $\pm$ 0.55	0.19 (0.17-0.21)	0.64 (0.50-0.94)	505.84 (33)	<.0001
8	300	2.78 $\pm$ 0.31	3.29 $\pm$ 0.40	0.14 (0.12-0.16)	0.72 (0.54-1.17)	234.02 (28)	<.0001
12	150	5.13 $\pm$ 1.28	4.40 $\pm$ 1.40	0.07 (0.02-0.09)	0.23 (0.17-0.95)	144.76 (13)	<.0001

^aThe mortality was close to 80% at the least application rate of 0.1 g/m² after exposure for 24, 36 and 48 h. Therefore, there were not enough data points to generate the probit estimates.

^bN=total number of insects used in generating the probit regression estimates.

^c χ^2 values for goodness-of-fit of model to data were significant ($P < 0.05$), indicating poor fit of model to data.

Table 2.5. Comparison of LC₉₉ values of *R. dominica* adults exposed to silica powder 1 and silica powder 2 treated concrete arenas.

Silica powder 1 vs. Silica powder 2 ^a	Ratio (95% CI)*
Silica powder 1 for 24 h vs. Silica powder 2 for 4 h	6.79 (3.41 – 13.52)
Silica powder 1 for 36 h vs. Silica powder 2 for 8 h	1.69 (1.04 – 2.74)
Silica powder 1 for 48 h vs. Silica powder 2 for 12 h	7.34 (4.16 – 12.94)

^aThe powder mentioned first had a higher LC₉₉ values.

*Significant ($P < 0.05$).

Table 2.6. Probit regression estimates and times required for 50 and 99 % mortality for *R. dominica* exposed to silica 1 and silica 2 powders treated concrete arenas at 1.5 and 0.25 g/m² application rates for different time periods at 28 °C and 65% r.h.

Powder	N ^a	Mean ± SE		Lethal time (95% CI) (h)		χ^2 (df)	P ^b
		Intercept	Slope	LT ₅₀	LT ₉₉		
Silica 1	160	-4.70 ± 0.71	3.94 ± 0.58	15.49 (13.42-	60.25 (44.29-103.10)	13.57 (14)	0.483
Silica 2	90	-2.70 ± 1.07	4.64 ± 1.43	3.82 (2.10-4.62)	12.13 (8.77-41.41)	5.34 (7)	0.619

^a N=total number of insects used in generating the probit regression estimates.

^b χ^2 values for goodness-of-fit of model to data were not significant ($P > 0.05$), indicating good fit of model to data.

Table 2.7. Probit regression estimates and concentrations required for 50 and 99 % reduction in adult progeny production for *R. dominica* exposed to silica 1 powder treated concrete arenas at various application rates for different time periods at 28 °C and 65% r.h.

Duration (h) ^a	N ^b	Mean ± SE		Effective concentration (95% CI) (g/m ²)		χ^2 (df)	P ^c
		Intercept	Slope	EC ₅₀	EC ₉₉		
4	90	1.36 ± 0.06	1.26 ± 0.10	0.08 (0.06-0.11)	5.88 (4.00-9.88)	541.37 (88)	<0.0001
8	70	1.54 ± 0.04	1.19 ± 0.07	0.05 (0.04-0.06)	4.64 (3.45-6.74)	112.08 (68)	0.0006
12	85	1.67 ± 0.05	1.12 ± 0.09	0.03 (0.02-0.05)	3.83 (2.72-6.17)	278.22 (83)	<0.0001
24	20	3.68 ± 0.57	2.22 ± 0.65	0.02 (0.001-0.05)	0.27 (0.19-0.65)	47.50 (18)	0.0002
36	14	4.08 ± 0.83	2.43 ± 0.90	0.02 (0.00002-0.05)	0.19 (0.15-1.29)	23.80 (12)	0.0217

^a Probit analysis could not be performed for 48 h data as no adult progeny was observed even at the lowest application rate of 0.1 g/m².

^b N=total number of insects used in generating the probit regression estimates.

^c χ^2 values for goodness-of-fit of model to data were significant ($P < 0.05$), indicating poor fit of model to data.

Table 2.8. Probit regression estimates and concentrations required for 50 and 99 % reduction in adult progeny production for *R. dominica* exposed to silica 2 powder treated concrete arenas at various application rates for different time periods at 28 °C and 65% r.h.

Duration (h) ^a	N ^b	Mean ± SE		Effective concentration (95% CI) (g/m ²)		χ^2 (df)	P ^c
		Intercept	Slope	EC ₅₀	EC ₉₉		
4	20	4.21 ± 0.30	3.19 ± 0.34	0.05 (0.04-0.06)	0.26 (0.23-0.31)	15.98 (18)	0.5937
8	15	4.85 ± 0.79	2.96 ± 0.85	0.02 (0.003-0.04)	0.14 (0.12-0.19)	17.62 (13)	0.1727

^a Reduction in progeny production was close to 100% for the least application rate of 0.1 g/m² after exposure for 12, 24, 36 and 48 h.

Therefore, there were not enough data points to generate the probit estimates.

^b N=total number of insects used in generating the probit regression estimates.

^c χ^2 values for goodness-of-fit of model to data were not significant ($P > 0.05$), indicating good fit of model to data.

Table 2.9. Comparison of EC₉₉ values of *R. dominica* adults exposed to silica powder 1 and silica powder 2 treated concrete arenas.

Silica powder 1 vs. Silica powder 2 ^a	Ratio (95% CI)*
Silica powder 1 for 4 h vs. Silica powder 2 for 4 h	22.89 (10.72 – 48.87)
Silica powder 1 for 8 h vs. Silica powder 2 for 8 h	33.27 (22.96 – 48.23)

^aThe powder mentioned first had a higher EC₉₉ value.

*Significant ($P < 0.05$).

Table 2.10. Probit regression estimates and times required for 50 and 99 % reduction in adult progeny production for *R. dominica* exposed to silica 2 powder treated concrete arenas at various application rates for different time periods at 28 °C and 65% r.h.

Powder	N ^a	Mean ± SE		Effective time (95% CL) (h)		χ^2 (df)	P ^b
		Intercept	Slope	ET ₅₀	ET ₉₉		
Silica 1	30	0.11 ± 0.08	1.20 ± 0.11	0.80 (0.52-1.10)	68.44 (44.45-125.50)	62.04 (28)	0.0002
Silica 2	21	4.85 ± 0.79	2.96 ± 0.85	0.06 (0.01-0.14)	19.91 (12.70-41.97)	12.97 (19)	0.840 ^c

^a N=total number of insects used in generating the probit regression estimates.

^b χ^2 values for goodness-of-fit of model to data were significant ($P < 0.05$), indicating poor fit of model to data.

^c χ^2 values for goodness-of-fit of model to data were not significant ($P > 0.05$), indicating good fit of model to data.

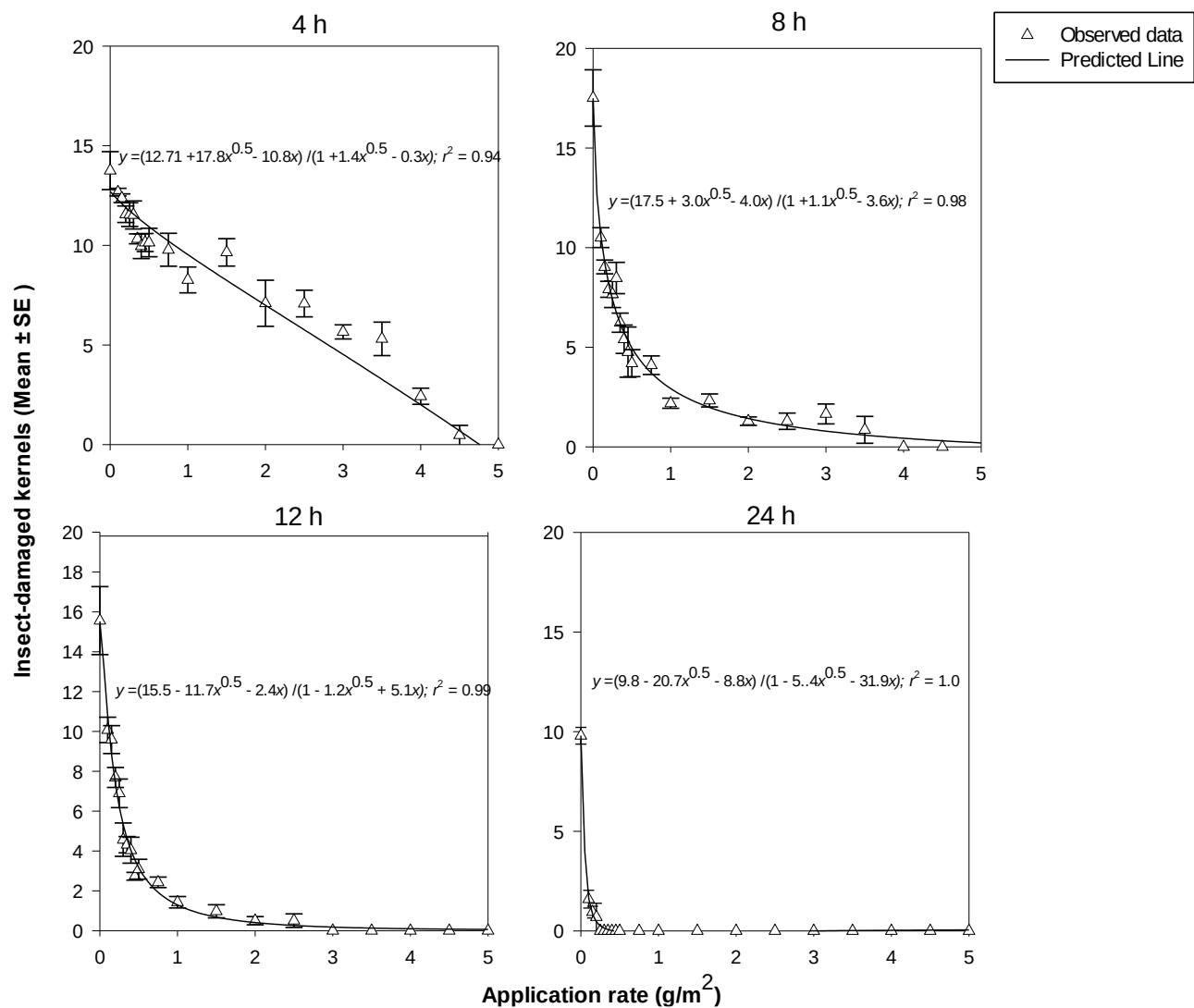


Figure 2.1. Percentage of kernels damaged by *R. dominica* at 42 d after exposure to concrete arenas treated with silica powder 1 for different application rates and exposure times.

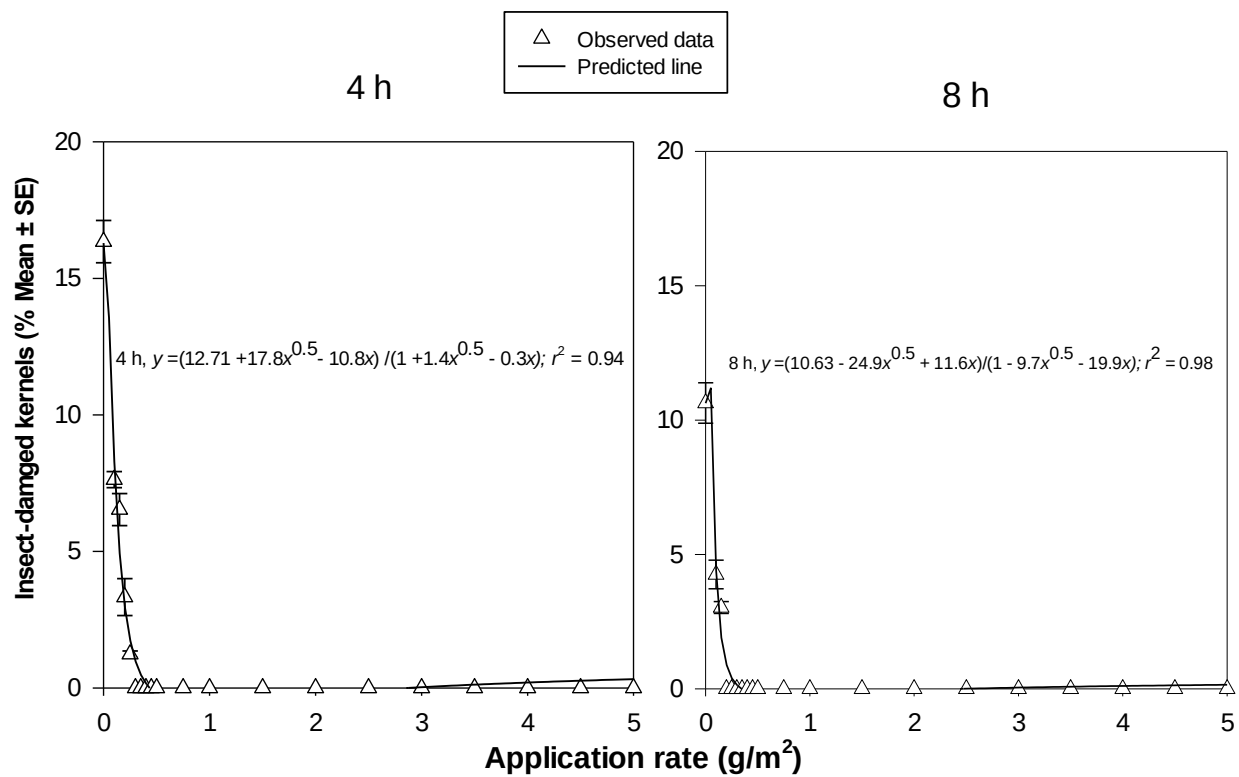


Figure 2.2. Percentage of kernel damaged by *R. dominica* at 42 d after exposure to concrete arenas treated with silica powder 2 for 4 and 8 h to various application rates.

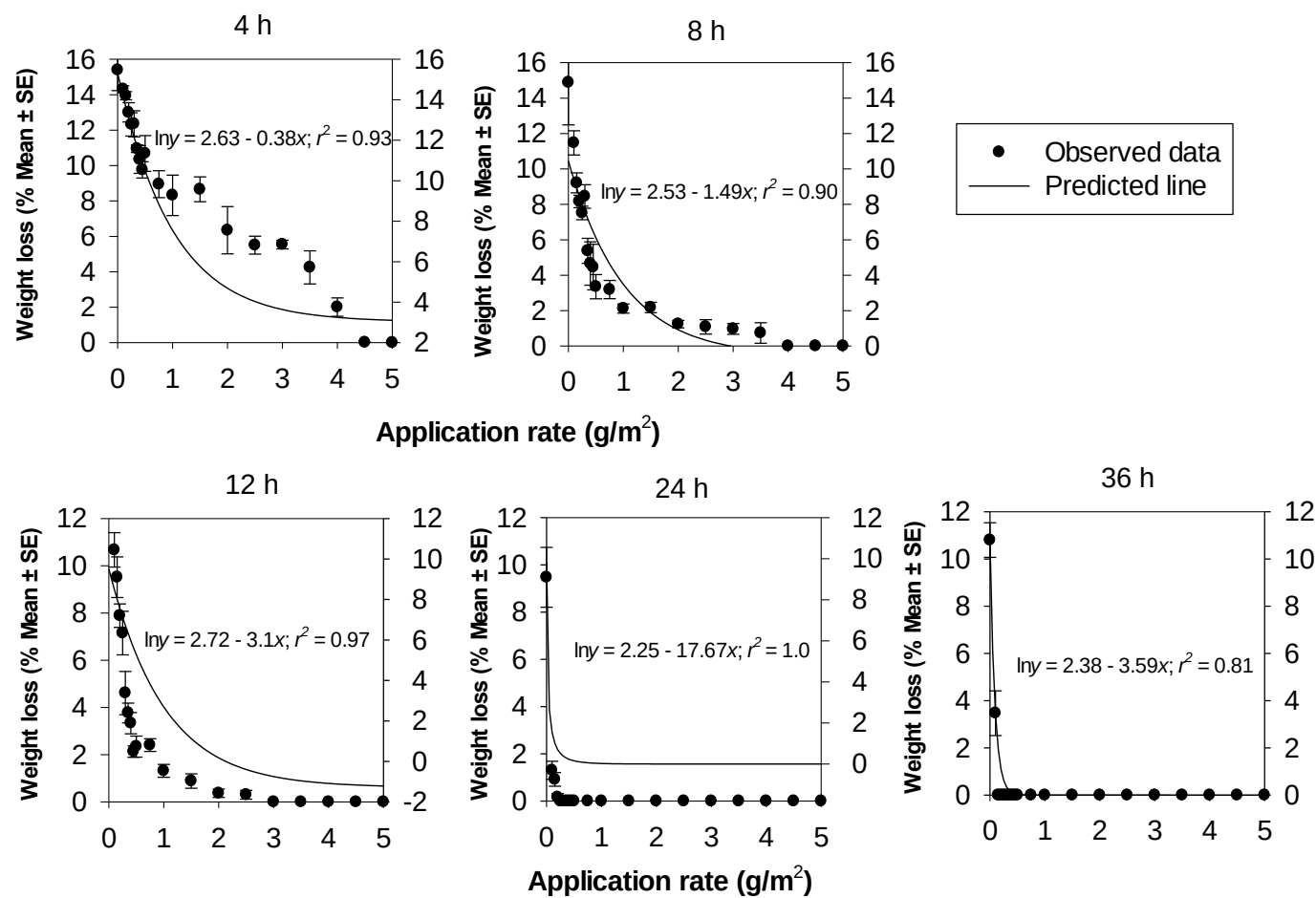


Figure 2.3. Weight loss caused by *R. dominica* at 42 d after exposure to concrete arenas treated with silica powder 1 for 4, 8, 12, 24, and 36 h to various application rates.

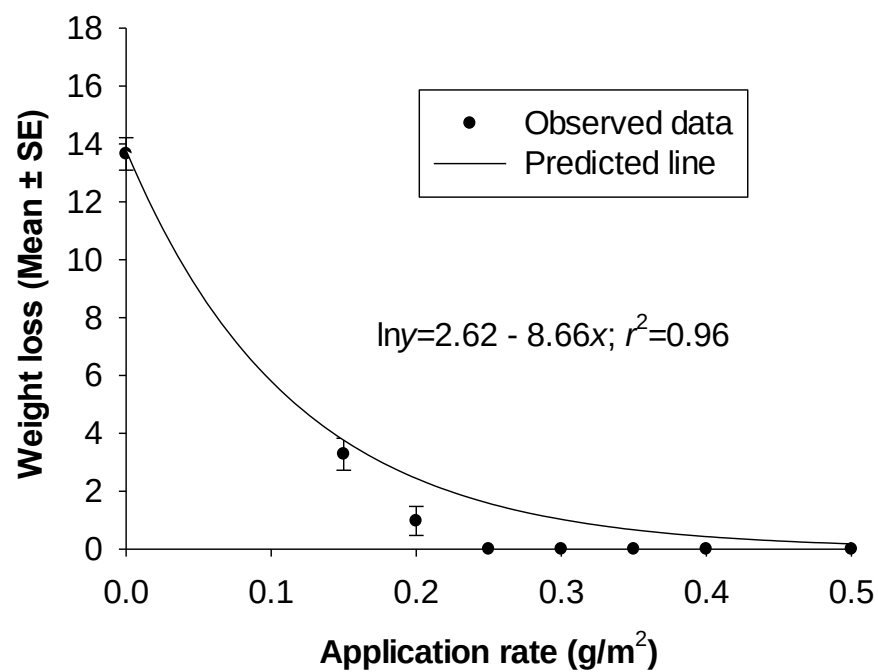


Figure 2.4. Weight loss caused by *R. dominica* at 42 d after exposure to concrete arenas treated at 0 to 0.50 g/m² of silica powder 2 for 4 h.

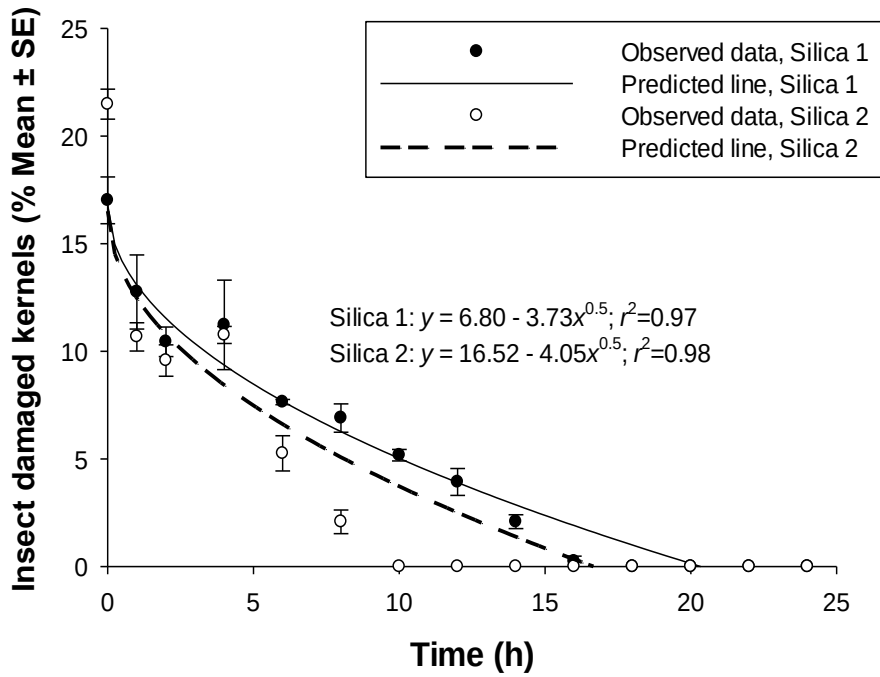


Figure 2.5. Percentage of kernels damaged by *R. dominica* at 42 d after exposure to concrete arenas treated at 1.5 g/m² of silica powder 1 and 0.25 g/m² of silica powder 2 for various exposure times.

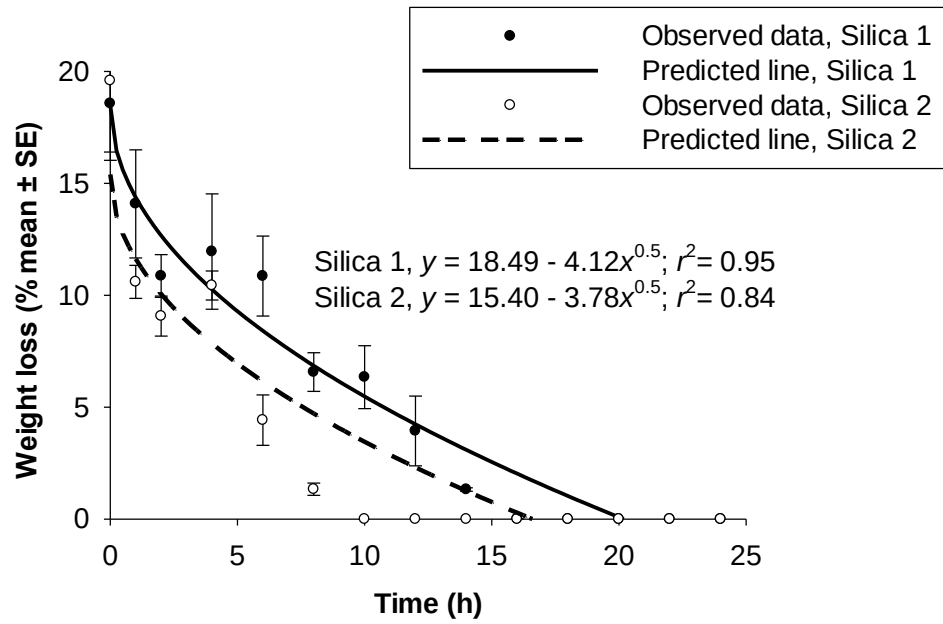


Figure 2.6. Percentage of weight loss caused by *R. dominica* at 42 d after exposure to concrete arenas treated at 1.5 g/m² of silica powder 1 and 0.25 g/m² of silica powder 2.

Chapter 3 - Insecticidal efficacy of two amorphous silica powders applied to concrete arenas against the rice weevil, *Sitophilus oryzae* (L.), (Coleoptera: Curculionidae)

3.1 Abstract

The efficacy of two amorphous silica powders, 1 and 2 was evaluated against the rice weevil, *Sitophilus oryzae* (L.). Ten adults of *S. oryzae* were exposed to concrete arenas treated with different application rates (0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g/m²) of silica powder 1 and 2 for 12, 24, 36, and 48 h. After exposure, the live and dead adults were transferred to 30 g of organic hard red spring wheat and mortality assessments were carried out after 14 d. The data on adult progeny production, insect-damaged kernels, and grain weight loss was determined at 42 d. The estimated LC₉₉ values were 15.81, 13.65, 9.35, and 5.35 g/m², respectively, when adults were exposed to silica powder 1 for 12, 24, 36, and 48 h and 13.31, 9.68, 4.67, and 4.65 g/m², respectively, when exposed to silica powder 2 for 12, 24, 36, and 48 h. The LT₉₉ values were 41.30 h for silica powder 1 and 28.74 h for silica powder 2. The EC₉₉ values to reduce 99% progeny production were 36.92, 9.28, 5.44, and 2.34 g/m², respectively, when adults were exposed to silica powder 1 for 12, 24, 36, and 48 h and 10.52, 4.96, 1.52, and 1.69 g/m², respectively, when exposed to silica powder 2 for 12, 24, 36, and 48 h. The ET₉₉ values to reduce 99% progeny production were 27.39 h for silica powder 1 and 18.36 h for silica powder 2. No insect-damaged kernels and grain weight loss caused by *S. oryzae* was significantly lower when adults were exposed to silica powder 2 compared to silica powder 1.

Keywords: Amorphous silica powders, Efficacy assessment, *Sitophilus oryzae*, Insect-damaged kernels, Grain weight loss.

3.2 Introduction

Inert dusts such as diatomaceous earths are considered as viable alternatives to residual insecticides owing to its low mammalian toxicity (Korunić et al., 1998; Subramanyam and Roesli, 2000), toxicity against various insect pests, no harmful residue and treated commodity (Losic and Korunić, 2018), ease in removal from treated grains, long-term stability (Korunić, 1999; Subramanyam and Roesli, 2000; Athanassiou et al., 2005; Vayias et al., 2006; Kılıç, 2022), and the absence of insect resistance (Golob, 1997; Korunić, 1998; Subramanyam and Roesli, 2000). Among various mechanism of action of diatomaceous earth formulations on insect pests, the adsorption of epicuticular waxes, leading to death by desiccation is the most widely accepted mechanism (Ebeling, 1971; Korunić et al., 1998; Subramanyam and Roesli, 2000). The efficacy of diatomaceous earth formulations is dependent upon the application rate, exposure time, treatment conditions, type of grain, and insect species and their life stages (Vayias and Athanassiou, 2004; Athanassiou et al., 2006; Kavallieratos et al., 2007; Zeni et al., 2021). For instance, the rice weevil, *Sitophilus oryzae* (L.), poses a significant threat to stored food grains and is recognized as a major challenge in terms of resistance to various insecticides, including phosphine (Daglish et al., 2014; Nguyen et al., 2015), primiphos methyl (Attia et al., 2020; Khan et al., 2022), and synthetic pyrethroids (Athanassiou et al., 2014; Daglish, 1998; Daglish and Nayak, 2018). However, *Sitophilus* spp. have been reported to exhibit slightly lower susceptibility to diatomaceous earth formulations compared to the flour beetles, *Tribolium* spp. and the lesser grain borer, *Rhyzopertha dominica* (F.) (Desmarchelier and Dines 1987; Golob, 1997; Korunic, 1998, 2006; Arthur, 2001 and 2002; Arnaud et al., 2005), and they have not demonstrated resistance to diatomaceous earth formulations. These findings highlight the

significance of utilizing diatomaceous earth formulations in the control of *S. oryzae* in stored product environments.

Wakil et al. (2010) evaluated the efficacy of dry dust and slurry treatments of Protect-It (applied at rates of 0, 3, 4, 5 and 6 g/m²) and DEBBM-P/WP (diatomaceous earth and bitterbarkomycin, plant extract, applied at rates of 0, 0.5, 1, 1.5 and 2 g/m²) against *S. oryzae* by exposing the adults to dry dust treated glass Petri dishes for 24, 48, 96, 144, and 288 h. Their observation revealed that applying dry dust treatments of Protect-It at a rate of 3 g/m² for 96 h and DEBBM-P/WP at a rate of 1.5 g/m² for 48 h proved effective against *S. oryzae*, resulting in 100% mortality of adults. For slurry treatments, achieving complete mortality required exposing adults to 6 g/m² of Protect-It for 144 h and 0.5 g/m² of DEBBM-P/WP for 96 h. Baliota and Athanassiou (2023) evaluated the efficacy of DE 5 and DE 6 (diatomaceous earth products from Greece), and zeolite by exposing the adults of *S. oryzae* to steel/concrete arenas treated at 0.5 and 1.0 g/m² application rates for 1, 3, 7 and 14 d. They observed that exposure of adults to both concrete arenas and steel surfaces at 1.0 g/m² of DE 6 and zeolite gave complete mortality of adults after 72 h. Erturk et al. (2020) evaluated the efficacy of Detech[®], a wettable powder formulation against *S. oryzae* by exposing the adults to concrete and wooden surfaces treated at application rates ranging from 0.25 to 2 g/m² exposed for 24 to 288 h. They reported complete mortality of adults in concrete surface treatments at 2 g/m² of Detech[®] exposed for 288 h. While they observed about 98% mortality of adults exposed same application rate and exposure period in their tests on wooden surfaces.

Diatomaceous earths containing amorphous silica are reported to be effective on stored product insects at reasonably lower rates than the older formulations (Korunić, 1998; Subramanyam and Roesli, 2000; Manivannan and Subramanyam, 2023a,b). In our recent study,

we evaluated the insecticidal activity of two amorphous silica powders, 1 and 2 that were supplied by Imerys, Lompoc, California, USA on *R. dominica*. We observed that the adults of *R. dominica* can be completely controlled at an application rate of 1.25 g/m² of silica powder 1 and 0.25 g/m² of silica powder 2 (Manivannan and Subramanyam, 2023a).

The efficacy of two silica powders 1 and 2 on the mortality and adult progeny production of *S. oryzae* adults was evaluated by exposing the adults to silica powder treated concrete arenas. The lethal concentrations (LCs) and lethal times (LTs) required for 50 and 99% mortality of *S. oryzae* and the effective concentrations (ECs) and effective times (ETs) required for 50 and 99% reduction of adult progeny production were estimated. We also determined the effect of silica powders 1 and 2 on the insect-damaged kernels and grain weight loss caused by *S. oryzae* at 42 d after exposure to silica powders 1 and 2. This was done by exposing the adults to concrete arenas treated with different application rates of silica powders 1 and 2 exposed for 12, 24, 36, and 48 h.

3.3 Materials and Methods

3.3.1 Amorphous silica powders

Two amorphous silica powders, 1 and 2 obtained from Imerys, Lompoc, California were used for the evaluation. Particle diameter of silica powder 1 was significantly smaller ($D_{90} = 26.40 \mu\text{m}$) compared to silica powder 2 ($D_{90} = 31.43 \mu\text{m}$). Silica powder 1 had a silicon dioxide content of 94% and silica powder 2 had 97% in its total composition. The oil adsorption capability of silica powders 1 and 2 were 231% and 386%, respectively. The pH values for silica powder 1 was 9.6, and 7.2 for silica powder 2 (Manivannan and Subramanyam, 2023a).

3.3.2 Insects

Insect cultures of *S. oryzae* were established from adults isolated from a laboratory strain maintained in the Department of Grain Science and Industry, Kansas State University since

1999. *S. oryzae* cultures were reared in 0.95 L mason glass jars containing organic hard red spring wheat (Great River Organic Milling Company, Fountain City, Wisconsin, USA) of 12.5% moisture. The glass jars were covered with 250 µm wire metal screens and 9 cm diameter filter paper lids and held inside temperature humidity chamber (Pervical Scientific, Inc., Model I-36 VL, Perry, Iowa, USA) at 28 °C and 65% r.h. with a 14:10 light:dark photoperiod.

3.3.3 Concrete arenas

The procedure used to prepare concrete arenas has been described in our previous published papers (Manivannan and Subramanyam, 2023a,b). Concrete poured Petri dishes were made by mixing Rockite concrete mix (Rockite, Hartline Products Co., Inc., Cleveland, Ohio, USA) with tap water in 2:1 ratio and pouring the mix to 9 cm and 1.5 cm height plastic Petri dishes (Fisher Scientific Co, LLC., Pittsburgh, Pennsylvania, USA). The concrete-poured dishes were left to air-dry on a laboratory workbench for 24 h. Following drying, the inner walls of the dishes were lined with polytetrafluoroethylene (Insect-a-Slip, BioQuip product, Inc, Rancho, Dominguez, California, USA) to create a barrier preventing the adults of *S. oryzae* aggregating to the edges of the Petri dishes and avoiding contact with the silica treated surface (Sehgal and Subramanyam, 2014; Tadesse and Subramanyam, 2018).

3.3.4 Bioassays

The insecticidal activity of the silica powders 1 and 2 against *S. oryzae* was determined by conducting three separate tests. In the first test, the adults of *S. oryzae* were exposed to concrete arenas treated at seven different application rates, 0 (control), 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 g/m² for 12, 24, 36, and 48 h. Each treatment combination was replicated five times. After transferring the required application rate of powder on to the dishes, the powder was distributed uniformly using a dissection needle. Ten adults of *S. oryzae* (1-4-week-old) were released onto

the dishes and secured with lids. All the dishes were inside a growth chamber at 28°C and 65% r.h. After the end of the exposure period, both the live and dead adults from each treatment replicate dish was carefully removed using a feather lite forceps and were transferred to 150 ml round bottomed containers consisting of 30 g of organic hard red winter wheat (12.5% wet basis). After the adults were transferred, the containers were placed inside a growth chamber at rearing conditions for 14 d to determine mortality. Insects that were immobile when sensitized with brush were regarded dead.

In the second test, the adults of *S. oryzae* were exposed to 7.0 g/m² of silica powder 1 and 5.0 g/m² of silica powder 2 for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h. Each powder-exposure period combination was replicated five times. A similar protocol was followed as described in the first experiment.

In the third test, the efficacy of two powders on the progeny production of *S. oryzae* was determined at 42 d. A similar procedure was followed as mentioned in the first and second tests. Each powder-exposure time combination was replicated five times. Progeny counts were taken at 42 d by subtracting the ten parental adults from the total counts. Furthermore, wheat samples obtained from each combination of powder application rate and exposure period were processed using a Boerner divider (Seedburo Equipment Company, Des Plaines, Illinois, USA) to extract a representative sample of approximately 3.5 g. This sample was then utilized to assess the count of insect-damaged and undamaged kernels as described by Tadesse et al. (2019) and Manivannan and Subramanyam (2023a). The percentage of grain weight loss was calculated using the formula provided by Adams and Schulten (1978) and Boxall (1986).

3.3.5 Data analysis

The mortality response data of *S. oryzae* adults exposed to different application rates of silica powder 1 and 2 for varying exposure periods were corrected for control mortality (Abbott, 1925). Probit analysis (SAS Institute, 2012) was employed to estimate the lethal concentrations needed for 50% and 99% mortality, as well as the effective concentrations required for 50% and 99% reduction in progeny production of *S. oryzae*. Additionally, the concentrations for 50% and 99% mortality (LT₅₀ and LT₉₉) and 50% and 99% reduction in progeny production (ET₅₀ and ET₉₉) were determined. The LC₉₉, LT₉₉, EC₉₉ and ET₉₉ values for silica 1 powder were compared with silica 2 powder using ratio tests (Robertson et al., 2007). The differences between any two LC₉₉/LT₉₉/EC₉₉ or ET₉₉ values were considered significant ($P \leq 0.05$) if the 95% confidence interval for the ratio test did not include 1 (Robertson et al., 2007).

The non-linear model, $y^{0.5} = a + bx^{0.5}$ was fitted to the percentage insect-damaged kernels and grain weight loss caused by *S. oryzae* after exposed to different application rates of silica powder 1 and silica powder 2 using Table Curve2D software (Jandel Scientific, San Rafael, California, USA). For the percentage insect-damaged kernels and grain weight loss data, a non-linear model, $y = a + bx^{0.5}$, were fitted as a function of exposure period to silica powders 1 and 2. Model comparisons were performed, and the *F*-test was utilized to identify any individual model that exhibited deviation from the pooled data (Draper and Smith, 1998). All analyses were carried out using SAS 9.4 software (SAS Institute, 2012), and the graphs were made using SigmaPlot 12.5 software (Systat, Software, Inc., San Jose, California, USA).

3.4 Results

3.4.1. Responses of *S. oryzae* adults to silica powders

The mean $\pm$ SE mortality of *S. oryzae* adults in the untreated controls ranged from 0 to $8.0 \pm 3.7\%$. Complete mortality of *S. oryzae* adults was achieved when adults were exposed for 36 h to 5.0 g/m^2 of silica powder 1. The mean $\pm$ SE percentage corrected mortality of *S. oryzae* adults exposed to silica powder 1 at rates ranging from 0.5 to 5.0 g/m^2 for 12 to 48 h, varied from 16.7 ± 3.3 to 100%. Complete mortality of *S. oryzae* adults was achieved with exposure to 5 g/m^2 of silica powder 2 for 24 h. The mean $\pm$ SE percentage corrected mortality of *S. oryzae* adults exposed to silica powder 2 at rates of 0.5 to 5.0 g/m^2 for 12, 24, 36, and 48 h, varied from 14.3 ± 4.1 to 100.0%. The calculated Chi-square (χ^2) values for the goodness-of-fit test of the model to the mortality response data of *S. oryzae* to silica powders 1 and 2 were statistically significant ($P < 0.05$), suggesting poor fit of the probit model to the data (Table 3.1 and 3.2). The estimated LC_{50} and LC_{99} values for silica powder 1 were 1.05 and 15.81 for 12 h, 0.95 and 13.65 for 24 h, 0.69 and 9.35 for 36 h, and 0.42 and 5.35 g/m^2 for 48 h exposures, respectively (Table 1). The corresponding LC_{50} and LC_{99} for silica powder 2 were 1.67, and 13.31 for 12 h, 0.76 and 9.68 for 24 h, and 0.27 and 4.67 g/m^2 for 36 h, and 0.35 and 4.65 exposure, respectively (Table 2). Silica powder 1 required significantly higher LC_{99} values compared to silica powder 2 when *S. oryzae* adults were exposed for 36 h [LC_{99} ratio (95% CI) = 5.85 (3.18 – 10.75) and 48 h LC_{99} ratio (95% CI) = 25.76 (11.09 – 59.86)] (Table 3.3). Ratio tests confirmed that the adults of *S. oryzae* were more susceptible to silica powder 2 than silica powder 1.

The mean $\pm$ SE of mortality in *S. oryzae* adults within the untreated control groups in the second set of tests varied from 6.0 ± 4.0 to $8.0 \pm 3.7\%$. Exposure to 7 g/m^2 of silica powder 1 for 24 h resulted in a mortality range of 18.0 ± 2.0 to 100%, while exposure to 5 g/m^2 of silica

powder 2 for 24 h led to a mortality range of 20.0 ± 7.1 to 100%. Complete mortality in adults was observed following exposure for 20 to 24 h to 7 g/m² and 5 g/m² of silica powders 1 and 2, respectively. The estimated Chi-square (χ^2) values for the goodness-of-fit test of the model to the mortality response data of *S. oryzae*, subjected to 7 g/m² of silica powder 1 and 5 g/m² of silica powder 2, were not statistically significant ($P > 0.05$), indicating a satisfactory fit of the probit model to the data (Table 3.4). The lethal times needed for 50% and 99% mortality of *S. oryzae* when exposed to 7 g/m² of silica powder 1 were 2.93 and 41.30 h, respectively. The lethal times required for 50% and 99% mortality of *S. oryzae* exposed to 5 g/m² of silica powder 2 were 6.73 and 28.74 h, respectively.

3.4.2 Adult progeny production

The mean $\pm$ SE number of *S. oryzae* adult progeny production in control groups varied from 120.6 ± 6.2 to 167.2 ± 18.8 . Following exposure to silica powder 1 at rates ranging from 0 to 5 g/m² for 12 to 48 h, the mean $\pm$ SE number of *S. oryzae* adult progeny produced ranged from 0 to 97 ± 3.4 . Exposure to silica powder 2 at rates of 0 to 5 g/m² for 12 to 48 h resulted in a mean $\pm$ SE number of *S. oryzae* adult progeny ranging from 0 to 75.2 ± 6.3 . Complete suppression of adult progeny production occurred when *S. oryzae* adults were exposed to an application rate of 5.0 g/m² of silica powders 1 and 2 for 24 h. The χ^2 values for the goodness-of-fit of the model to adult progeny production data for silica powder 1 were significant ($P < 0.05$), indicating a poor fit of the probit models to the data. In contrast, the χ^2 values for goodness-of-fit of the model to adult progeny production data for silica powder 2 at 36 and 48 h of exposure were not significant ($P > 0.05$), suggesting a good fit of the probit models to the data. The effective concentrations (ECs) needed for 50% and 99% suppression of adult progeny production in *S. oryzae* exposed to silica powder 1 were 0.79 and 36.92 g/m² for 12 h, 0.42 and 9.28 g/m² for 24 h, 0.35 and 5.44

g/m² for 36 h, and 0.13 and 2.34 g/m² for 48 h of exposure (Table 3.5). For silica powder 2, the ECs required to reduce 50% and 99% of progeny production were 0.68 and 10.52 g/m² for 12 h, 0.32 and 4.96 g/m² for 24 h, 0.12 and 1.52 g/m² for 36 h, and 0.16 and 1.69 g/m² for 48 h (Table 3.6). Silica powder 1 required significantly higher application rates to achieve 99% mortality of *S. oryzae* compared to silica powder 2 when adults were exposed for 12 h [EC₉₉ ratio (95% CI) = 3.51 (1.73 – 7.13), 24 h [EC₉₉ ratio (95% CI) = 1.87 (1.24 – 2.81), 36 h [EC₉₉ ratio (95% CI) = 3.57 (2.33 – 5.46) and 48 h [EC₉₉ ratio (95% CI) = 1.36 (1.04 – 1.85)] (Table 3.7). This indicated that silica powder 2 was more effective on *S. oryzae* than silica powder 1.

The mean $\pm$ SE number of *S. oryzae* adult progeny produced in the untreated control groups ranged from 130.4 ± 14.8 to 133.0 ± 4.0 . When adults were exposed to 7 g/m² of silica powder 1 for 24 h, the mean $\pm$ SE number of *S. oryzae* progeny produced ranged from 0 to 75.2 ± 9.6 . Similarly, the mean $\pm$ SE number of *S. oryzae* progeny produced when adults were exposed to 5 g/m² of silica powder 2 for 24 h ranged from 0 to 49.4 ± 4.8 . Notably, complete suppression of progeny production was achieved when adults were exposed for 18 to 24 h to 7 g/m² of silica powder 1 and after 20 to 24 h of exposure to 5 g/m² of silica powder 2. The estimated Chi-square (χ^2) values for the goodness-of-fit of the model to progeny production data were significant ($P < 0.05$), indicating a poor fit of the probit model to the data (Table 3.8). The effective times (ETs) required for 50% and 99% reduction of adult progeny production of *S. oryzae* exposed to 7 g/m² of silica powder 1 were 2.52 and 27.39 h. The effective times required for 50% and 99% reduction of progeny production of *S. oryzae* exposed to 5 g/m² of silica powder 2 were 1.36 and 18.38 h. Silica powder 1 required significantly longer time to reduce the progeny production of *S. oryzae* by 99% compared to silica powder 2 [ET₉₉ ratio (95% CI) = 1.49 (1.14 – 1.94).

3.4.3 Percentage of insect-damaged kernels

The insect damaged kernels (mean $\pm$ SE) in controls varied from 10.6 ± 1.5 to $12.4 \pm 0.9\%$. The insect-damaged kernels (mean $\pm$ SE) observed in treatments after exposure for 12 to 48 h to 0 to 5 g/m^2 of silica powder 1 varied from 0 to $12.3 \pm 1.6\%$. The insect-damaged kernels (mean $\pm$ SE) caused by *S. oryzae* after exposure to 0 to 5 g/m^2 of silica powder 2 for 12 to 48 h varied from 0 to $8.5 \pm 0.6\%$. Complete inhibition of insect-damaged kernels was noticed at an application rate of 5.0 g/m^2 of silica powder 1 for 24 h. While no insect-damaged kernels were observed when *S. oryzae* adults were exposed to 5 g/m^2 of silica powder 2 for 12 h. The fitted models effectively elucidated the correlation between insect-damaged kernels and the application rates of silica powder 1 ($r^2_{\text{range}} = 0.75 - 0.98$) (Fig. 3.1) and silica powder 2 ($r^2_{\text{range}} = 0.91 - 0.99$) (Fig. 3.2) exposed for 12 to 48 h. The nonlinear model comparisons for insect-damaged kernels caused by *S. oryzae* exposed to silica powder 2 at 12 and 24 h ($F = 2.67$; $df = 2, 10$; $P = 0.1177$) and at 36 and 48 h ($F = 0.60$; $df = 2, 10$; $P = 0.5698$) revealed no significant differences ($P > 0.05$) between the specified time intervals. Significant differences ($P < 0.05$) were observed in all remaining pairwise comparisons (Table 3.9).

The insect-damaged kernels observed in the controls ranged from 11.5 ± 2.3 to $13.5 \pm 1.0\%$. The insect-damaged kernels (mean $\pm$ SE) induced by *S. oryzae* following an exposure to 7 g/m^2 of silica powder 1 for 24 h varied from 0 to $6.4 \pm 0.7\%$. The insect-damaged kernels (mean $\pm$ SE) caused by *S. oryzae* after exposure to 5 g/m^2 of silica powder 2 for 24 h ranged from 0 to $9.0 \pm 1.1\%$. No insect-damaged kernels were observed when *S. oryzae* adults were exposed for 18 to 24 h at 7 g/m^2 of silica powder 1 and for 16 to 24 h at 5 g/m^2 of silica powder 2. The fitted models effectively elucidated the correlation between insect-damaged kernels and the exposure to silica powder 1 ($r^2 = 0.92$) and silica powder 2 ($r^2 = 0.98$) (Fig. 3.3). The nonlinear model

comparison for insect-damaged kernels caused by *S. oryzae* after exposure to silica powder 1 and 2 ($F = 1.46$; $df = 2, 22$; $P = 0.2546$) were not significantly different from each other.

3.4.4 Grain weight loss

The grain weight loss (mean $\pm$ SE) in controls after 42 d varied from 11.1 ± 2.0 to $14.1 \pm 2.2\%$. The grain weight loss (mean $\pm$ SE) caused by *S. oryzae* after exposure for 12 to 48 h to 0 to 5 g/m^2 of silica powder 1 varied from 0 to $13.7 \pm 2.2\%$. The grain weight loss (mean $\pm$ SE) caused by *S. oryzae* after exposure for 12 to 48 h to 0 to 5 g/m^2 of silica powder 2 varied from 0 to $8.2 \pm 0.6\%$. Grain weight loss was 0% when adults of *S. oryzae* were exposed to an application rate of 5.0 g/m^2 for 24 h. Grain weight loss was completely reduced by exposing adults for 12 h to 5 g/m^2 of silica powder 2. The decrease in percentage grain weight loss with increasing rates of silica powder 1 and 2 followed a nonlinear trend. The fitted models effectively elucidated the correlation between the grain weight loss and the application rates of silica powder 1 ($r^2_{\text{range}} = 0.78 - 0.98$) (Fig 3.4) and silica powder 2 ($r^2_{\text{range}} = 0.91 - 0.98$) (Fig 3.5) exposed for 12 to 48 h. The nonlinear model comparisons for grain weight loss caused by *S. oryzae* exposed to silica powder 2 at 12 and 24 h ($F = 4.89$; $df = 2, 10$; $P = 0.0331$) and at 36 and 48 h ($F = 1.060$ $df = 2, 10$; $P = 0.3824$) were not significant ($P > 0.05$). Significant differences ($P < 0.05$) were observed in all remaining pairwise comparisons (Table 3.10).

The grain weight loss (mean $\pm$ SE) observed in the controls ranged from 13.0 ± 2.8 to $15.4 \pm 1.2\%$. The grain weight loss (mean $\pm$ SE) caused by *S. oryzae* after exposure for 24 h to 7 g/m^2 of silica powder 1 varied from 0 to $6.5 \pm 0.7\%$. The grain weight loss (mean $\pm$ SE) caused by *S. oryzae* after exposure for 24 h to 5 g/m^2 of silica powder 2 varied from 0 to $9.8 \pm 1.2\%$. No grain weight loss was observed when *S. oryzae* adults were exposed to 7 g/m^2 of silica powder 1 for 18 to 24 h, and for 16 to 24 h at 5 g/m^2 of silica powder 2. The fitted models effectively

elucidated the correlation between grain weight loss and exposure period of silica powder 1 ($r^2 = 0.85$) and silica powder 2 ($r^2 = 0.88$) (Fig. 3.6). The nonlinear model comparison for grain weight loss caused by *S. oryzae* after exposure to silica powder 1 and 2 for 24 h was not significantly different from each other ($F = 0.66$; $df = 2, 22$; $P = 0.5278$).

3.5 Discussion

Diatomaceous earths affect insect pests by dehydration-induced surface enlargement of the insect integument (Zacher and Kunicke, 1931), destruction of digestive tract (Smith, 1969), and obstruction of spiracles and trachea (Webb, 1945) and adsorption of epicuticular waxes in insects, leading to death by desiccation (Ebeling, 1971; Korunić et al., 1998; Subramanyam and Roesli, 2000). Mortality of insects occurs when they lose $> 60\%$ of their water content due to the action of inert dusts (Ebeling, 1971). However, studies have shown that insects supplied with food after exposure to diatomaceous earth powders could recover the desiccation effects by restoring water content due to food consumption (Arthur, 2000). Therefore, insects exposed to silica powders were transferred to wheat after the specified exposure periods, and mortality was assessed after 14 d (Tesfaye and Subramanyam, 2019; Manivannan and Subramanyam, 2023a). This directly pertains to the potential recovery of insects from desiccation effects of silica powders through the replenishment of water content via food consumption, particularly after exposure to dust-treated concrete surfaces in grain processing environments.

We observed absolute mortality of *S. oryzae* by exposing the adults to concrete arenas treated with 5 g/m^2 of silica powder 1 and 2. However, silica powder 1 required a 36 h exposure for complete mortality, whereas exposure of adults to silica powder 2 for 24 h resulted in 100% mortality of *S. oryzae*. These results suggested that silica powder 2 exhibit better mortality responses on *S. oryzae* than silica powder 1. In our earlier study with these two silica powders,

we observed that 100% mortality of *R. dominica* can be achieved at much lower rate of 0.5 g/m² of silica powder 1 and silica powder 2 by exposing adults for 24 and 4 h, respectively (Manivannan and Subramanyam, 2023a). These findings imply that there is a species-specific variation in the insecticidal activity between silica powder 1 and 2, with *R. dominica* demonstrating more susceptibility than *S. oryzae*. Likewise, Balliota and Athanassiou (2023), also observed the increased susceptibility of *R. dominica* in comparison to *S. oryzae* and *T. confusum* when exposed to steel and concrete surfaces treated with two diatomaceous earths, DE 5, and DE 6, and zeolites at 0.5 or 1 g/m² rates for 1, 3, 7, and 14 d.

The estimated Chi-square (χ^2) values for goodness-of-fit test of model to mortality response data of *S. oryzae* to silica powders, 1 and 2 were significant ($P < 0.05$), suggesting poor fit of the model to the data. Furthermore, the estimated slope values were lower than 2, indicating the tested population of *S. oryzae* were heterogeneous (Georghiou and Metcalf, 1961; Yu, 2008; Rodrigues et al., 2023). This variation in adult mortality responses could be attributed to heterogeneity. Since we used unsexed 1 to 4-week-old adults for the experiments, the differences in adult activity might have led to differential pick up (Le Patourel et al., 1989; Malia et al., 2016) leading to variation in mortality responses. In the case of heterogeneity, the variances and the covariances were adjusted using a heterogeneity factor (HF) estimated as described by Subramanyam et al. (2014) and E et al. (2017). A 15.81, 13.65, 9.35, and 5.35 g/m² of silica powder 1 and 13.31, 9.68, 4.67, and 4.65 g/m² of silica powder 2 was required to achieve 99% mortality of *S. oryzae* when adults were exposed for 12, 24, 36, and 48 h, respectively. Tadesse et al. (2019) evaluated the efficacy of two non-diatomaceous earth powders from Ethiopia, filter cake and Triplex against *S. oryzae* exposed to concrete treated arenas. Their study reported LC₉₉ values of 8.49 and 21.38 g/m² for 99% mortality of *S. oryzae* when adults

were exposed for 12 h to filter cake and Triplex, respectively. We observed that the lethal concentrations required for 99% mortality of *S. oryzae* adults was significantly higher ($P < 0.05$) for silica powder 1 compared to silica powder after 36 and 48 h exposure. The ratio tests confirmed that silica powder 2 exhibited greater effectiveness than silica powder 1 against *S. oryzae*. The lethal times required for 99% mortality of *S. oryzae* was not significantly different between silica powder 1 and silica powder 2, since both the powders were tested at two different applications rates of 7 g/m² and 5 g/m², respectively exposed for 0 to 24 h.

There was no adult progeny production in either powder when *S. oryzae* adults were subjected to an application rate of 5.0 g/m² for 24 h. However, at this application rate (5.0 g/m²) of silica powder 1, complete mortality of *S. oryzae* was not observed after a 24 h exposure. These results suggested that the adults that survived the desiccation effect of the silica powder 1 at this rate were unable to oviposit, and/or the larvae failed to develop into pupae and then into adults because of silica powder 1. Moreover, since we used unsexed 1 to 4-week-old adults for the experiment, it is possible that there were insufficient pairs to reproduce and generate offspring after surviving the desiccation effects of silica powder 1. The estimated EC₉₉ values for silica powder 1 was significantly higher ($P < 0.05$) than silica powder 2 after 12, 24, 36, and 48 h exposure. The ratio tests validated the greater efficacy of silica powder 2 compared to silica powder 1 in reducing adult progeny production in *S. oryzae*. In our earlier tests with these two silica powders 1 and 2 against *R. dominica* we observed that silica powder 2 had superior efficacy than silica powder 1 (Manivannan and Subramanyam, 2023a). However, the EC₉₉ values reported for *R. dominica* were much lower and ranged between 0.19 to 5.88 g/m² when adults were exposed for 12 to 48 h to silica powder 1 and were 0.26 and 0.14 when adults were exposed for 4 and 8 h to silica powder 2, respectively. While in the present study, the estimated

EC₉₉ values for 99% reduction in adult progeny reduction of *S. oryzae* ranged from 2.34 to 36.92 g/m² for silica powder 1 and ranged from 1.69 to 10.52 g/m² exposed for 12, 24, 36, and 48 h.

No grain weight loss or insect-damaged kernels were noticed when adults were exposed for 24 h to 5.0 g/m² of silica powder 1 and for 12 h to 5.0 g/m² of silica powder 2. In a previous study focused on *R. dominica*, we found that exposure to 0.15 g/m² of silica powder 1 for 36 h and 0.1 g/m² of silica powder 2 exposed for 12 h resulted in no insect-damaged kernels and grain weight loss (Manivannan and Subramanyam, 2023a). Comparing the present study with *S. oryzae* with our earlier research against *R. dominica* underscores the species-specific differences in the insecticidal activity of the two amorphous silica powders. The variations in the insecticidal activity of diatomaceous earth powders among insect species can be attributed to both morphology (such as insect body size, differences in epidermal lipids, and the presence of dermal hairs) and physiology (including tolerance to dehydration, agility, and feeding habits) (Ebeling, 1971).

Moreover, the efficacy of inert dusts against stored product insects may differ depending on factors such as the content of amorphous silica, particle size, oil adsorption capability, pH values (< 8.5), clay quantity, and other impurities (Korunić, 1998; Korunić, 2013; Liška et al., 2017). However, the particle diameter of silica powder 1 was significantly smaller than silica powder 2, this factor did not influence the responses of *S. oryzae* adults. Silica powder 2, with a favorable pH value < 8.5, demonstrated a better response on *S. oryzae* adults compared to silica powder 1, which had a pH of 9.6. In addition, the increased efficacy of silica powder 2 over silica powder 1 against *S. oryzae* could also be attributed to the differences in oil adsorption capability. Silica powder 2 with a higher adsorption capacity (386%) in comparison to silica

powder 1 (231%) resulted in better mortality response, no adult progeny production, and complete inhibition of insect-damaged kernels and grain weight loss.

The findings of this study indicate that silica powder 2 exhibits higher efficacy against *S. oryzae* adults compared to silica powder 1, achieving better results at lower application rates and exposure durations. The enhanced effectiveness of silica powder 2 is attributed to its favorable lower pH value and high oil adsorption capability. Given the rising demand for chemical-free food, the utilization of diatomaceous earth powders with efficiency at reduced application rates and exposure times is imperative. Nevertheless, further field-oriented research is necessary to assess the insecticidal efficacy of these powders in empty floor bins, providing insights into their practical feasibility and long-term stability.

3.6 References

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Table 3.1. Probit regression estimates and concentrations required for 50 and 99% mortality for *S. oryzae* exposed to silica powder 1 treated concrete arenas at various application rates for different exposure times at 28 °C and 65% r.h.

Duration (h)	N ^a	Mean $\pm$ SE		Lethal concentration (g/m ² , 95% CI)		χ^2 (df)	P ^b
		Intercept	Slope	LC ₅₀	LC ₉₉		
12	300	-0.04 $\pm$ 0.07	1.98 $\pm$ 0.18	1.05 (0.87-1.23)	15.81 (10.91-26.72)	5.31 (28)	<0.0001
24	300	1.23 $\pm$ 0.12	1.72 $\pm$ 0.21	0.95 (0.80-1.10)	13.65 (9.90-21.11)	118.18 (28)	<0.0001
36	250	0.33 $\pm$ 0.06	2.06 $\pm$ 0.19	0.69 (0.56-0.81)	9.35 (6.67-15.18)	91.73 (23)	<0.0001
48	200	1.80 $\pm$ 0.14	2.30 $\pm$ 0.21	0.42 (0.30-0.53)	5.53 (3.74-10.58)	76.08 (18)	<0.0001

^a N=total number of insects used in generating the probit regression estimates.

^b χ^2 values for goodness-of-fit of model to data were significant ($P < 0.05$), indicating poor fit of model to data.

Table 3.2. Probit regression estimates and concentrations required for 50 and 99% mortality for *S. oryzae* exposed to silica powder 2 treated concrete arenas at various application rates for different exposure times at 28 °C and 65% r.h.

Duration (h) ^a	N ^b	Mean $\pm$ SE		Lethal concentration (95% CI) (g/m ²)		χ^2 (df)	P ^c
		Intercept	Slope	LC ₅₀	LC ₉₉		
12	300	-0.56 $\pm$ 0.13	2.57 $\pm$ 0.30	1.67 (1.36-1.97)	13.31 (8.89-25.19)	363.54 (28)	<.0001
24	250	0.26 $\pm$ 0.07	2.13 $\pm$ 0.19	0.76 (0.62-0.88)	9.38 (6.69-15.22)	100.29 (23)	<.0001
36	200	1.06 $\pm$ 0.19	1.89 $\pm$ 0.35	0.27 (0.12-0.41)	4.67 (2.84-13.64)	114.95 (18)	<.0001
48	150	0.94 $\pm$ 0.12	2.08 $\pm$ 0.52	0.35 (0.12-0.51)	4.65 (2.43-36.30)	112.93 (13)	<.0001

^a N=total number of insects used in generating the probit regression estimates.

^b χ^2 values for goodness-of-fit of model to data were significant ($P < 0.05$), indicating poor fit of model to data.

Table 3.3. Comparison of LC₉₉ values of *S. oryzae* adults exposed to silica powder 1 and silica powder 2 treated concrete arenas.

Powder comparison	Ratio (95% CI)
Silica powder 1, 12 h ^a vs. Silica powder 2 12, h	1.19 (0.77 – 1.84)
Silica powder 1, 24 h ^a vs. Silica powder 2, 24 h	1.45 (0.86 – 2.46)
Silica powder 1, 36 h ^a vs. Silica powder 2, 36 h	5.85 (3.18 – 10.75)*
Silica powder 1, 48 h ^a vs. Silica powder 2, 48 h	25.76 (11.09 – 59.86)*

^a The powder mentioned first had a higher LC₉₉ values.

*Significant ($P < 0.05$).

Table 3.4. Probit regression estimates and times required for 50 and 99 % mortality for *S. oryzae* exposed to silica 1 and silica 2 powders treated concrete arenas at 7 and 5 g/m² application rates for different exposure times at 28 °C and 65% r.h.

Powder	N ^a	Mean ± SE		Lethal time (95% CI) (h)		χ^2 (df)	P ^b
		Intercept	Slope	LT ₅₀	LT ₉₉		
Silica 1	150	-0.95 ± 0.25	2.03 ± 0.36	2.93 (2.08-3.78)	41.30 (21.87-147.67)	18.18 (13)	0.1508
Silica 2	150	-3.07 ± 0.55	3.70 ± 0.67	6.73 (5.87-7.94)	28.74 (18.89-68.37)	6.86 (13)	0.9090

^aN=total number of insects used in generating the probit regression estimates.

^b χ^2 values for goodness-of-fit of model to data were not significant ($P > 0.05$), indicating good fit of model to data.

Table 3.5. Probit regression estimates and concentrations required for 50 and 99 % reduction in adult progeny production for *S. oryzae* exposed to silica 1 powder treated concrete arenas at various application rates for different exposure times at 28 °C and 65% r.h.

Duration (h) ^a	N ^b	Mean $\pm$ SE		Effective concentration (95% CI) (g/m ²)		χ^2 (df)	P ^c
		Intercept	Slope	EC ₅₀	EC ₉₉		
12	300	0.14 $\pm$ 0.06	1.40 $\pm$ 0.14	0.79 (0.61-0.97)	36.92 (21.67-81.39)	101.05 (28)	<0.0001
24	300	0.66 $\pm$ 0.05	1.72 $\pm$ 0.14	0.42 (0.31-0.51)	9.28 (6.87-13.97)	72.94 (28)	<0.0001
36	300	0.89 $\pm$ 0.07	1.96 $\pm$ 0.21	0.35 (0.24-0.46)	5.44 (3.96-8.71)	118.12 (28)	<0.0001
48	300	1.64 $\pm$ 0.06	1.86 $\pm$ 0.20	0.13 (0.08-0.19)	2.34 (1.88-3.32)	38.00 (28)	0.0098

^aN=total number of insects used in generating the probit regression estimates.

^b χ^2 values for goodness-of-fit of model to data were significant ($P < 0.05$), indicating poor fit of model to data.

Table 3.6. Probit regression estimates and concentrations required for 50 and 99 % reduction in adult progeny production for *S.*

oryzae exposed to silica 2 powder treated concrete arenas at various application rates for different exposure times at 28 °C and 65% r.h.

Duration (h)	N ^a	Mean ± SE		Effective concentration (95% CI) (g/m ²)		χ^2 (df)	P
		Intercept	Slope	EC ₅₀	EC ₉₉		
12	300	0.33 ± 0.06	1.95 ± 0.15	0.68 (0.56-0.79)	10.52 (7.86-15.53)	92.57 (28)	<0.0001
24	300	0.97 ± 0.04	1.96 ± 0.13	0.32 (0.25-0.38)	4.96 (4.02-6.45)	44.90 (28)	0.0226
36	300	1.94 ± 0.07	2.09 ± 0.25	0.12 (0.07-0.17)	1.52 (1.27-1.98)	17.05 (28)	0.9476*
48	300	1.81 ± 0.06	2.24 ± 0.23	0.16 (0.10-0.21)	1.69 (1.42-2.15)	35.12 (28)	0.1665*

^aN=total number of insects used in generating the probit regression estimates.

* χ^2 values for goodness-of-fit of model to data were not significant ($P > 0.05$), indicating good fit of model to data.

Table 3.7. Comparison of EC₉₉ values of *S. oryzae* adults exposed to silica powder 1 and silica powder 2 treated concrete arenas.

Powder comparison	Ratio (95% CI)*
Silica powder 1, 12 h ^a vs. Silica powder 2 12, h	3.51 (1.73 – 7.13)
Silica powder 1, 24 h ^a vs. Silica powder 2, 24 h	1.87 (1.24 – 2.81)
Silica powder 1, 36 h ^a vs. Silica powder 2, 36 h	3.57 (2.33 – 5.46)
Silica powder 1, 48 h ^a vs. Silica powder 2, 48 h	1.36 (1.04 – 1.85)

^a The powder mentioned first had a higher LC₉₉ values.

*Significant ($P < 0.05$).

Table 3.8. Probit regression estimates and times required for 50 and 99 % reduction of adult progeny production of *S. oryzae* exposed to silica 1 and silica 2 powders treated concrete arenas at 7 and 5 g/m² application rates for different exposure times at 28 °C and 65% r.h.

Powder	N ^a	Mean ± SE		Effective time (95% CI) (h)		χ^2 (df)	P ^b
		Intercept	Slope	ET ₅₀	ET ₉₉		
Silica 1	400	-0.90 ± 0.11	2.25 ± 0.13	2.52 (2.19-2.84)	27.39 (22.78-34.47)	90.04 (38)	<0.0001
Silica 2	430	-0.27 ± 0.09	2.06 ± 0.12	1.36 (1.11-1.60)	18.38 (15.71-22.27)	62.26 (41)	0.0116

^aN=total number of insects used in generating the probit regression estimates.

^b χ^2 values for goodness-of-fit of model to data were not significant ($P < 0.05$), indicating poor fit of model to data.

Table 3.9. Pairwise comparison of non-linear models fit to insect-damaged kernels data of *S. oryzae* adults exposed for different exposure times to silica powders 1 and 2.

Powder	Exposure times compared	<i>F</i> - value	df	<i>P</i> -value
Silica 1	12 h vs 24 h	4.10	2, 10	0.0500
	12 h vs 36 h	31.99	2, 10	0.0001
	12 h vs 48 h	57.72	2, 10	<0.0001
	24 h vs 36 h	9.10	2, 10	0.0056
	24 h vs 48 h	22.00	2, 10	0.0002
	36 h vs 48 h	9.75	2, 10	0.0048
Silica 2	12 h vs 24 h	2.67	2, 10	0.1177 [#]
	12 h vs 36 h	21.89	2, 10	0.0002
	12 h vs 48 h	26.12	2, 10	0.0001
	24 h vs 36 h	4.98	2, 10	0.0316
	24 h vs 48 h	6.71	2, 10	0.0142
	36 h vs 48 h	0.60	2, 10	0.5698 [#]

[#] Not significant ($P > 0.05$)

Table 3.10. Pairwise comparison of non-linear models fit to grain weight loss data of *S. oryzae* adults exposed for different exposure times to silica powders 1 and 2.

Powder	Exposure times compared	<i>F</i> - value	df	<i>P</i> -value
Silica 1	12 h vs 24 h	4.16	2, 10	0.0484
	12 h vs 36 h	40.50	2, 10	<0.0001
	12 h vs 48 h	54.34	2, 10	<0.0001
	24 h vs 36 h	13.21	2, 10	0.0016
	24 h vs 48 h	21.79	2, 10	0.0002
	36 h vs 48 h	3.47	2, 10	0.0719
Silica 2	12 h vs 24 h	4.89	2, 10	0.0331 [#]
	12 h vs 36 h	24.41	2, 10	0.0001
	12 h vs 48 h	12.04	2, 10	0.0022
	24 h vs 36 h	144.09	2, 10	<0.0001
	24 h vs 48 h	3.46	2, 10	0.0070
	36 h vs 48 h	1.06	2, 10	0.3824 [#]

[#] Not significant ($P > 0.05$)

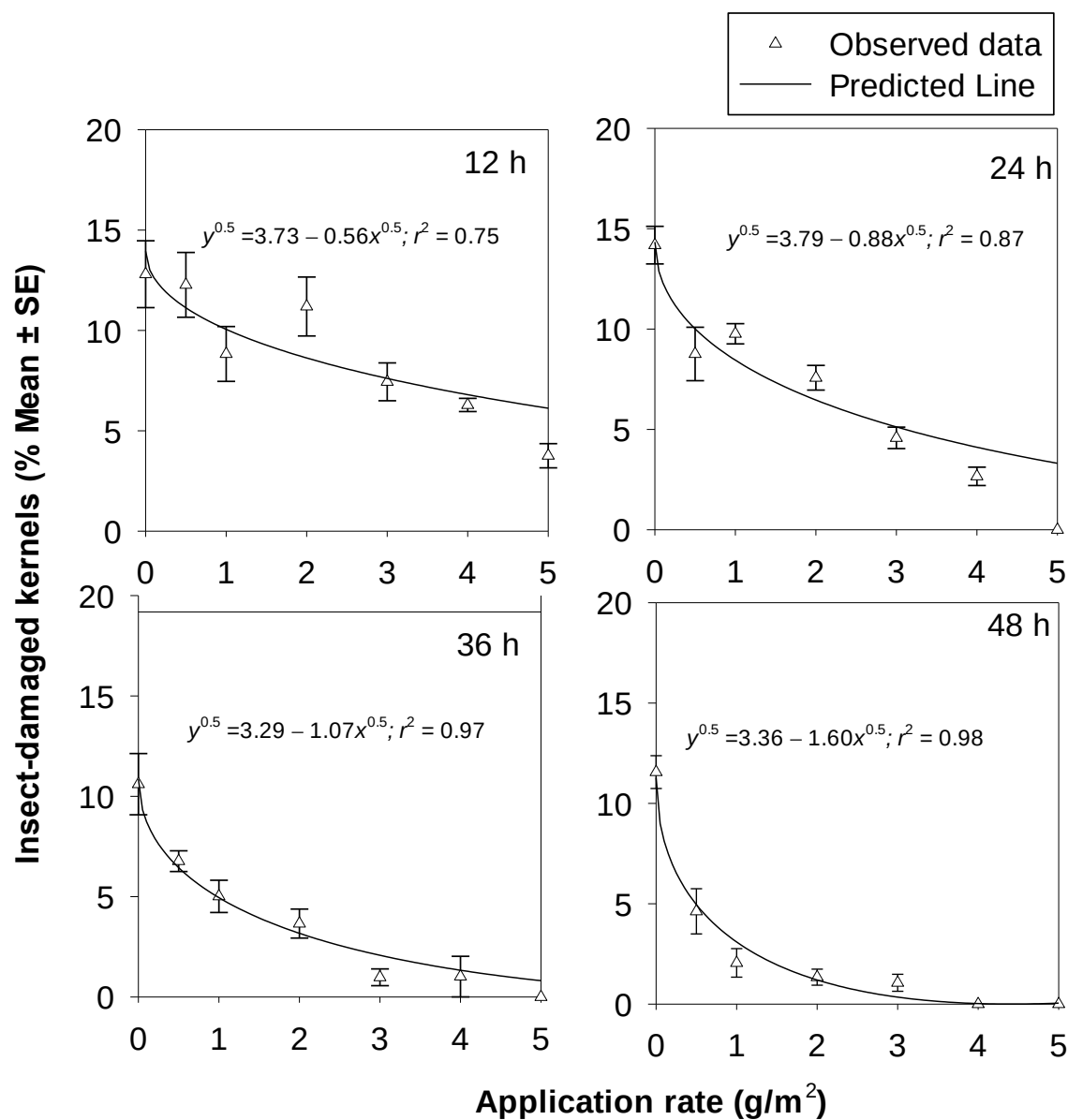


Figure 3.1. Percentage of kernels damaged by *S. oryzae* at 42 d after exposure to concrete arenas treated with silica powder 1 for different application rates and exposure times.

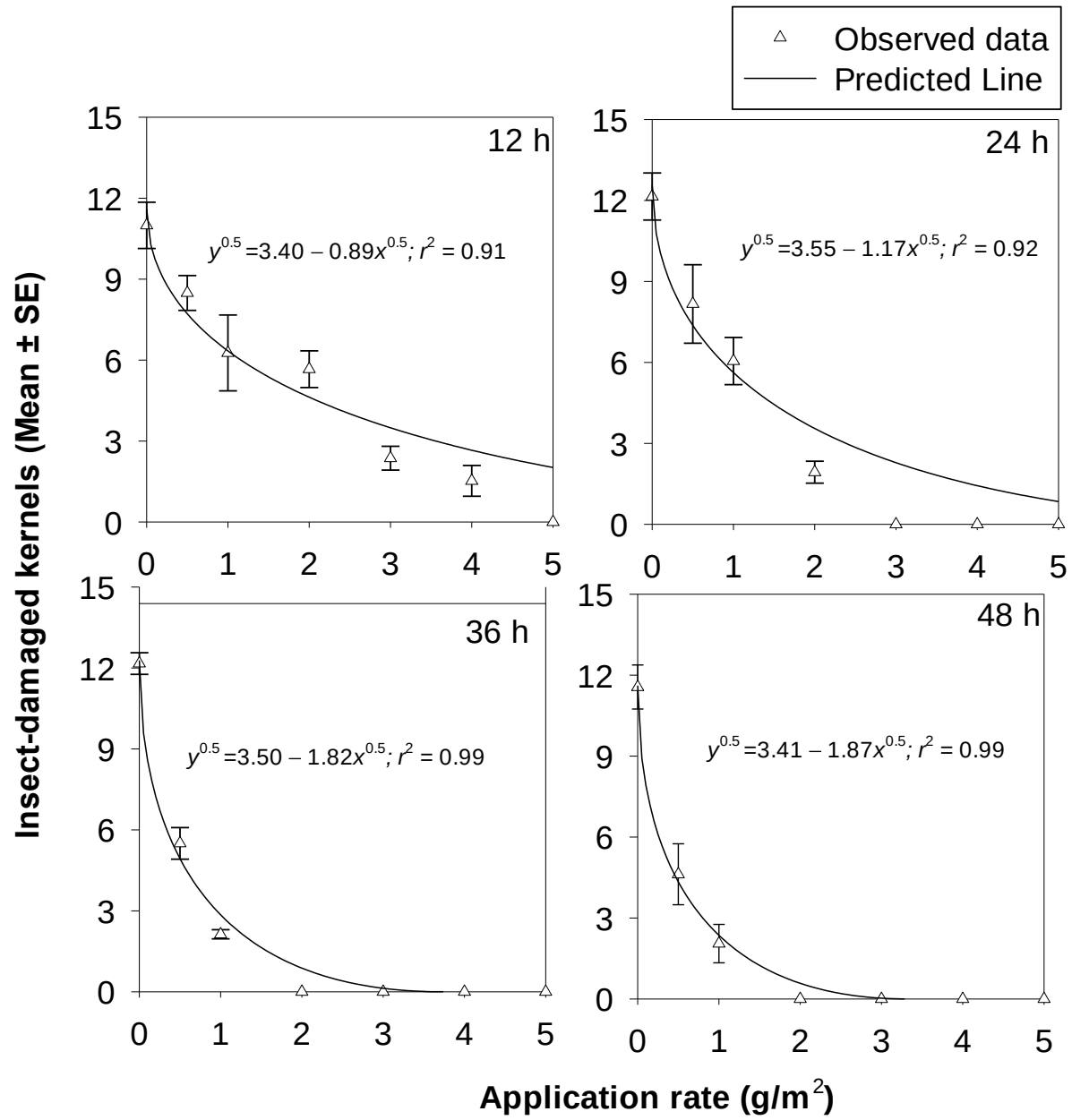


Figure 3.2. Percentage of kernel damaged by *S. oryzae* at 42 d after exposure to concrete arenas treated with silica powder 2 for different application rates and exposure times.

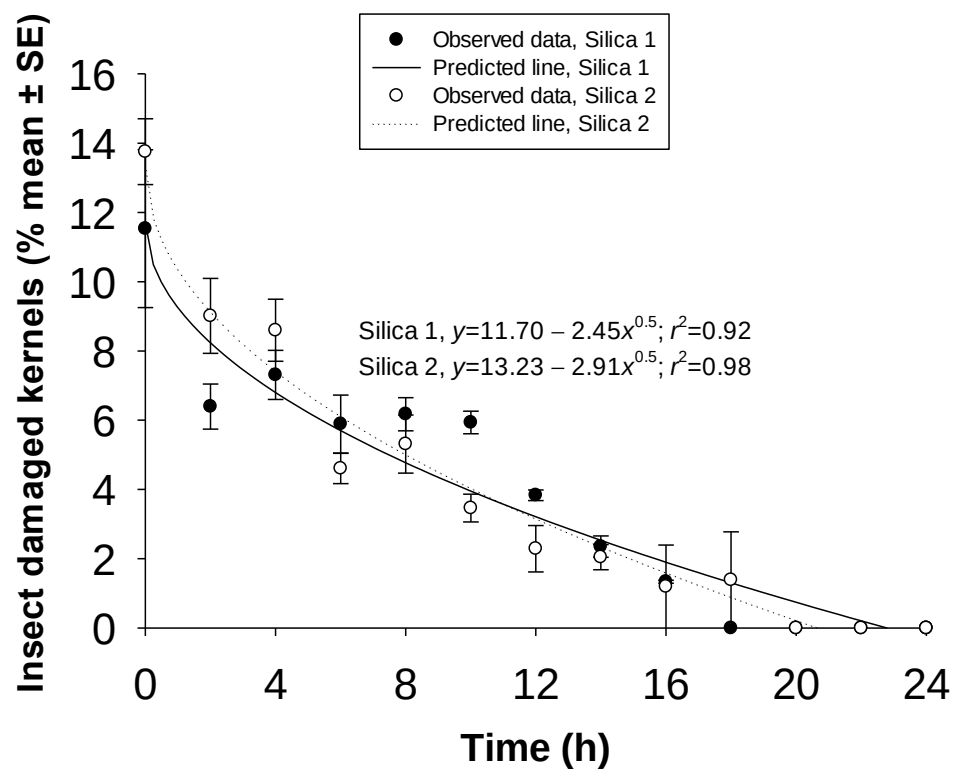


Figure 3.3. Percentage of damaged kernels caused by *S. oryzae* at 42 d after exposure to concrete arenas treated at 7 g/m² of silica powder 1 and 5 g/m² of silica powder 2 for various exposure times.

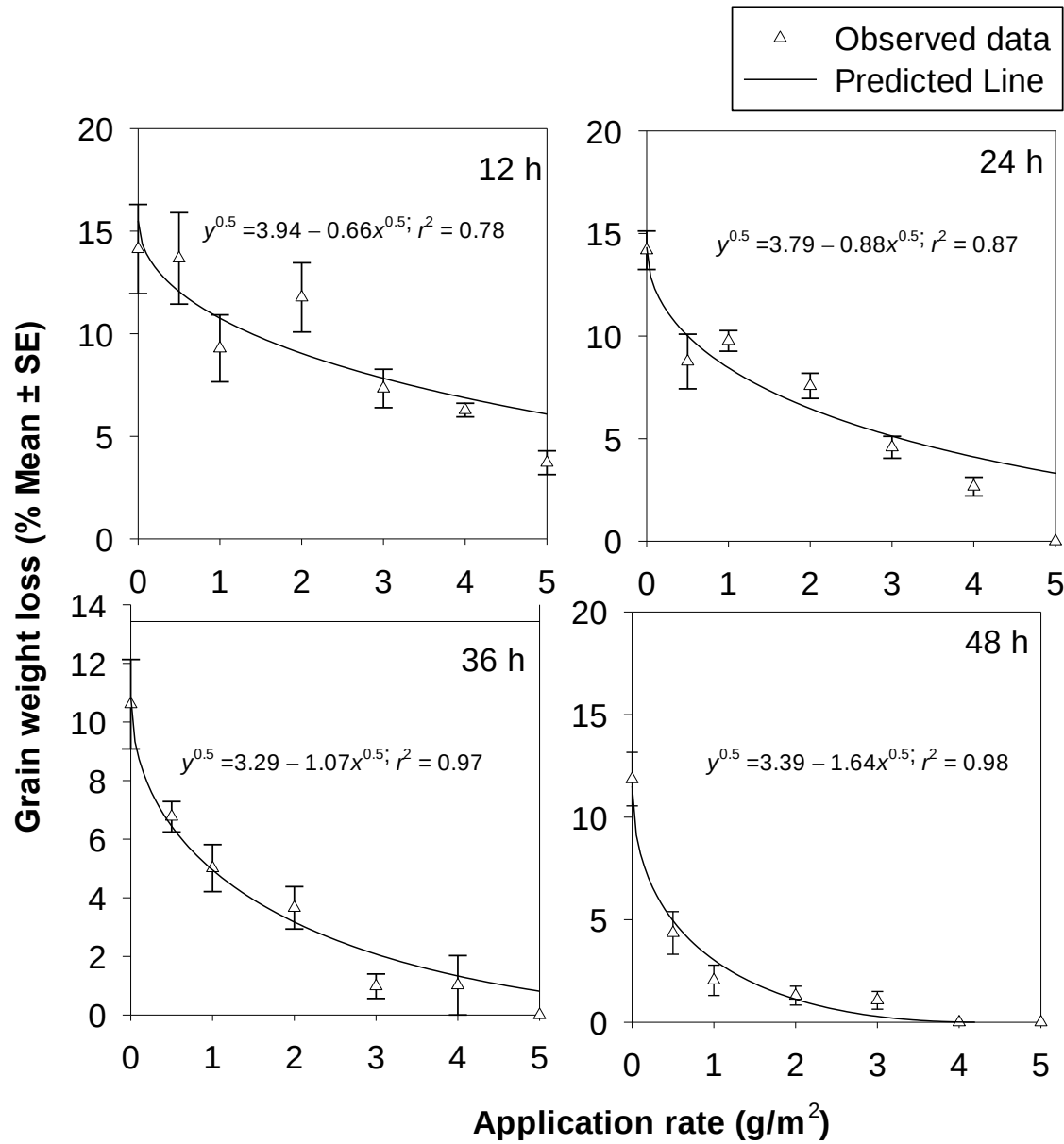


Figure 3.4. Weight loss caused by *S. oryzae* at 42 d after exposure to concrete arenas treated with silica powder 1 for various application rates and exposure times.

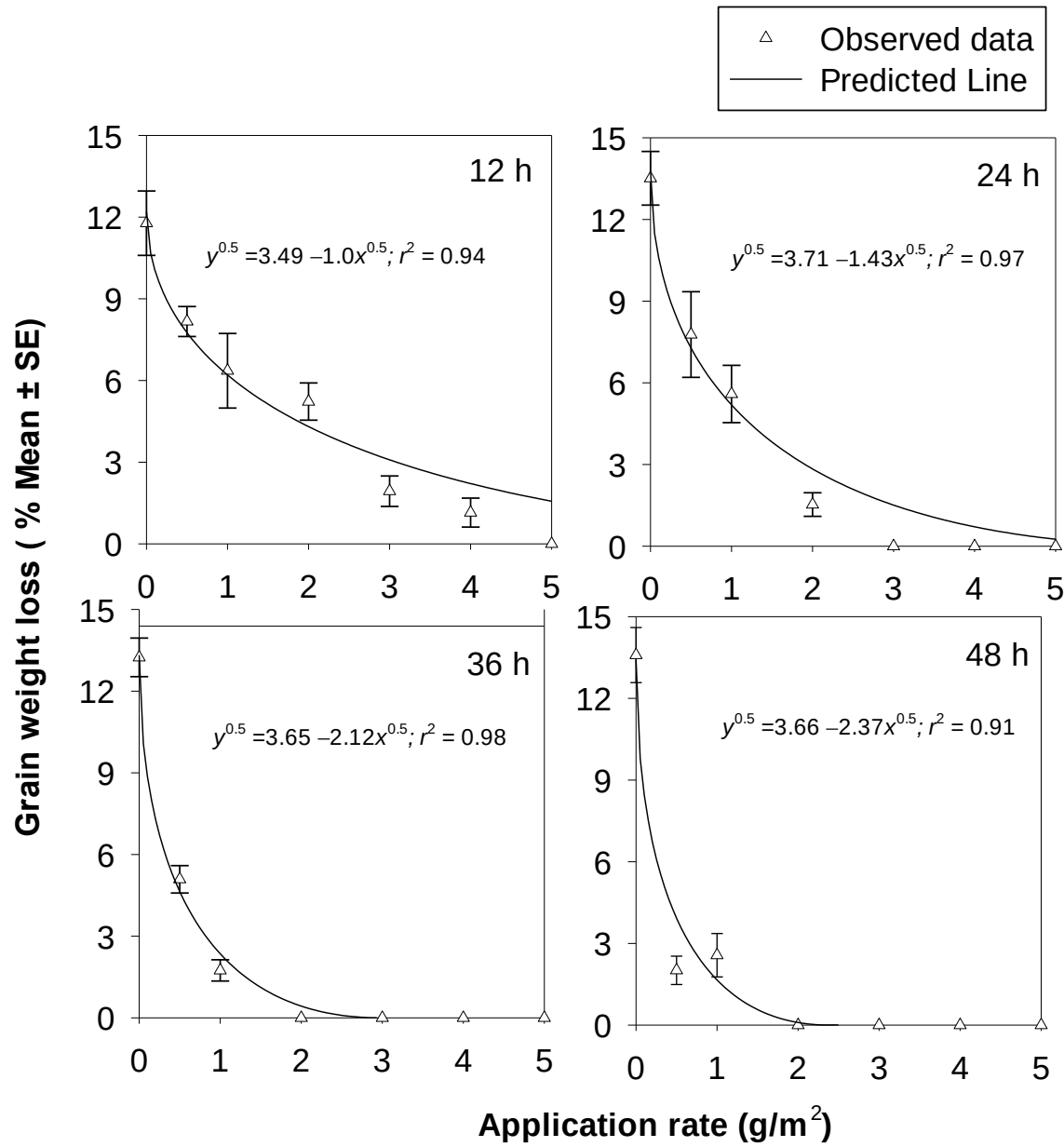


Figure 3.5. Weight loss caused by *S. oryzae* at 42 d after exposure to concrete arenas treated with silica powder 2 for various application rates and exposure times.

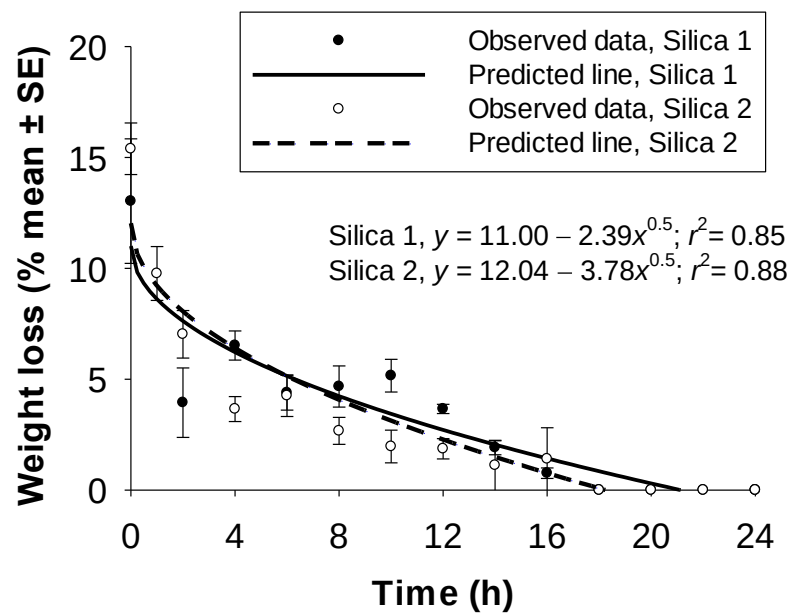


Figure 3.6. Weight loss caused by *S. oryzae* at 42 d after exposure to concrete arenas treated at 7 g/m² of silica powder 1 and 5 g/m² of silica powder 2 for various exposure times.

Chapter 4 - Insecticidal toxicity of two amorphous silica powders against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

4.1 Abstract

The contact toxicity of two amorphous silica powders 1 and 2 procured from Imery's chemicals, Lompoc, California, USA, were evaluated against the red flour beetle, *Tribolium castaneum* (Herbst). The efficacy of the silica two powders was determined by exposing 10 adults of *T. castaneum* to twelve different concentrations of silica powder 1 and 2 for 12, 24, 36, and 48 h. Mortality assessments were made after 14 d, and data on adult progeny production was recorded at 42 d. Complete mortality of *T. castaneum* was observed when adults were exposed to concentrations of 1.5 to 5 g/m² of silica powder 1 for 36 h. Conversely, in tests with silica powder 2, complete mortality was only achieved when adults were exposed for 48 h to concentrations ranging from 0.75 to 5 g/m². Silica powder 1 exhibited greater efficacy in inhibiting adult progeny production in *T. castaneum*, particularly at a concentration of 2.0 g/m² after 24 h exposure. Overall, silica powder 1 displayed superior performance in terms of adult mortality and the suppression of *T. castaneum* adult progeny production. This advantage can be attributed to the smaller particle size of silica powder 1 when compared to silica powder 2.

Keywords: Amorphous silica powders, *T. castaneum*, adult mortality, progeny production, particle size.

4.2 Introduction

The development of insect resistance to conventional insecticides and the growing demand for chemical residue free food has increased the search for viable alternatives that are safer for application in stored product environments. Among various non-chemical alternatives used for safeguarding stored food products, there has been a renewed focus on the use of inert dusts in recent times. Inert dusts are dry powders that are chemically unreactive (Subramanyam and Roesli, 2000) and are now an integral part of the integrated pest management of stored product insects (Korunić, 1999). Inert dusts exhibit strong insecticidal activity with low mammalian toxicity [Korunić, 1996 and 1998, Cook and Armitage, 1999]. Inert dusts applied in stored product environments have been shown to augment the efficacy of other pest management methods such as the aeration, fumigation, and heat treatments, with success stories reported in Australia and USA (Winks and Russell, 1994; Field et al., 1997). Inert dusts, specifically diatomaceous earth and zeolites are considered as viable alternatives to chemical insecticides [Subramanyam and Roesli, 2000, Baliota and Athanassiou, 2023]. Many studies have reported the efficacy of inert dusts on stored insect insects when admixed with grains (Arthur and Puterka, 2003; Athanassiou et al., 2003; Athanassiou et al., 2008; Demissie et al., 2008; Nukenine et al., 2010; Jean et al., 2015; Fazlabad et al., 2017; Liska et al., 2017; Tadesse et al., 2019). Newer formulations of diatomaceous earths are more effective on stored product insects than the older formations which required higher application rates often affecting the physical properties of grains (Korunić, 1998; Subramanyam and Madamanchi, 1998). However, studies pertaining to the use of inert dusts for surface treatments including bins walls and floors, are very limited (Baliota and Athanassiou, 2023).

Silica dusts containing amorphous silica exhibit remarkable efficiency at lower application rates when compared to other formulations (Korunić, 1998) and hence are applied as structural treatments. Inert dusts affect insects by absorbing the lipids from the insects' epicuticle, ultimately resulting in desiccation and death (Subramanyam and Roesli, 2000; Korunić, 1998; Ebeling, 1971; Shawir et al., 1988; Mewis and Ulrichs, 2001; Korunić, 2013; Mutambuki, 2013; Malia et al., 2016). The susceptibility of stored-product insects to diatomaceous earth is known to vary among species (Korunić, 1998; Subramanyam and Madamanchi, 1998; Carlson and Ball, 1962; Desmarchelier and Dines, 1987). Stored product insects such as the *Cryptolestes* species are generally more susceptible to diatomaceous earth products, while the *Sitophilus* species slightly less susceptible, while *Oryzaephilus* species, the lesser grain borer, *Rhyzopertha dominica* (F.), and flour beetles, *Tribolium* species are the more tolerant ones (Korunić, 1998; Desmarchelier and Dines, 1987; Golob, 1997; Arthur, 2001). Similarly, various researchers have indicated flour beetles, *Tribolium spp.*, as the most tolerant insect species among the stored product insects [Desmarchelier and Dines, 1987; Maceliski and Korunić, 1972; Fields and Muir, 1995]. Yao, 2014 evaluated the efficacy of zeolite against five stored product insects such as the lesser grain borer, *Rhyzopertha dominica* (F.), rice weevil, *Sitophilus oryzae* (L.), maize weevil, *Sitophilus zeamais* (Motschuslky), sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) and *T. castaneum*. In his research, it was discovered that among the five species exposed to fine zeolite-treated concrete arenas at both 5 g/m² and 10 g/m², *T. castaneum* adults were the least susceptible. He observed that a minimum of 24 h exposure was required to achieve 100% mortality of *T. castaneum* adults at the two tested concentrations.

The diatomaceous earth formulation, Protect-It, registered for use on raw grains and in floor surfaces, has shown to manage many stored product insects [Korunić, 1996 and 1998].

Surface application of Protect-It at a label rate of 0.5 g/m² required an exposure time of 48 or 72 h to achieve complete mortality of the red flour beetle, *Tribolium castaneum* (Herbst) and the confused flour beetle, *Tribolium confusum* (DuVal), when the adults were not provided food after the treatment (Arthur, 2001). A recent study from Greece (Baliota and Athanassiou, 2023) showed that *T. confusum* adults exposed to two diatomaceous earths, DE5 and DE 6 and zeolite treated concrete surfaces at 0.5 and 1.0 g/m² gave achieved complete mortality of adults after 3 days of exposure. In contact toxicity bioassays conducted in concrete arenas (Frederick and Subramanyam, 2016), DiaFil® 610 demonstrated 100% control of *Tribolium castaneum* (Herbst) at application rates of 2.5 and 5.0 g/m² for 24 h exposure period. These studies indicate *Tribolium spp.* as one of the tolerant insect groups to inert dusts and any research on inert dusts formulations showing better efficacy on *T. castaneum* at lower application rates can be crucial in the effective management of *T. castaneum*. Moreover, the insecticidal activity of inert dusts can vary based on the geographical location, diatom species, pH, particle size distribution, internal surface areas, lipid adsorption capability etc., (Korunić, 1997; Fields and Korunić, 2000). Studies by Vayias et al. (2004) also showed that the smaller diatomaceous earth particles exhibit better insecticidal activity on certain beetle species. In this study, two candidate amorphous diatomaceous earths, supplied by Imery's chemicals, Lompoc, California, USA, referred to here as silica powders 1 and 2 were evaluated for their contact toxicity against *T. castaneum* using concrete arenas to simulate empty bin floors. The objective of this study was to determine the effect of silica powders 1 and 2 on the adult mortality and the progeny production of *T. castaneum* exposed for different concentrations and exposure periods.

4.3 Materials and Methods

4.3.1 Silica powders

Two amorphous silica powders 1 and 2 with different elemental composition, size and shape characteristics supplied by Imery's chemicals, Lompoc California, USA, were used for the contact toxicity bioassays against *T. castaneum*. The elemental composition, particle size diameter, and shape characteristics of the two silica powders have been described in detail elsewhere (Manivannan and Subramanyam, unpublished data). The silicon dioxide content in silica powder 1 was 94% and 97% for silica powder 2. The particles with diameter of utmost 26.40 and 31.43 μm dominated the silica powder 1 and silica powder 2 respectively (Manivannan and Subramanyam, unpublished data).

4.3.2 Test insects

Insects used in the experiments originated from colonies maintained in the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA since 1999. Insect cultures of a laboratory strain of *T. castaneum* were reared in 0.94 L glass mason jars containing 250 g of organic whole wheat flour (Great River Organic Milling Company, Fountain City, Wisconsin, USA) supplemented with 5% brewer's yeast (by weight). The glass jars were covered with wire metal screens (250 μm) and filter paper lids (7 cm diameter) and held in growth chamber under 28°C and 65% r.h. with a 14:10 light:dark photoperiod.

4.3.3 Concrete arenas

Rockite concrete mix (Rockite, Hartline Products Co., Inc., Cleveland, Ohio, USA) was used made into a slurry by mixing with tap water in 2:1 ratio. Concrete dishes were prepared by pouring the slurry into plastic Petri dishes of 9 cm diameter and 1.5 cm height (Fisher Scientific Co, LLC., Pittsburgh, Pennsylvania, USA). The concrete poured dishes were allowed to dry on a

laboratory working bench for 24 h. After drying, the inner walls of the dishes were coated with polytetrafluoroethylene (Insect-a-Slip, BioQuip product, Inc, Rnach, Domnigeuz, California, USA) to avoid insects crawling on the sides of the dishes thus avoiding contact with treated arenas (Sehgal and Subramanyam, 2014; Tadesse and Subramanyam, 2018a). The concrete arenas were used to simulate empty bin or silo floors.

4.3.4 Contact toxicity tests

Three different experiments were carried out to determine the effect of silica powders 1 and 2 on *T. castaneum* adults. In the first experiment, the concrete arenas were treated with silica powders 1 and 2 at twelve different concentrations 0, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 g/m². After adding the required concentration of powders onto the dishes, a dissection needle was used to distribute the powders on the concrete arenas. Ten unsexed 1-4-week-old adults of *T. castaneum* were introduced to each concentration of the silica powders, for 12, 24, 36, and 48 h. There were five replications for each powder concentration-exposure combination. The concrete arenas were secured with lids and then placed inside the growth chamber at 28°C and 65% r.h. After the intended exposure period, insects from each concrete arena were carefully transferred to individual 150 ml round bottom plastic containers containing 30 g of whole wheat flour and 5% brewer's yeast. After 14 d, the wheat flour was sieved using 595 µm sieve (US standard sieve 30) openings to isolate the adults. The data on insect mortality was determined by recording the number of dead insects from the total exposed. The insect that did not move when prodded with camel's hairbrush were regarded as dead.

In the second experiment, the effect of silica powders 1 and 2 on the progeny production of *T. castaneum* was evaluated after 42 d. The protocol followed was similar to those mentioned in the first experiment. At 42 d, the adults were separated from the diet by sieving using a 595

µm sieve openings. The number of progeny production data were recorded by subtracting the ten parental adults from the total progeny counts.

In the third experiment, ten unsexed 1-4-week-old adults of *T. castaneum* were exposed to 5.0 g/m² and 6.0 g/m² of silica powder 1 and silica powder 2, respectively, for 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h. Five replications were maintained for each powder-exposure period combination. In the third experiment, adults after intended exposure were handled similar to first and second experiments including mortality assessment and adult progeny production data.

4.3.5 Data analysis

Control mortality was absent across all exposure times, and as a result, the mortality data was not corrected for control mortality. The non-linear model, $y = a + be^{-x}$ were fitted to the percentage mortality data of *T. castaneum* as a function of concentration of silica powder 1 and silica powder 2 for different exposure periods where possible, using Table Curve2D software (Jandel Scientific, San Rafael, California, USA). The non-linear model, $\ln y = a + bx^{0.5}$ was fitted to the progeny production data as a function of concentration of silica powders 1 and 2 for different exposure periods where possible, using Table Curve2D software. The non-linear model, $y^{0.5} = a + bx^{0.5}$ was fitted for adult mortality and progeny production data as a function of time period for silica powder 1 at 5.0 g/m² and silica powder 2 at 6.0 g/m². The parameters a and b were estimated by fitting equations to the adult mortality and progeny production data as a function of concentration and exposure period. Each possible pairwise comparison between powders and exposure periods of individual powders was performed by comparing the individual models with pooled models (Draper and Smith, 1998). Any two models were significant ($P < 0.05$) from one another if the F -test showed individual models to deviate from the pooled model.

The data analysis was performed using SAS 9.4 software (SAS Institute, 2012ssssss). The graphs were plotted using SigmaPlot 12.5 software (Systat, Software, Inc., San Jose, California, USA).

4.4 Results

4.4.1 Responses of *T. castaneum* adults to silica powders

The mean $\pm$ SE percentage mortality of *T. castaneum* adults exposed to 0.5 to 5 g/m² of silica powder 1 in concrete arenas for 12, 24, 36, and 48 h ranged from 12.0 $\pm$ 4.9 to 100%. Mortality of *T. castaneum* increased with increasing concentration and exposure time. The minimum concentrations required to achieve complete mortality of *T. castaneum* when adults were exposed to concrete arenas treated with silica powder 1 for 24, 36, and 48 h were 5.0, 1.5, and 1.5 g/m², respectively. However, complete mortality of *T. castaneum* adults was not attained at any of the tested concentrations when they were exposed to 0.5 to 5 g/m² of silica powder 1 for 12 h (Table 4.1). The mean $\pm$ SE percentage mortality of *T. castaneum* adults exposed to 0.5 to 5 g/m² of silica powder 2 for 12, 24, 36, and 48 h ranged from 14.0 $\pm$ 5.1%. None of the tested concentrations resulted in complete mortality of *T. castaneum* when the adults were exposed to 0.5 to 5 g/m² of silica powder 2 for 12, 24, and 36 h. While the minimum concentration required for complete mortality of *T. castaneum* adults exposed for 48 h to concrete arenas treated with silica powder 2 was 0.75 g/m² (Table 4.1).

The increase in the percentage mortality of *T. castaneum* at increasing concentrations of silica powder 1 and 2 were non-linear. The fitted models satisfactorily explained the relationship between the mortality of *T. castaneum* and the concentrations of silica powder 1 (r^2 , range = 0.75 – 0.87) (Fig 4.1) and silica powder 2 (r^2 , range = 0.63 – 0.94) (Fig 4.2), at 12, 24, 36, and 48 h. Comparison of non-linear models fitted for percentage insect mortality were significantly higher for silica powder 1 compared to silica powder 2 after 12 ($F = 9.92$; $df = 2, 20$; $P = 0.0010$), 24 (F

= 64.79; df = 2, 20; $P = 0.0000$), and 36 h ($F = 12.24$; df = 2, 20; $P = 0.0003$). This indicated that silica powder 1 was more efficacious on *T. castaneum* adults compared to silica powder 2.

The non-linear model fitted to percentage insect mortality data to silica powder 1 was not significantly different compared to silica powder 2 after 48 h exposure ($F = 0.10$; df = 2, 20; $P = 0.9009$). Mortality responses of *T. castaneum* exposed to silica powder 1 at 24 and 36 h ($F = 0.95$; df = 2, 20; $P = 0.4047$), 24 and 48 h ($F = 1.29$; df = 2, 20; $P = 0.2976$), and 36 and 48 h ($F = 0.10$; df = 2, 20; $P = 0.9072$) were not significantly different ($P < 0.05$) from one another. However, all the remaining pairwise comparisons between exposure times for silica powder 1 ($F_{\text{range}} = 16.41 - 24.17$; df = 2, 20; $P = 0.0000$) and silica powder 2 ($F_{\text{range}} = 7.81 - 56.70$; df = 2, 20; $P_{\text{range}} = 0.0000 - 0.0031$) were significant (Table 4.2).

Complete mortality was observed when *T. castaneum* adults were exposed for 22 h at 5.0 g/m² of silica powder 1. However, complete mortality was achieved when *T. castaneum* adults were exposed to 6.0 g/m² of silica powder 2 for 24 h. The fitted models satisfactorily explained the relationship between percentage insect mortality and the exposure period of silica powder 1 ($r^2 = 0.98$) and silica powder 2 ($r^2 = 0.98$) (Fig 4.3). Comparison of non-linear models fitted for the percentage insect mortality data as a function of exposure period showed significant difference between the silica powder 1 and 2 ($F = 10.41$; df = 2, 22; $P = 0.0007$).

4.4.2 Adult progeny production

The mean $\pm$ SE number of *T. castaneum* adult progeny produced in controls ranged from 77.2 ± 6.6 to 96.4 ± 9.8 . The mean $\pm$ SE number of *T. castaneum* adult progeny produced after exposure to 0.5 to 5.0 g/m² of silica powder 1 for 12 to 48 h exposure times ranged from 0 to 39.8 ± 7.3 . The minimum concentrations required to achieve complete inhibition of adult progeny production of *T. castaneum* when adults were exposed to concrete arenas treated with

silica powder 1 for 24, 36, and 48 h were 2, 0.75, and 0.75 g/m². However, complete inhibition of adult progeny production of *T. castaneum* was not attained at any of the tested concentrations when they were exposed to 0.5 to 5 g/m² of silica powder 1 for 12 h (Table 4.3). The mean $\pm$ SE number of *T. castaneum* adult progeny produced after exposure to 0.5 to 5.0 g/m² of silica powder 2 ranged from 0 to 67.2 ± 9.3 . The minimum concentrations required to achieve complete inhibition of adult progeny production of *T. castaneum* when adults were exposed to concrete arenas treated with silica powder 2 for 36 and 48 h was 3 and 0.75 g/m². However, complete inhibition of adult progeny production of *T. castaneum* was not attained at any of the tested concentrations when they were exposed to 0.5 to 5 g/m² of silica powder 1 for 12 and 24 h (Table 3).

The decrease in the adult progeny production of *T. castaneum* at increasing concentrations of silica powder 1 and 2 were non-linear. The fitted models satisfactorily explained the relationship between the adult progeny production of *T. castaneum* and the concentrations of silica powder 1 (r^2 , range = 0.62 – 1.0) (Fig 4.4) and silica powder 2 (r^2 , range = 0.91 – 1.0) (Fig 4.5), exposed for 12, 24, 36, and 48 h. Comparison of non-linear models fitted for adult progeny production were significantly higher for silica powder 2 compared to silica powder 1 after 12 ($F = 44.17$; $df = 2, 20$; $P = 0.0000$), 24 ($F = 55.82$; $df = 2, 20$; $P = 0.0000$), 36 h ($F = 5.00$; $df = 2, 20$; $P = 0.0174$), and 48 h ($F = 30.04$; $df = 2, 20$; $P = 0.0000$). This indicated that silica powder 1 was more effective against *T. castaneum* adults compared to silica powder 2 in inhibiting the adult progeny production. Mortality responses of *T. castaneum* exposed to silica powder 1 at 24 and 36 h were not significantly different ($F = 0.07$; $df = 2, 20$; $P = 0.9294$).

However, all the remaining pairwise comparisons between exposure times for silica powder 1 (F ,

range = 12.75 – 151.54; df = 2, 20; $P = 0.0000 - 0.0002$) and silica powder 2 (F , range = 30.15 – 2173.74; df = 2, 20; $P = 0.0000$) were significant (Table 4.4).

Complete reduction in adult progeny production of *T. castaneum* was observed in the third experiment when adults were exposed for 18 to 24 h at 5.0 g/m² of silica powder 1. A complete reduction in adult progeny production of *T. castaneum* was observed when adults were exposed for 18 to 24 h to 6.0 g/m² of silica powder 2. The fitted models satisfactorily explained the relationship between adult progeny production and the exposure period of silica powder 1 ($r^2 = 0.91$) and silica powder 2 ($r^2 = 0.95$) (Fig 4.6). Comparison of non-linear models fitted for the adult progeny production data as a function of exposure period showed no significant difference between the silica powder 1 and 2 ($F = 0.93861$; df = 2, 22; $P = 0.40628$).

4.5 Discussion

Many studies have investigated the effectiveness of inert dusts against the adults of *T. castaneum* (Arthur, 2000; Frederick and Subramanyam, 2016; Fields and Korunić, 2000; Le Patourel, 1986; Dowdy, 1999; Rigaux, 2001; Baldassari et al., 2008). The primary mode of action of inert dusts on insects involves the adsorption of the waxy cuticular layer leading to death by desiccation (Subramanyam and Roesli, Mewis and Ulrichs, 2001, Ebeling, 1971; Korunić, 1999, Prasantha et al., 2003). Mortality occurs when insects lose more than 60% of water content from their body due to desiccation (Ebeling, 1971). In a study conducted by Rigaux et al. (2001), it was demonstrated that adult mortality in *T. castaneum* occurs when the insect body loses approximately 30 to 36% from its initial water content of 52-53%. Mortality assessment of *T. castaneum* adults carried out following exposure to silica powders 1 and 2 revealed that the insects became brittle, primarily due to desiccation and a subsequent reduction in water content from their body.

The contact toxicity results showed that *T. castaneum* adults exhibited higher susceptibility to silica powder 1 compared to silica powder 2. We observed complete mortality of *T. castaneum* adults exposed for 36 h to 1.5 to 5 g/m² of silica powder 1. Conversely, complete mortality was achieved when adults were exposed for 48 h to 0.75 to 5 g/m² of silica powder 2. Fields et al. (2002) evaluated the efficacy of four diatomaceous earths, Dryacide, Insecto, Perma-Guard, and Protect-It against *T. castaneum* adults at 1.0 g/m² exposed for 24 h followed by placement on untreated wheat for 7 days. They found that Dryacide was the most effective formulation leading to 96% mortality, followed by Protect-It which gave 55% mortality of adults, while the Insecto, Perma-Guard were least effective achieving mortalities of 7 and 13%, respectively. Our findings suggest that *T. castaneum* is a relatively tolerant species when it comes to inert dusts, requiring a minimum 36 h exposure for complete adult mortality. This result is consistent with previous studies that have identified *Tribolium spp.* as the most resilient stored product insect species when treated to inert dusts (Korunić, 2013; Desmarchelier and Dines, 1987; Maceliski and Korunić, 1972; Fields and Muir, 1995; Yao, 2014).

The efficacy of inert dusts against stored product insects can vary based on several factors including the amorphous silica content, particle size, oil sorption capacity, pH values (< 8.5), clay quantity, and other impurities (Liska et al., 2017, Korunić, 1998; Korunić, 2013). The manufacturer-provided elemental composition data for the two powders were related with the mortality response of the two silica powders. Regarding the silicon dioxide content, both powders exceeded 94%. Building on a prior hypothesis derived from our studies with *R. dominica* (Manivannan and Subramanyam, unpublished data), it is presumed that the silicon dioxide levels in silica powders 1 and 2 may have influenced the adsorption of epicuticular lipids from the insect's integument, ultimately causing death by desiccation (Subramanyam et al.,

2000, Astuti et al., 2019). Comparing the particle diameters of the two silica powders, it was noted that silica powder 1 had significantly smaller particle size (D_{50} and D_{90}) in comparison to silica powder 2. Given this smaller particle size, silica powder 1 had a higher likelihood of adhering to the insect's body, resulting in increased mortality. This finding aligns with previous research emphasizing the importance of smaller particle sizes for improved insecticidal activity of inert dust against many insect pests (Alexander et al., 1944; Athanassiou et al., 2004 and 2016; Kavallieratos et al., 2010; Rumbos et al., 2012). Inert dusts with higher oil adsorption capacity also demonstrated better insecticidal activity (Stadler et al., 2012). Despite the fact that silica powder 2 had an oil absorption capacity 0.5 times higher than that of silica powder 1, we did not observe silica powder 2 surpassing silica powder 1 in terms of mortality response on *T. castaneum* adults.

We achieved complete mortality of *T. castaneum* when the adults were exposed to 5.0 g/m² of silica powder 1 for 20 h. While complete control of *T. castaneum* was achieved when then adults were exposed for 24 h to 6.0 g/m² of silica powder 2, indicating lower efficacy of silica powder 2 compared to silica powder 1. Tadesse and Subramanyam (2018b) in studies with non-diatomaceous earth inert dusts from Ethiopia such as filter cake and Triplex powders, indicated complete control of *S. zeamais* adults can be achieved at 7.5 g/m² of filter cake and 10 g/m² of triplex powders after exposure for 24 h. However, *T. castaneum* is the most tolerant beetle species among the stored product insects. The concentrations required for complete mortality of adults of *T. castaneum* reported in the present study are lower than those observed by Tadesse and Subramanyam (2018b) in their studies with *S. zeamais*. This difference in mortality response could be attributed to the lower silicon dioxide contents in filter cake (< 54%) and Triplex (< 70%) as compared to 94 and 97% observed for silica powders 1 and 2 in our

study. The adult progeny production of *T. castaneum* was inversely related to the powder concentration. We observed that the adult progeny production was completely inhibited when adults were exposed to 2.0 to 5.0 g/m² of silica powder 1 for 24 h. On the other hand, complete reduction of adult progeny production was observed when adults were exposed to 3 to 5 g/m² of silica powder 2 exposed for 36 h. Tadesse and Subramanyam (2018b) reported complete inhibition of adult progeny production of *S. zeamais* when adults were exposed to 7.5 to 10 g/m² of filter cake and Triplex powders for 24 h. Complete reduction in adult progeny production of *T. castaneum* was observed when adults were exposed for to 5.0 g/m² of silica powder 1 and 6.0 g/m² for silica powder 2 for 18 to 24 h. These results further affirmed that silica powder 1 with smaller particle size diameter was significantly effective in reducing the adult progeny production compared to silica powder 2.

Our study provides a comprehensive evaluation of the efficacy of silica powders 1 and 2 against *T. castaneum* in concrete arenas simulating empty bin or silo floors. We observed a concentration-time dependent relationship between the application of these inert dusts and *T. castaneum* mortality. Silica powder 1 with its smaller particle size, proved more effective in reducing adult progeny production compared to silica powder 2. Interestingly, despite differences in oil adsorption capacity, silica powder 1 outperformed silica powder 2 in terms of mortality response and inhibition of adult progeny production, suggesting that factors beyond oil adsorption capability play a pivotal role in the insecticidal action of these powders. Our results highlight the potential of these silica powders as a valuable tool for the control of *T. castaneum* for treating empty bin floors prior to storage of new grains.

4.6 References

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Table 4.1. Minimum application rate (g/m²) required for 100% mortality of *T. castaneum* exposed to two amorphous silica powders treated concrete arenas for different time periods.

Silica powder	Application rate (g/m ²)			
	12 h	24 h	36 h	48 h
Silica 1	— [*]	5.0	1.5	1.5
Silica 2	— [*]	— [*]	— [*]	0.75

*Complete mortality was not achieved in any of the tested application rates.

Table 4.2. Pairwise comparison of non-linear models fitted to application rate and 14 d mortality data of *T. castaneum* adults exposed to silica powders 1 and 2.

Powder	Exposure times compared	F- value	df	P- value
Silica 1	12 h vs 24 h	16.4086	2, 20	0.0001*
	12 h vs 36 h	23.9973	2, 20	0.0000*
	12 h vs 48 h	24.1673	2, 20	0.0000*
	24 h vs 36 h	0.9469	2, 20	0.4047
	24 h vs 48 h	1.2886	2, 20	0.2976
	36 h vs 48 h	0.0098	2, 20	0.9072
Silica 2	12 h vs 24 h	7.81	2, 20	0.0031*
	12 h vs 36 h	70.49	2, 20	0.0000*
	12 h vs 48 h	56.70	2, 20	0.0000*
	24 h vs 36 h	55.11	2, 20	0.0000*
	24 h vs 48 h	41.54	2, 20	0.0000*
	36 h vs 48 h	8.80	2, 20	0.0018*

* Significant ($P < 0.05$)

Table 4.3. Minimum application rate (g/m²) required for complete reduction of adult progeny production of *T. castaneum* exposed to two amorphous silica powders treated concrete arenas for different time periods.

Silica powder	Application rates (g/m ²)			
	12 h	24 h	36 h	48 h
Silica 1	— [*]	2.0	0.75	0.75
Silica 2	— [*]	— [*]	3.0	0.75

^{*} Complete reduction in adult progeny not achieved in any of the tested application rates.

Table 4.4. Pairwise comparison of non-linear models fitted to application rate and 42 adult progeny production data of *T. castaneum* adults exposed to silica powders 1 and 2.

Powder	Exposure times compared	<i>F</i> - value	df	<i>P</i> -value
Silica 1	12 h vs 24 h	23.99	2, 20	0.0000*
	12 h vs 36 h	12.75	2, 20	0.0003*
	12 h vs 48 h	151.74	2, 20	0.0000*
	24 h vs 36 h	0.07	2, 20	0.9294
	24 h vs 48 h	15.52	2, 20	0.0001*
	36 h vs 48 h	13.58	2, 20	0.0002*
Silica 2	12 h vs 24 h	15.60	2, 20	0.0001*
	12 h vs 36 h	127.44	2, 20	0.0000*
	24 h vs 36 h	67.44	2, 20	0.0000*
	24 h vs 48 h	2173.74	2, 20	0.0000*
	36 h vs 48 h	120.13	2, 20	0.0000*

* Significant ($P < 0.05$)

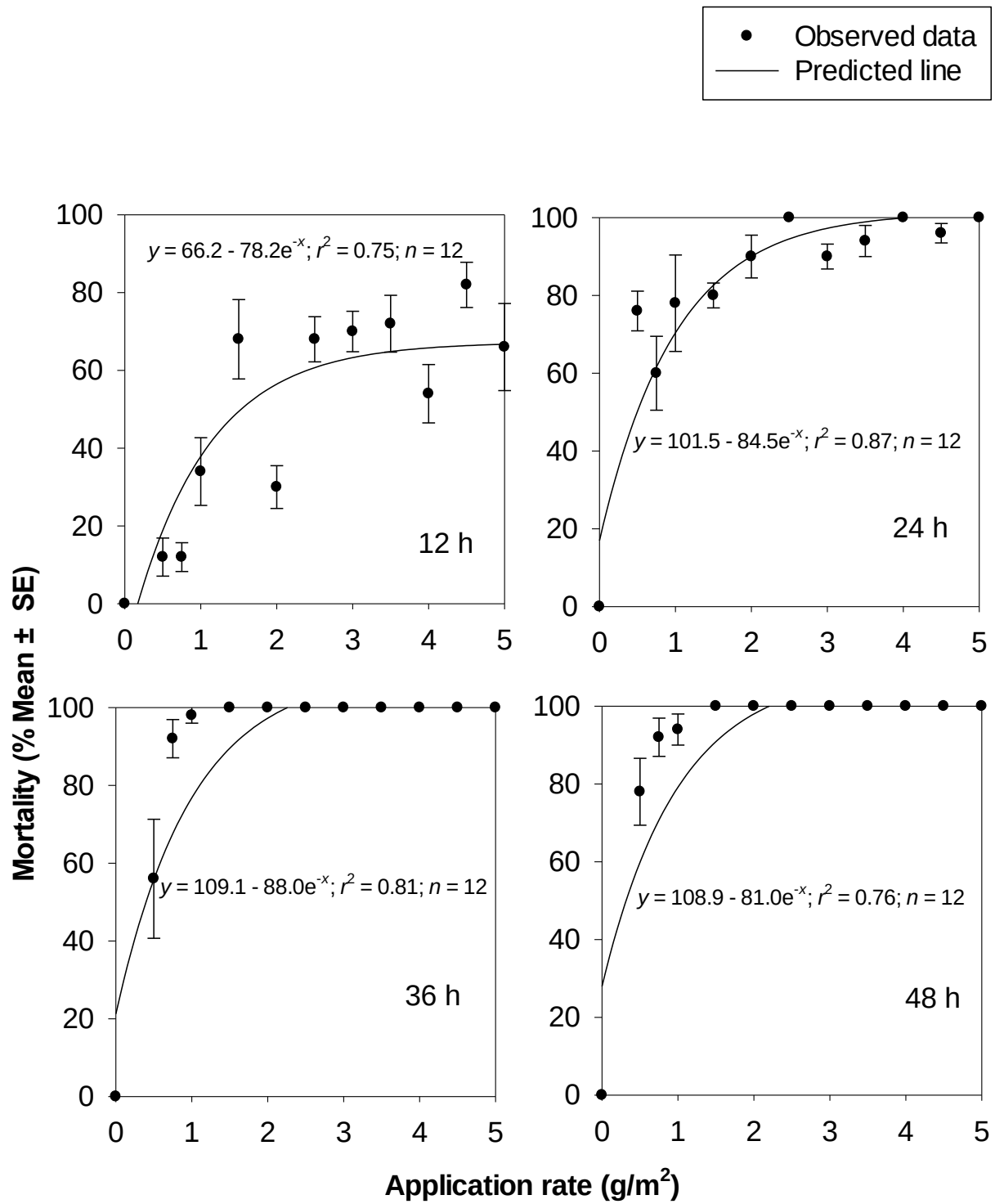


Figure 4.1. Percentage mortality of *T. castaneum* adults at 14 d after exposure to concrete arenas treated with silica powder 1 for different application rates and exposure times.

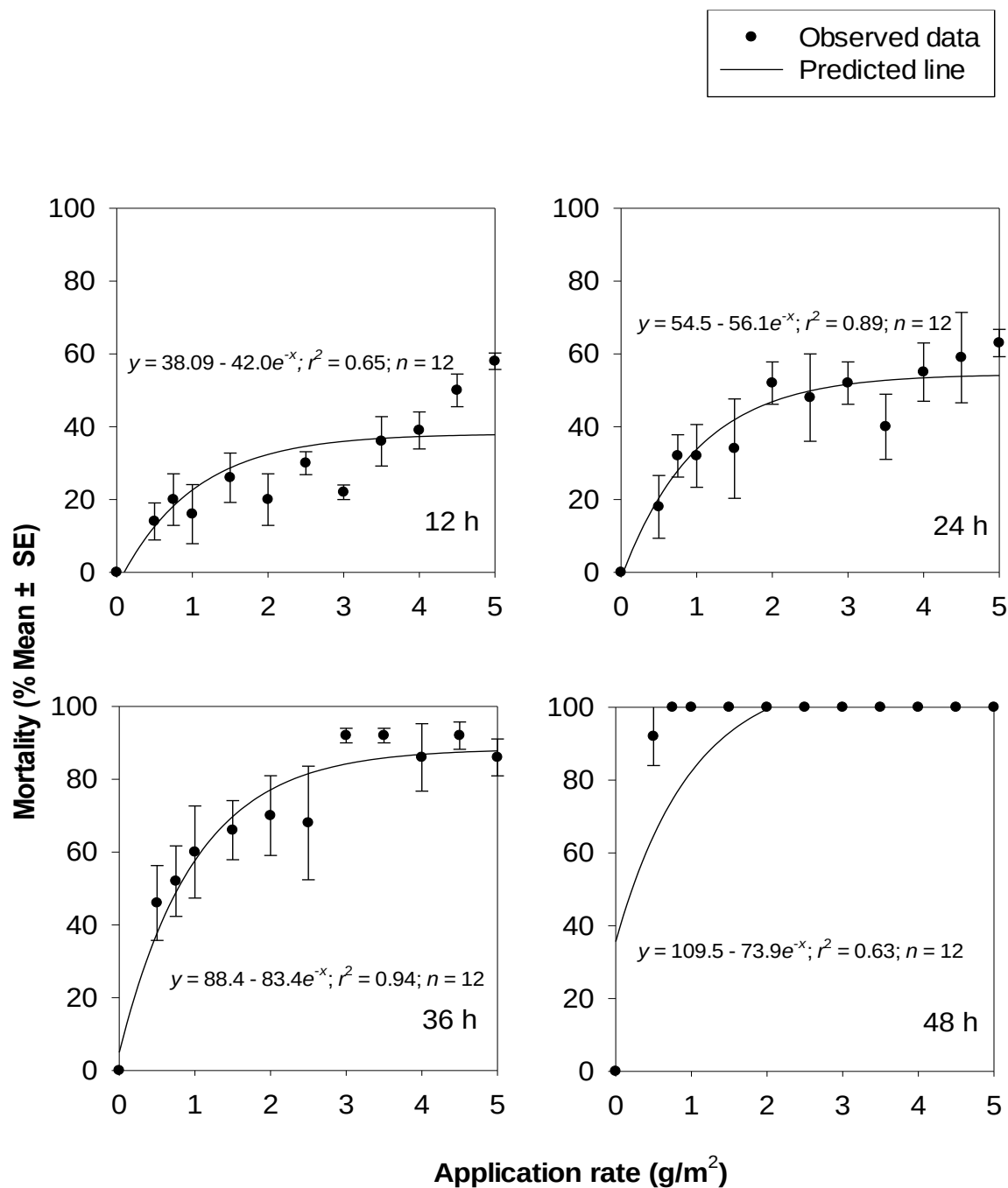


Figure 4.2. Percentage mortality of *T. castaneum* adults at 14 d after exposure to concrete arenas treated with silica powder 2 for different application rates and exposure times.

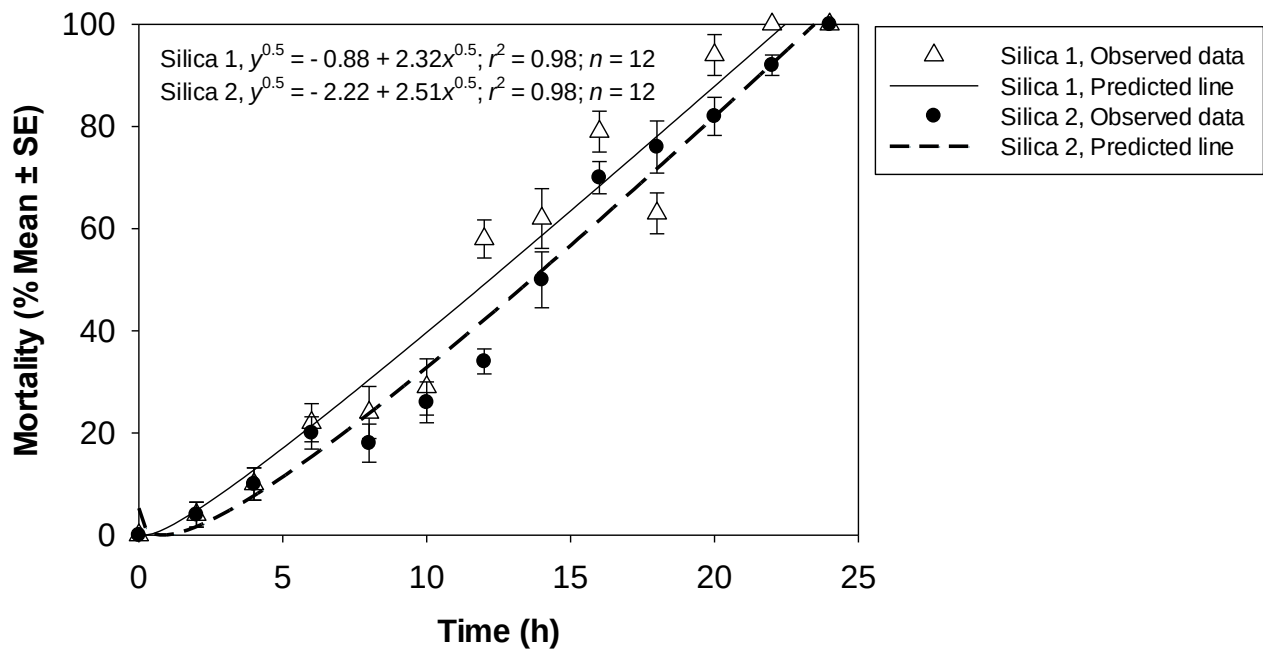


Figure 4.3. Percentage mortality of *T. castaneum* adults at 14 d after exposure to concrete arenas treated with 5.0 g/m² of silica powder 1 and 6.0 g/m² of silica powder 2 for various exposure times.

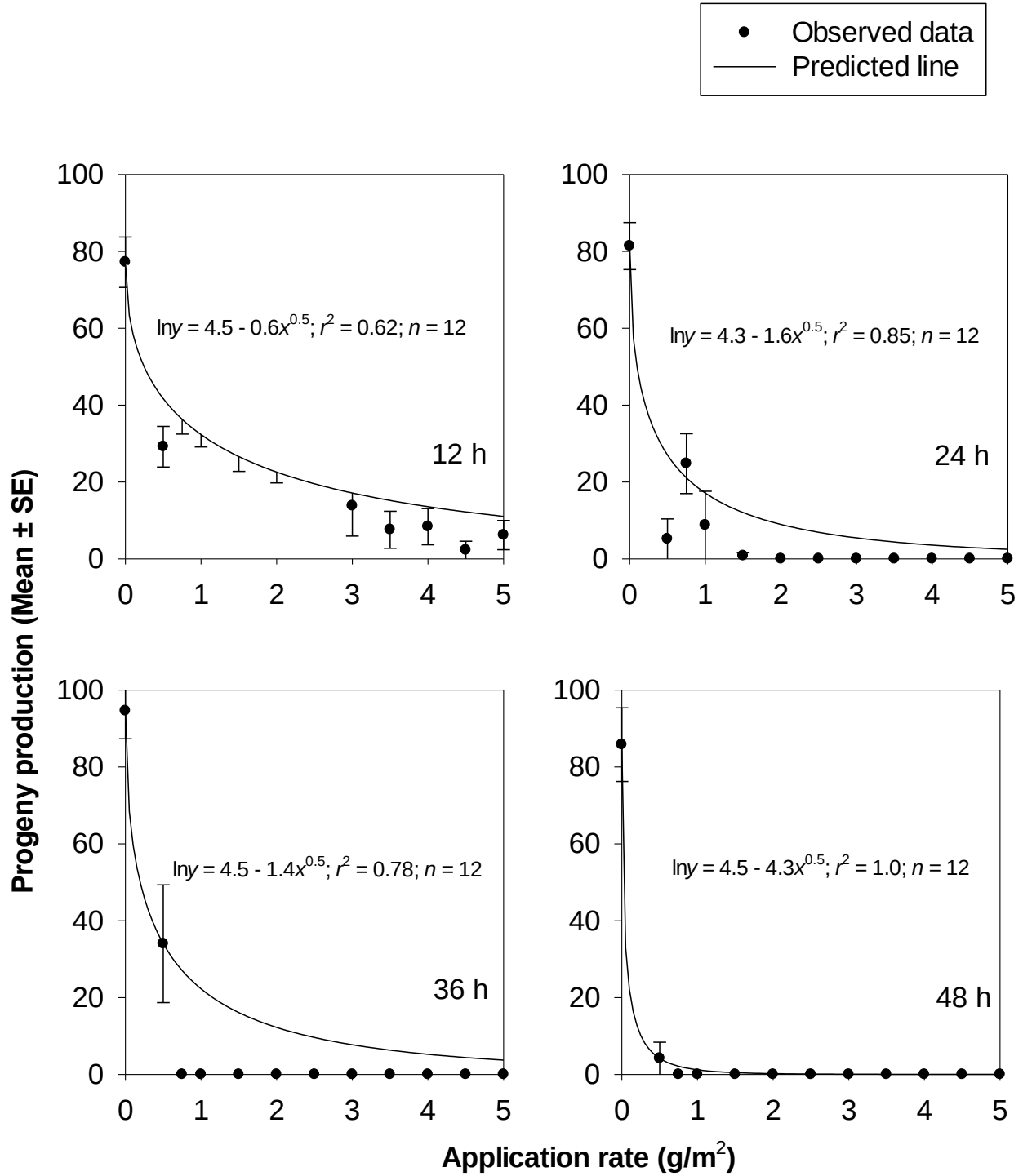


Figure 4.4. Adult progeny production from *T. castaneum* adults at 42 d after exposure to concrete arenas treated with silica powder 1 for different application rates and exposure times.

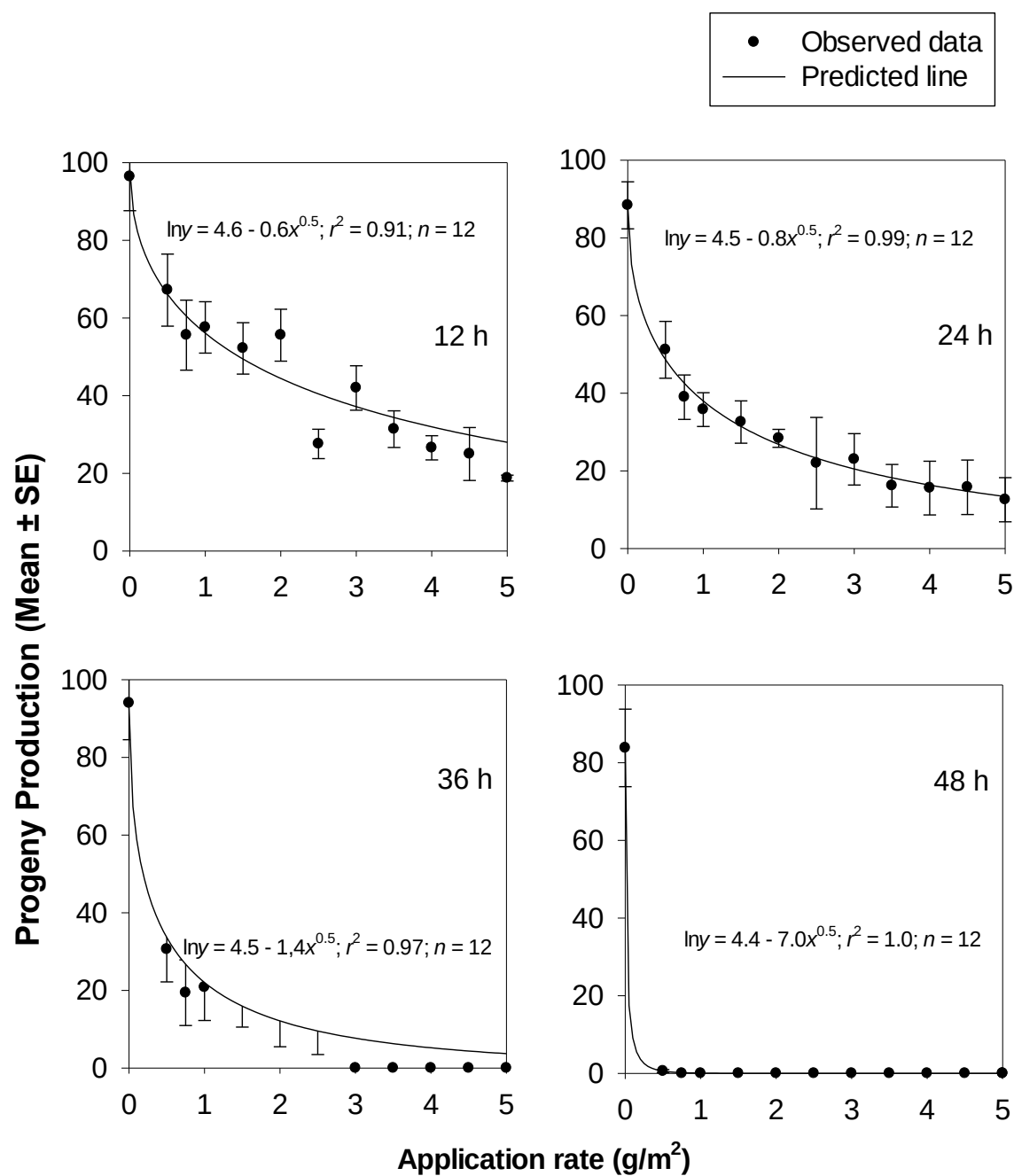


Figure 4.5. Adult progeny production from *T. castaneum* adults at 42 d after exposure to concrete arenas treated with silica powder 2 for different application rates and exposure times.

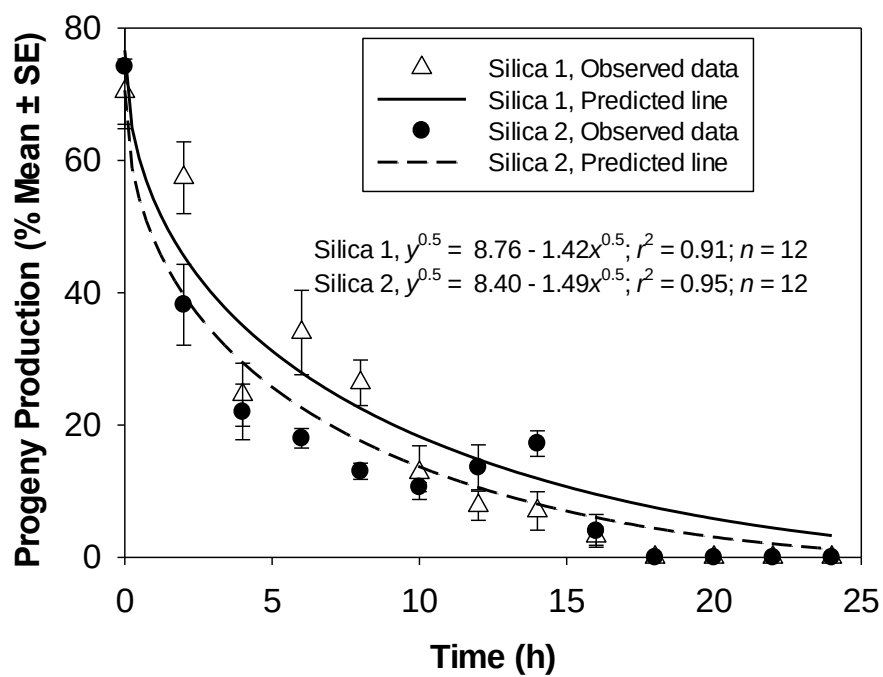


Figure 4.6. Adult progeny production from *T. castaneum* adults at 42 d after exposure to concrete arenas treated with 5.0 g/m² of silica powder 1 and 6.0 g/m² of silica powder 2 for various exposure times.

Chapter 5 - Insecticidal activity of two amorphous diatomaceous earth powders against the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae)

5.1 Abstract

The increased demand for residue-free food grains has led to exploring diatomaceous earth formulations as potential alternatives to synthetic insecticides. In the present study, we evaluated the efficacy of two amorphous silica powders 1 and 2 with different elemental composition, particle diameter, and shape characteristics against the confused flour beetle, *Tribolium confusum* Jacquelin du Val. The insecticidal activity of the two silica powders was determined by exposing *T. confusum* adults to silica powder treated concrete arenas at different application rates (0, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 g/m²) for 12, 24, 36, and 48 h. Mortality assessments were made after 14 d, and adult progeny counts were made at 42 d. Silica powder 2 demonstrated greater efficacy and produced 100% adult mortality and completely inhibited adult progeny production when adults were exposed for 24 h to an application rate of 4.0 g/m². Complete mortality and complete suppression of adult progeny production was noticed after 36 h exposure to 3.5 g/m² of silica powder 1. Exposure of *T. confusum* adults to 6 g/m² of silica powder 1 and 4 g/m² of silica powder 2 gave complete mortality after 24 and 20 h, respectively. No adult progeny production was observed when adults were exposed for 24 h to 6 g/m² and 4 g/m² of silica powder 1 and silica powder 2, respectively.

Keywords: Amorphous silica powders, *T. confusum*, mortality, progeny production, oil adsorption capability, efficacy assessment.

5.2 Introduction

The popularity of inert dusts such as diatomaceous earths for management of stored product insect pests in recent years has been on the rise, driven by a growing demand for insecticide residue free food grains. Inert dusts are chemically unreactive powders (Subramanyam and Roesli, 2000), with a strong insecticidal activity. Inert dusts exhibit low mammalian toxicity (Korunić et al., 1996; 1998), and can offer long term protection to food grains without leaving any harmful residues (Losic and Korunić, 2018). Diatomaceous earth formulations derived from the fossils of phytoplanktons or diatoms (Korunić, 1998; Losic and Korunić, 2018) are recognized as promising alternatives to synthetic insecticides worldwide (Subramanyam and Roseli, 2000). Diatomaceous earth products are used to treat food grains (Zeni et al., 2021) or empty grain bins and floors to manage stored product insects. The insecticidal activity of diatomaceous earth formulations has been studied and reported for many stored product insects. The action of diatomaceous earth products on stored product insects involves the absorption of the waxy epicuticular layers in insects leading to death by desiccation (Ebeling, 1971; Shawir et al., 1988; Subramanyam and Roesli, 2000), as well as the obstruction of respiratory pathway by blocking spiracles and trachea in insects (Webb, 1946). Diatomaceous earth formulations are effective at higher application rates (Kavallieratos et al., 2018), and have been shown to adversely affect grain physical properties when used as protectants (Korunić et al., 1996; 1998; Losic and Korunić, 2018). Therefore, it is important to find amorphous silica powders derived from diatomaceous earths that are effective on stored product insects at lower application rates (Subramanyam et al., 1998; Manivannan and Subramanyam, 2023a,b; Manivannan et al., 2024), potentially mitigating any detrimental impact on the physical properties of treated grains.

The efficacy of diatomaceous earth formulations on stored product insects is known to vary based on the application rate, exposure period, treatment conditions, type of commodity and insect species and insect life stage (Korunić, 1998; Subramanyam et al., 1998; Vayias et al., 2004; Athanassiou et al., 2006, 2011; Kavallieratos et al., 2007; Zeni et al., 2021; Agrafioti et al., 2023). Studies have shown that flat bodied insects such as the *Cryptolestes* spp. are highly susceptible to diatomaceous earth formulations (Subramanyam and Roesli, 2000), while the flour beetles, *Tribolium* spp. and the lesser grain borer, *Rhyzopertha dominica* (F.) are the most tolerant insect species (Golob, 1997; Korunić, 1998; Arthur, 2001, 2002).

Arthur (2000a) documented the effectiveness of Protect-It applied to filter papers held inside plastic Petri dishes at the rate of 0.5 g/m² when exposed for 72 h at 27 °C and, at 40 and 57% r.h. resulting in complete mortality of the red flour beetle, *Tribolium castaneum* (Herbst) and the confused flour beetle, *Tribolium confusum* Jacquelin du Val. Exposure of *T. confusum* adults to two enriched diatomaceous earth formulations with different particle sizes (DE 5 and DE 6), on concrete arenas at 0.5 and 1.0 g/m² was found to induce complete mortality of adults after 3 days (Baliota and Athanassiou, 2023). These varied results indicate that the efficacy of diatomaceous earth formulations on stored product insects could be attributed to the variation in geographical origin, diatom species, pH, particle size distribution, internal surface areas, and lipid absorption capability (Korunić, 1999; Fields and Korunić, 2000). Furthermore, many studies have reported that *Tribolium* spp. to be more tolerant to diatomaceous earth formulations (Fields and Korunić, 2000; Athanassiou et al., 2004; Korunić et al., 2020) compared to other stored product insect species such as rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.); rice weevil, *Sitophilus oryzae* (L.); and *R. dominica*. Reports on new diatomaceous earth formulations effective at lower application

rates and shorter exposure periods can have a significant impact in the management of *Tribolium* spp.

In this study, we assessed the effectiveness of two new amorphous silica powders, referred to as silica powder 1 and silica powder 2, obtained from Imery's, Lompoc, California, USA, against *T. confusum*. Drawing from our prior research with the same silica powders, we observed that the adults of *T. castaneum* were more susceptible to silica powder 1 treated concrete surfaces at lower application rates and shorter exposure periods compared to silica powder 2 (Manivannan and Subramanyam, 2023b). Considering the variability in the efficacy of silica powders among insect species, our current study investigated the effect of two candidate amorphous silica powders against *T. confusum* adults. We exposed *T. confusum* adults to silica powder treated concrete surfaces at different application rates and exposure periods and evaluated its efficacy on adult mortality and adult progeny suppression.

5.3 Materials and Methods

5.3.1 Silica powders

The two silica powders, 1 and 2, with different elemental compositions, particle size diameter, and shape characteristics, described earlier by Manivannan and Subramanyam (2023a) were used in the present study to determine efficacy against *T. confusum*. Briefly, the silicon dioxide content in silica powder 1 was 94% while it was 97% for silica powder 2. The pH values for silica powder 1 and silica powder 2 were 9.6 and 7.2, respectively. The particle diameter, i.e. D₉₀ (90% of particles within the size range) of silica powder 1 was 26.4 µm and 31.4 µm for silica powder 2. The oil absorption capability of silica powder 1 and silica powder 2 were 231 and 386%, respectively.

5.3.2 Insects

The Cultures of *T. confusum* used for the experiments originated from insect colonies maintained in the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA since 1999. Cultures using a laboratory strain of *T. confusum* were reared in 0.95 L mason glass jars containing organic whole wheat flour (Great River Organic Milling Company, Fountain City, Wisconsin, USA) supplemented with 5% (by wt) of brewer's yeast. The glass jars were covered with 250 µm wire metal screens and 9 cm diameter filter paper lids and held inside controlled temperature humidity chamber (Pervical Scientific, Inc., Model I-36 VL, Perry, Iowa, USA) at 28°C and 65% r.h. with a 14:10 light:dark photoperiod.

5.3.3 Concrete arenas

The procedure used for making concrete arenas to test the insecticidal activity of the two silica powders were similar to those described by Manivannan and Subramanyam (2023 a,b). Briefly, concrete arenas were prepared by mixing Rockite concrete mix (Rockite, Hartline Products Co., Inc., Cleveland, Ohio, USA) with tap water at the ratio of 2:1 and poured into plastic Petri dishes of 9 cm diameter and 1.5 cm height (Fisher Scientific Co, LLC., Pittsburgh, Pennsylvania, USA). The concrete poured dishes were allowed to dry on a laboratory working bench for 24 h. Once the Petri dishes were dried, the inner walls of the dishes were coated with polytetrafluoroethylene (Insect-a-Slip, BioQuip product, Inc, Rancho, Dominguez, California, USA) to prevent insects from crawling onto the sides of the dishes and thus avoiding contact with the treated concrete surfaces (Sehgal and Subramanyam, 2014; Tadesse and Subramanyam, 2018). The concrete arenas were used to simulate empty bin floors.

5.3.4 Bioassays

Three different experiments were performed to evaluate the efficacy of the two silica powders against *T. confusum*. In the first experiment, the concrete arenas were treated with silica powders 1 and 2 at 12 application rates of 0 (control), 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 g/m² for 12, 24, 36, and 48 h. Once the application rates of silica powders were transferred onto the concrete arenas, a dissection needle was used to distribute the powders inside concrete arenas. Ten unsexed, 1-4-week-old adults of *T. confusum* from cultures were exposed to each application rate of the silica powders for 12, 24, 36, and 48 h. Five replications were maintained for each powder, application rate, and exposure time combination. The silica powder treated arenas were secured with lids, and then placed inside temperature humidity chamber at 28°C and 65% r.h. After the intended exposure period, both live and dead adults from each concrete arena were picked up using featherlight forceps and transferred to 150 ml round bottomed plastic containers containing 30 g of whole wheat flour and 5% brewer's yeast. The plastic containers were held inside growth chambers at 28°C and 65% r.h. for 14 d. After 14 d, the data on adult mortality was determined. The insects that did not move when prodded with camel's fine brush were considered dead.

In the second experiment, adults of *T. confusum* were exposed to 6.0 g/m² and 4.0 g/m² of silica powder 1 and silica powder 2, respectively, for 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h. Five replications were maintained for each silica powder-exposure period combination. The protocol followed was similar to that mentioned in the first experiment. After 14 d, the data on adult mortality was determined for insects exposed to 6.0 g/m² of silica powder 1 and 4.0 g/m² of silica powder 2 for 0 – 24 h.

In the third experiment, two different tests were performed to evaluate the effect of silica powders 1 and 2 on the adult progeny production of *T. confusum*. In the first test, protocol similar to those mentioned in the first experiment were followed to study the effect of different application rates and exposure times of silica powders 1 and 2 on adult progeny production of *T. confusum*. Five replications were maintained for each powder, application rate, and exposure period combination. The progeny counts were taken at 42 d by separating the adults from the diet by passing the diet using a 595 μm circular round-holed aluminum sieve. The actual adult progeny counts were enumerated after subtracting the introduced 10 parental adults. The second test followed a protocol similar to those mentioned above in the second experiment. In the second test, the adult progeny counts were recorded at 42 d after exposing the adults of *T. confusum* for 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h to 6 g/m^2 of silica powder 1 and 4 g/m^2 of silica powder 2. Each powder and exposure period combination was replicated five times.

5.3.5 Data analysis

The non-linear model, $y = a + bx^{0.5}$, was fit to percentage adult mortality data of *T. confusum* as a function of silica powder 1 and silica powder 2 application rates (0 to 5 g/m^2) exposed for 12, 24, 36, and 48 h using Table Curve2D software (Jandel Scientific, San Rafael, California, USA). The non-linear model, $\ln y = a - bx$, was fit to the progeny production data as a function of silica powders 1 and 2 application rates (0 to 5 g/m^2) exposed for 12, 24, 36, and 48 h. A non-linear model, $y^{0.5} = a + bx^{0.5}$, was fit to percentage adult mortality and progeny production data as a function of exposure time (0 – 24 h), at application rates of 6 g/m^2 of silica powder 1 and 4 g/m^2 of silica powder 2. Each possible pairwise comparison between powders and exposure periods of individual powders was performed by comparing the individual models

with a pooled model (Draper and Smith, 1998). Any two models were considered significantly different ($P < 0.05$) from one another if the F -test showed individual models to deviate from the pooled model. The data analysis was performed using SAS 9.4 software (SAS Institute, 2012). The graphs were plotted using SigmaPlot 12.5 software (Systat, Software, Inc., San Jose, California, USA).

5.4 Results

5.4.1 Responses of *T. confusum* adults to silica powders

In the first experiment, the mean $\pm$ SE percentage mortality in untreated controls ranged from 2.0 ± 2.0 to $4.0 \pm 2.5\%$. The mean percentage mortality of *T. confusum* adults exposed for 12, 24, 36, and 48 h to 0.5 to 5 g/m² of silica powder 1 in concrete arenas ranged from 0 to 100%. Complete mortality of *T. confusum* adults was not achieved when the adults were exposed for 12 and 24 h to 0.5 to 5.0 g/m² of silica powder 1. The minimum application rates required to achieve complete mortality of *T. confusum* was 3.5 and 1.5 g/m² when exposed for 36 and 48 h, respectively. The mean $\pm$ SE percentage mortality of *T. confusum* adults exposed for 12 to 48 h to 0.5 to 5 g/m² of silica powder 2 ranged from 24.0 ± 5.1 to 100%. Complete mortality of *T. confusum* was not achieved at any of the tested application rates of silica powder 2 when exposed for 12 h. The minimum application rates required to achieve complete mortality of *T. confusum* after exposure for 24, 36, and 48 h to silica powder 2 were 4.0, 2.5, and 3.0 g/m², respectively (Table 5.1). Mortality of *T. confusum* increased with increasing application rates of silica powders 1 and 2, and the mortality as a function of application rate at each exposure period followed a non-linear trend. The fitted non-linear model satisfactorily explained the relationship between the percentage mortality of *T. confusum* as a function of application rates of silica powder 1 ($r^2_{\text{range}} = 0.62 - 0.94$) (Fig 5.1) and silica powder 2 ($r^2_{\text{range}} = 0.79 - 0.93$) (Fig 5.2),

after 12, 24, 36, and 48 h exposure. Mortality responses of *T. confusum* exposed to silica powder 1 at 24 and 48 h ($F = 2.85$; $df = 2, 20$; $P = 0.0813$), 36 and 48 h ($F = 0.13$; $df = 2, 20$; $P = 0.8831$), and silica powder 2 at 12 and 24 h ($F = 1.35$; $df = 2, 20$; $P = 0.2809$) and 36 h and 48 h ($F = 0.71$; $df = 2, 20$; $P = 0.5020$) were not significantly different from one another. However, all the other pairwise comparisons were significant ($P < 0.05$) (Table 5.2).

In the second experiment, no adult mortality was observed in untreated controls after a 24 h exposure. Complete mortality of *T. confusum* was not observed when adults were exposed to 6.0 g/m² of silica powder 1 for 1 to 24 h. Complete mortality of *T. confusum* was achieved when adults were exposed to 4.0 g/m² of silica powder 2 for 20 to 24 h. This indicated that silica powder 2 was more effective on *T. confusum* adults compared to silica powder 1. The fitted non-linear model satisfactorily explained the relationship between percentage mortality of *T. confusum* adults and the exposure period of silica powder 1 ($r^2 = 0.97$) and silica powder 2 ($r^2 = 0.99$) (Fig 5.3). Comparison of non-linear models fitted for the percentage mortality data of *T. confusum* as a function of exposure period showed no significant difference between the silica powders 1 and 2 ($F = 1.6420$; $df = 2, 22$; $P = 0.2164$).

5.4.2 Adult progeny production

In the first test, the mean $\pm$ SE number of *T. confusum* adult progeny produced in controls ranged from 43.4 ± 9.6 to 89.8 ± 7.8 per container after 12 – 48 h exposure. The mean $\pm$ SE number of *T. confusum* adult progeny produced after exposure to 0.5 to 5.0 g/m² of silica powder 1 for 12, 24, 36, and 48 h ranged from 0 to 41.8 ± 8.2 per container. Complete inhibition of *T. confusum* adult progeny production was not achieved at any of the application rates (0.5 to 5.0 g/m²) of silica powder 1 and silica powder 2 after a 12 h exposure. The minimum application rates of silica powder 1 required to achieve complete suppression of adult progeny production of

T. confusum was 4.5, 1.5 and, 1.0 g/m² when exposed for 24, 36, and 48 h, respectively. The mean $\pm$ SE number of *T. confusum* adult progeny produced after exposure to 0.5 to 5.0 g/m² of silica powder 2 for 12, 24, 36, and 48 h ranged from 0 to 43.0 $\pm$ 8.1 per container. The minimum application rates of silica powder 2 required to achieve complete inhibition of adult progeny production of *T. confusum* after 24, 36, and 48 h of exposure were 2.5, 2.5 and, 2.0 g/m², respectively (Table 5.3). The decreasing trend in the adult progeny production of *T. confusum* at increasing application rates of silica powders 1 and 2 were non-linear. The fitted non-linear model satisfactorily explained the relationship between the *T. confusum* adult progeny production data as a function of application rates of silica powder 1 ($r^2_{\text{range}} = 0.80 - 0.92$) (Fig 5.4) and silica powder 2 ($r^2_{\text{range}} = 0.85 - 0.98$) (Fig 5.5), when exposed for 12, 24, 36, and 48 h. Pairwise comparisons of the fitted non-linear models showed that the progeny production data of *T. confusum* adults exposed to silica powder 1 at 24 and 36 h ($F = 3.19$; $df = 2, 20$; $P = 0.0626$) and 36 and 48 h ($F = 1.83$; $df = 2, 20$; $P = 0.1856$) were not significantly different from one another. However, all the other pairwise comparisons for silica powder 1 and silica powder 2 were significant ($P < 0.05$) (Table 5.4).

In the second test, the mean $\pm$ SE number of *T. confusum* adult progeny produced in controls ranged from 48.4 $\pm$ 5.5 to 49.8 $\pm$ 5.5 per container after 24 h exposure. The mean $\pm$ SE number of *T. confusum* adult progeny produced after exposure to 6.0 g/m² of silica powder 1 and 4.0 g/m² of silica powder 2 for 1 to 24 h ranged from 0 to 18.4 $\pm$ 2.0 per container and 0 to 28.8 $\pm$ 4.3 per container, respectively. Complete reduction in adult progeny production of *T. confusum* was observed when adults were exposed for 24 h to 6.0 g/m² of silica powder 1 and 4.0 g/m² of silica powder 2. The fitted non-linear model satisfactorily explained the relationship between adult progeny production data as a function of exposure period of silica powder 1 ($r^2 = 0.99$) and

silica powder 2 ($r^2 = 0.99$) (Fig 5.6). Comparison of the non-linear models fitted for the adult progeny production data for silica powder 1 at 6.0 g/m² and silica powder 2 at 4.0 g/m² as a function of exposure period showed no significant difference between the silica powder 1 and 2 ($F = 0.3221$; $df = 2, 22$; $P = 0.7280$).

5.5 Discussion

Our investigation highlights the potential of two amorphous silica powders, denoted as 1 and 2, applied to concrete surfaces against *T. confusum*. It is worth noting that *T. confusum* has demonstrated relative tolerance to diatomaceous earth powders, as documented in previous studies (Aldryhim, 1990; Arthur, 2000ab; Fields and Korunić, 2000). Consequently, the favorable results observed in our study with the two silica powders are promising. The mechanism by which diatomaceous earth powders act against stored product insects involves the absorption of epicuticular waxes, ultimately leading to death by desiccation (Ebeling, 1971; Subramanyam and Roesli, 2000). We observed a discernible difference in susceptibility between the two silica powders with respect to adult mortality and the inhibition of adult progeny production. Although we did not measure the water loss from the insects' bodies post-treatment, the increased fragility of the dead insects suggests a desiccation-induced effect (Ebeling, 1971).

Adults of *T. confusum* were more susceptible to silica powder 2 than silica powder 1. Complete mortality occurred after a 36 h exposure to 3.5 g/m² of silica powder 1, while 4.0 g/m² of silica powder 2 achieved the same result in just 24 h. In a previous study (Manivannan and Subramanyam, 2023b), *T. castaneum*, a congeneric species, displayed complete mortality after 36 h when adults were exposed to 1.5 g/m² of silica powder 1, but 48 h exposure was required when adults were exposed to 0.75 g/m² of silica powder 2. These findings indicate silica powder 1 was effective against both *Tribolium* spp. after 36 h exposure or more. However, *T. confusum*

adults proved more susceptible to silica powder 2 requiring a 24 h exposure compared to a 48 h exposure needed for *T. castaneum* (Manivannan and Subramanyam, 2023b). The variation between these two studies confirms the species-specific difference in insecticidal activity of the two silica powders, and further affirms the fact that *T. castaneum* is less susceptible species to diatomaceous earth powders as observed by many researchers (Maceljski and Korunić, 1972; Desmarchelier and Dines, 1987; Fields and Muir, 1995; Korunić, 2013; Yao, 2014).

The application of Fossil Shield, a diatomaceous earth powder, to plywood plates at 25 ± 1 °C and 62 ± 2 % r.h. at rates of 2 and 4 g/m² resulted in complete mortality of *T. confusum* after a 9 d exposure (Mewis and Ulrichs, 2001). Similarly, complete mortality of *T. confusum* adults was observed by exposing them for 7 days to Insecto and Protect-It treated wheat at an application rate of 1.0 g/kg at 30 °C (Kavallieratos et al., 2007). The results of these studies indicate the need for longer exposure times to achieve complete mortality of *T. confusum*. In the present study we observed 100% mortality of adults after 36 h of exposure to the two amorphous silica powders, 1 and 2 at application rates of 3.5 g/m² or more.

The effectiveness of diatomaceous earth formulations against stored product insects may vary due to several factors, including the content of amorphous silica, particle size, oil sorption capacity, pH values (< 8.5), quantity of clay, and other impurities (Korunić, 1998; Korunić, 2013; Liška et al., 2017). Therefore, the differences in findings between the above referenced studies and our current research may be attributed to the lower silicon dioxide content of Fossil Shield (Mewis and Ulrichs, 2001) which was 73%, whereas that of Insecto and Protect-It (Kavallieratos et al., 2007) were reported to be 86.7% and 83.7%, respectively. The silicon dioxide content of the powders we used exceeded 94%.

Given that diatomaceous earth formulations with particle sizes smaller than 45 μm were deemed more effective (Vayias et al., 2009), we inferred the efficacy of the silica powders based on their particle diameter. As both powders had D_{90} values less than 45 μm , we anticipated that the smaller sized powder particles can be easily picked up by insects leading to enhanced insecticidal activity against *T. confusum*. Furthermore, diatomaceous earth formulations with higher oil absorption capability (Korunić, 1998; Stadler et al., 2012) and pH values < 8.5 , demonstrate better insecticidal activity (Korunić, 1998). Silica powder 2 had a high oil absorption capability of 386% compared to silica powder 1 which had an oil absorption capability of 284%. This could be a major factor responsible for the higher efficacy of silica powder 2 against *T. confusum* adults. A similar observation was reported by Manivannan and Subramanyam (2023a), against *R. dominica* when adults were exposed to concrete surfaces treated with the two amorphous silica powders, 1 and 2. Additionally, silica powder 2 had a pH value of 7.2, which is lower than the desired value of < 8.5 for better efficacy of diatomaceous earth formulations on stored product insects.

Our study found an inverse relationship between adult progeny production in *T. confusum* and the application rates of silica powders 1 and 2. Complete suppression of progeny was not observed even at 5.0 g/m^2 after a 12 h exposure to both the powders, but after a 24 h exposure, silica powder 2 at 2.5 g/m^2 and silica powder 1 at 4.5 g/m^2 achieved complete suppression of adult progeny production. Manivannan and Subramanyam (2023b) reported complete inhibition of adult progeny production of *T. castaneum* after a 24 h exposure to 2.0 g/m^2 of silica powder 1 and after a 36 h exposure to 3.0 g/m^2 of silica powder 2. The varying effectiveness of these two powders between our earlier research and the present study, suggests species-specific susceptibility among stored product insects to these silica powder treatments. Moreover, the

superior efficacy of silica powder 2 over silica powder 1 in inhibiting adult progeny production in *T. confusum*, could be possibly due to its higher silicon dioxide content, lower pH, and greater oil absorption capability.

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Table 5.1. Minimum application rates (g/m²) required for 100% mortality of *T. confusum* adults exposed for time periods to two amorphous silica powders treated concrete arenas.

Silica powder	Application rate (g/m ²)			
	12 h	24 h	36 h	48 h
Silica 1	— ^a	— ^a	3.50	1.50
Silica 2	— ^a	4.00	2.50	3.00

—^a Complete mortality was not achieved at any application rate.

Table 5.2. Pairwise comparison of non-linear models fit to mortality data of *T. confusum* adults as function of silica powder application rates at different exposure times.

Powder	Exposure times compared	<i>F</i> - value	df	<i>P</i> -value
Silica 1	12 h vs 24 h	4.00	2, 20	0.0345*
	12 h vs 36 h	15.74	2, 20	0.0001
	12 h vs 48 h	5.85	2, 20	0.0010*
	24 h vs 36 h	30.32	2, 20	0.0000*
	24 h vs 48 h	2.852	2, 20	0.0813
	36 h vs 48 h	0.13	2, 20	0.8831
Silica 2	12 h vs 24 h	1.35	2, 20	0.2809
	12 h vs 36 h	9.84	2, 20	0.0011*
	12 h vs 48 h	13.61	2, 20	0.0002*
	24 h vs 36 h	4.20	2, 20	0.0298*
	24 h vs 48 h	7.50	2, 20	0.0037*
	36 h vs 48 h	0.71	2, 20	0.5020

* Significant ($P < 0.05$)

Table 5.3. Minimum application rates (g/m²) required for complete reduction of adult progeny production of *T. confusum* exposed for different time periods to two amorphous silica powders treated concrete arenas.

Silica powder	Application rate (g/m ²)			
	12 h	24 h	36 h	48 h
Silica 1	— ^a	4.50	1.50	1.00
Silica 2	— ^a	2.50	2.50	2.00

—^a Complete reduction in adult progeny not achieved at any application rate.

Table 5.4. Pairwise comparison of non-linear models fit to 42 days adult progeny production data of *T. confusum* as a function of silica powder application rates at different exposure times.

Powder	Exposure times compared	<i>F</i> - value	df	<i>P</i> -value
Silica 1	12 h vs 24 h	8.06	2, 20	0.0027*
	12 h vs 36 h	30.65	2, 20	0.0000*
	12 h vs 48 h	17.47	2, 20	0.0000*
	24 h vs 36 h	3.19	2, 20	0.0626
	24 h vs 48 h	5.80	2, 20	0.0103*
	36 h vs 48 h	1.83	2, 20	0.1856
Silica 2	12 h vs 24 h	7.71	2, 20	0.0033*
	12 h vs 36 h	18.95	2, 20	0.0000*
	24 h vs 36 h	23.91	2, 20	0.0000*
	24 h vs 48 h	19.99	2, 20	0.0000*
	36 h vs 48 h	1097	2, 20	0.0006*

* Significant ($P < 0.05$)

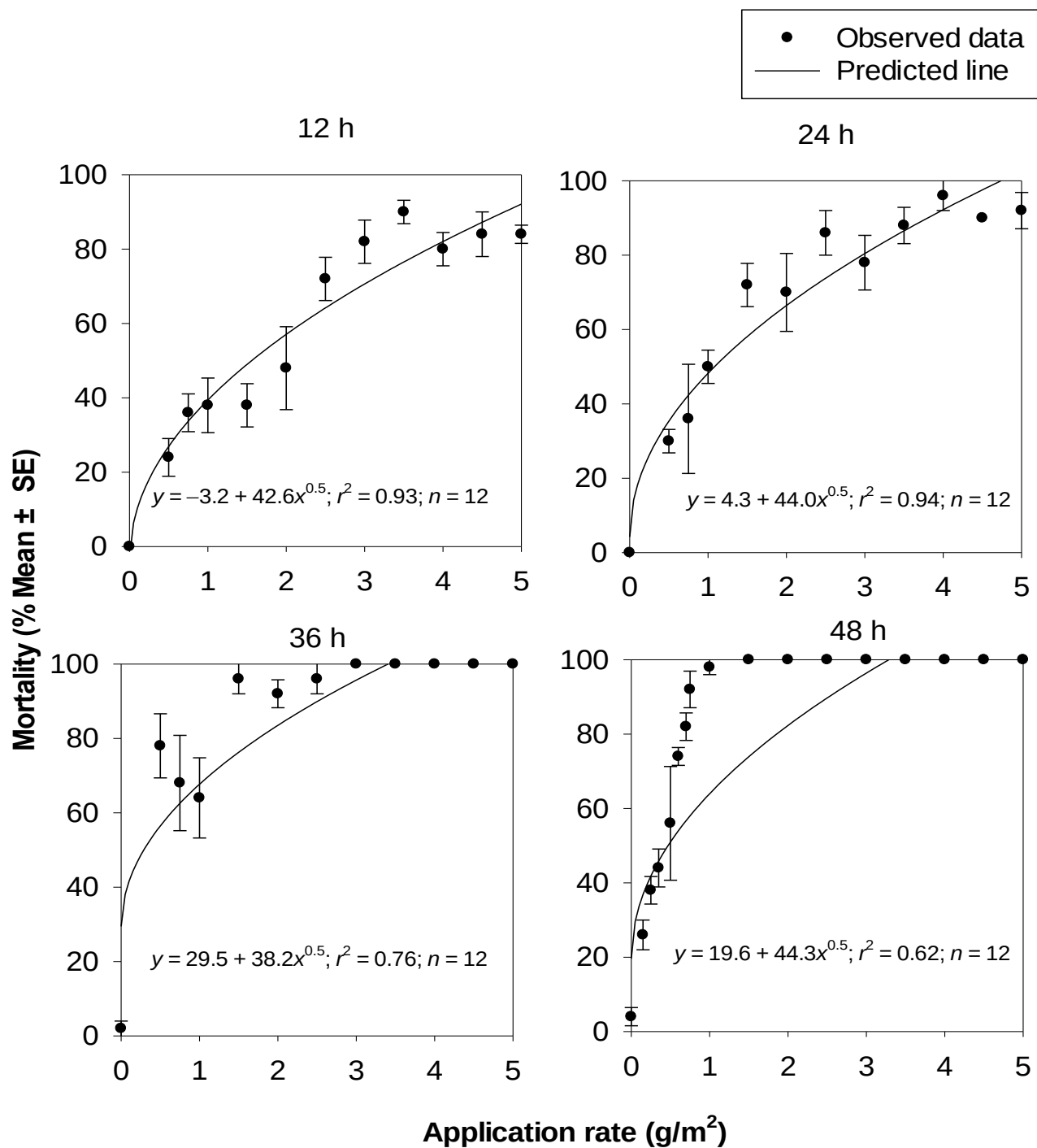


Figure 5.1. Percentage mortality of *T. confusum* adults at 14 days as a function of silica powder application rates at different exposure times.

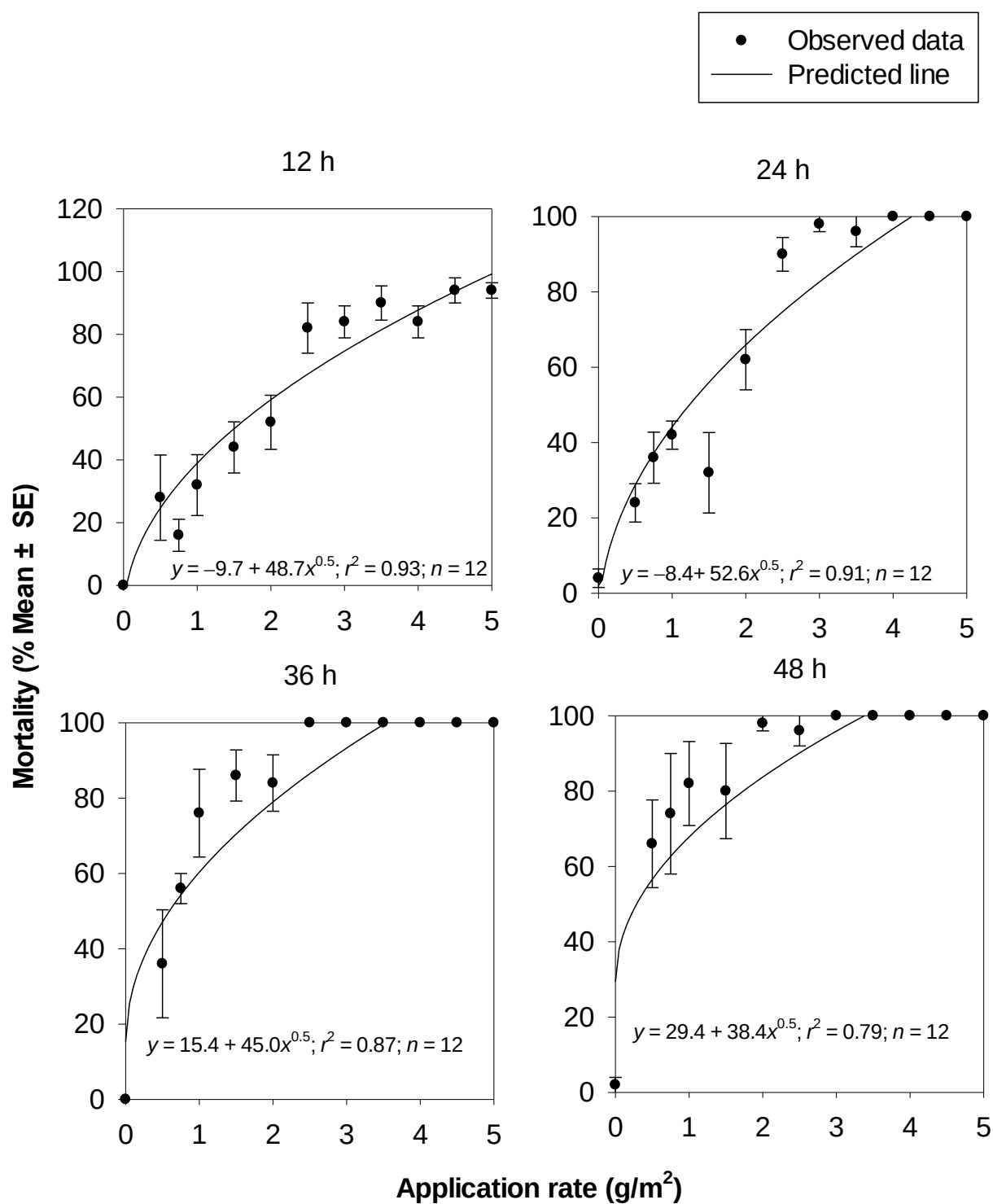


Figure 5.2. Percentage mortality of *T. confusum* adults at 14 days as a function of silica powder 2 application rates at different exposure times.

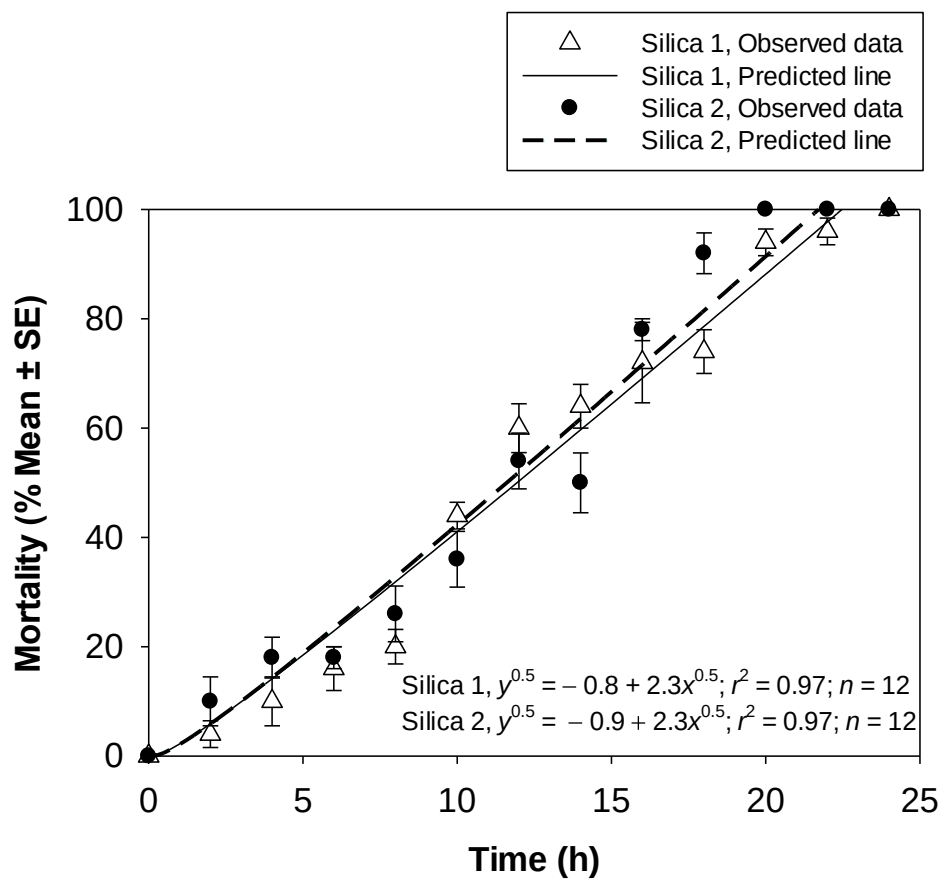


Figure 5.3. Percentage mortality of *T. confusum* adults at 14 days as a function of exposure time on concrete arenas treated with 6.0 g/m² of silica powder 1 and 4.0 g/m² of silica powder 2.

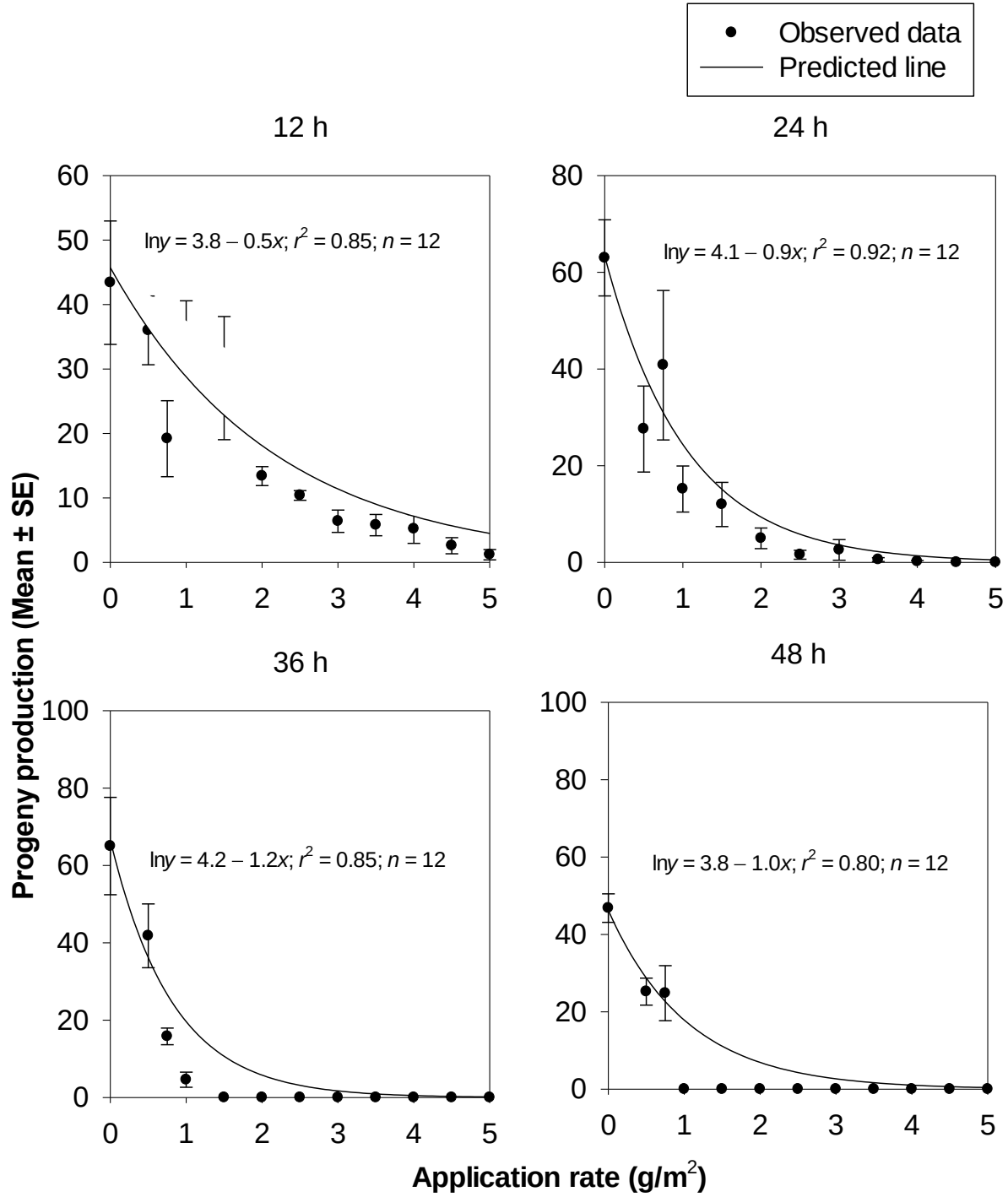


Figure 5.4. Adult progeny production of *T. confusum* adults at 42 days as a function of silica powder 1 application rates at different exposure times.

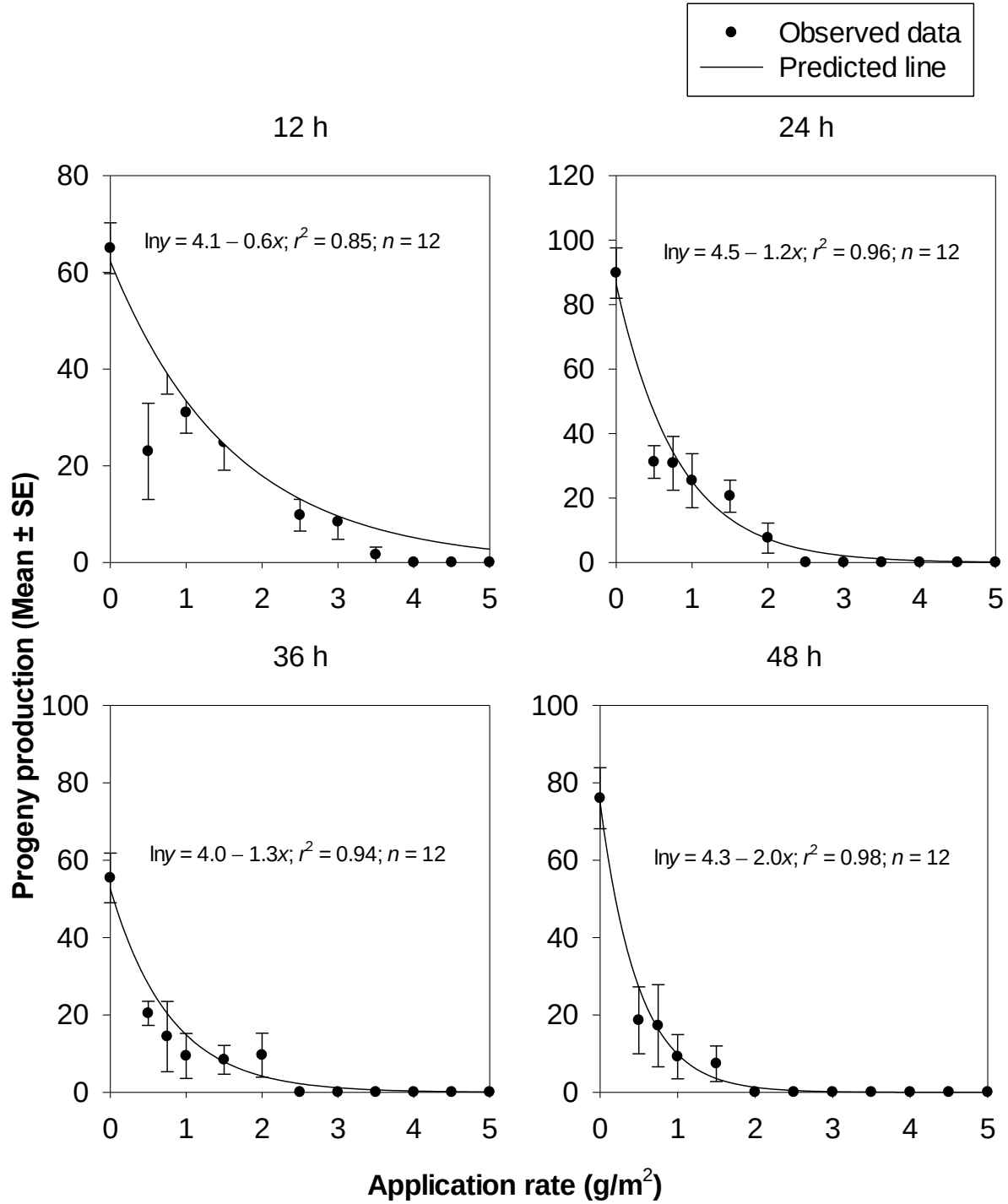


Figure 5.5. Adult progeny production of *T. confusum* adults at 42 days as a function of silica powder 2 application rates at different exposure times.

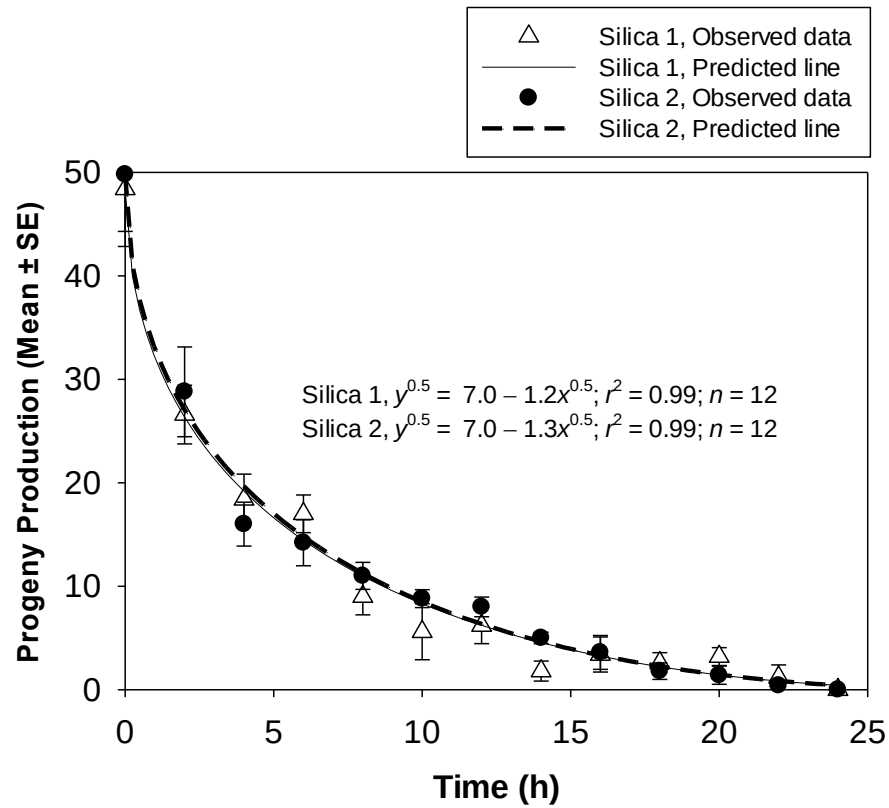


Figure 5.6. Adult progeny production of *T. confusum* adults at 42 days as a function of exposure time on concrete arenas treated with 6.0 g/m² of silica powder 1 and 4.0 g/m² of silica powder 2.

Chapter 6 - Efficacy of two amorphous silica powders against the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L), (Coleoptera: Silvanidae)

6.1 Abstract

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) is a storage pest challenging to control due to its small size and resistance to chemical insecticides. Effective and environmentally safe alternatives are needed for managing this beetle in grain processing environments. This study investigates the efficacy of two amorphous silica powders 1 and 2 from Imerys, Lompoc, California, USA, in controlling *O. surinamensis*. The effect of the two silica powders 1 and 2 was evaluated by exposing *O. surinamensis* adults to concrete arenas treated with eleven different application rates of silica powder 1 and 2 exposed for 12, 24, 36, and 48 h. Adult mortality was determined after 14 d and progeny counts were taken at 42 d. The results showed that silica powder 2 was effective to induce complete mortality and adult progeny production on *O. surinamensis* when adults were exposed for 12 h to 4.0 g/m² rate. Conversely, silica powder 1 required 36 h exposure to an application rate of 3.5 g/m² for complete mortality and suppression of adult progeny production. Silica powder 2 consistently proved effective against *O. surinamensis* than silica powder 1. The findings suggest the potential of these silica powders for treating empty floor bins to mitigate *O. surinamensis* populations in grain storage facilities.

Keywords: *Oryzaephilus surinamensis*, silica powders, adult mortality, progeny production, Oil adsorption capability.

6.2 Introduction

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), (Coleoptera: Silvanidae), is a secondary insect pest of stored grains. *O. surinamensis* primarily feeds on broken and damaged grains introduced into grain storage environments and exhibits a rapid developmental cycle, leading to significant and potentially severe infestations (Mathlein, 1971; Howe, 1973; Prickett et al., 1990). Due to its smaller size, it can hide in cracks and crevices within storage facilities, making it challenging to control with chemical insecticides. Additionally, it has developed resistance to many insecticides (Greening et al. 1974; Heather and Wilson 1983; Wallbank and Collins 2003; Gautam et al., 2020). This necessitates alternative methods for managing *O. surinamensis* in grain processing environments that are both effective and environmentally safe (Gharsan et al., 2022). Diatomaceous earth formulations demonstrate efficacy against various insect pests, including *O. surinamensis* (Allen, 2000; Arthur, 2001; Mohitazar et al., 2009; Schöller, and Reichmuth, 2010; El-Sayed et al., 2013; Li, 2018). Diatomaceous earth formulations are now being explored for their potential in insecticide-resistant management strategies (Balliota and Athanassiou, 2023). Diatomaceous earths are composed of fossilized remains of unicellular photosynthetic algae called diatoms (Korunić, 1998; Subramanyam and Roesli, 2000; Kılıç, 2022). The mode of action of diatomaceous earth on insect pests primarily relies on a physical mechanism, involving the adsorption of the waxy cuticular layer of insects, leading to death by desiccation (Ebeling, 1971; Subramanyam and Roesli, 2000). Consequently, resistance to diatomaceous earth is uncommon.

The efficacy of diatomaceous earth on insect pests is dependent upon the rate, treatment time, commodity, insect species and their stage (Korunić, 1998; Subramanyam et al., 1998; Vayias and Athanassiou, 2004; Athanassiou et al., 2007; Kavallieratos et al., 2007; Zeni et al.,

2021). Researchers have shown variable results on the susceptibility of *O. surinamensis* to diatomaceous earth formulations (Arthur, 2001). For instance, Golob (1997) stated *O. surinamensis* to be more susceptible to diatomaceous earth compared to internal feeders such as the rice weevil, *Sitophilus oryzae* (L.) and the lesser grain borer, *Rhyzopertha dominica* (F.). While Korunić (1998) reported that *Cryptolestes* spp. are more susceptible to diatomaceous earths, followed by *S. oryzae*, *O. surinamensis*, the flour beetles, *Tribolium* spp. and *R. dominica*. Arthur (2001) found that *O. surinamensis* exhibited susceptibility to Protect-It™ when adults were treated with wheat mixed at a rate of 0.3 g/kg and exposed for 72 h at temperatures of 27 and 32°C, with 40, 57, and 75% r.h.

Silica-based diatomaceous earths exhibit higher effectiveness at lower application rates in comparison to older formulations (Subramanyam et al., 1998). Previous studies on two amorphous silica powders, 1 and 2, from the US revealed variations in application rates needed for achieving complete mortality and inhibition of adult progeny production across different stored product insect species, *R. dominica*, *T. castaneum* and *T. confusum* (Manivannan and Subramanyam, 2023a,b; Manivannan and Subramanyam, unpublished data). In this context, the present study was aimed to determine the efficacy of two amorphous silica powders, 1 and 2 supplied by Imerys, Lompoc, California, USA on the mortality and progeny production of *O. surinamensis* exposed 12, 24, 36, and 48 h to different application rates of silica powder 1 and 2.

6.3 Materials and Methods

6.3.1 Silica powders

The amorphous silica powders 1 and 2 sourced from Imerys, Lompoc, California, USA were used for the experiments. Both the powders varied in their physical properties and elemental composition (Manivannan and Subramanyam, 2023a). Silica powder 1 had a D₉₀ of

26.40 μm) (diameter fraction where particles were within the range) which was significantly smaller ($P < 0.05$) than silica powder 2 with D_{90} value of 31.43 μm . The silicon dioxide content of silica powder 1 was 94% and silica powder 2 had 97%. The oil adsorption capability of silica powders 1 and 2 were 231% and 386%, respectively. Silica powder 1 had a pH value of 9.6, and silica powder 2 had a pH value of 7.2 (Manivannan and Subramanyam, 2023a).

6.3.2 Insects

O. surinamensis insect cultures were established for insect colonies maintained at the Department of Grain Science and Industry. Insect cultures were reared in 0.94 L glass mason jars containing 200 g of organic rolled oats (Great River Organic Milling, Arcadia, Wisconsin, USA). Adults of 1-to 4-week-old adults were used for the experiments. The insect culture jars were maintained at rearing conditions of 28 °C and 65% r.h. with a 14:10 light:dark photoperiod.

6.3.3 Concrete arenas

Concrete poured Petri dishes were prepared by mixing Rockite concrete mix (Rockite, Hartline Products Co., Inc., Cleveland, Ohio, USA with tap water in 2:1 ratio. The detailed procedure for preparation of concrete dishes have been described in detail in our earlier publications (Manivannan and Subramanyam, 2023a,b). The concrete poured dishes were dried for 24 h and the inner side of the Petri dish was coated with polytetrafluoroethylene (Insect-a-Slip, BioQuip product, Inc, Rancho, Dominguez, California, USA) to avoid escaped of insects to the side of the dishes during the experiments.

6.3.4 Insecticidal activity

Three tests were conducted to test the efficacy of the two amorphous silica powders 1 and 2 against *O. surinamensis*. In the first test, the adults of *O. surinamensis* were exposed for 12, 24, 36, and 48 h to eleven different application rates, 0 (control), 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0, 2.0,

3.0, and 4.0 g/m² of silica powder 1 and 2. Once the application rate of powders were loaded onto the dishes, a dissection needle was used to distribute the powders uniformly. Ten adults of *O. surinamensis* (1-4-week-old) were introduced to each Petri dish and exposed for 12, 24, 36, and 48 h. Each treatment combination had five replications. After the exposure period, the all the adults were removed from the dish and transferred to 150 ml round bottomed plastic containers with 20 g of rolled oats and held inside the growth chamber (Pervical Scientific, Inc., Model I-36 VL, Perry, Iowa, USA) to determine mortality after 14 d. In the second test, the adults of *O. surinamensis* were exposed to 3.5 g/m² of silica powder 1 and 2 for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h. Procedure followed was similar to those described in the first test. Each treatment combination was repeated five times. In the third test, we determined the efficacy of two amorphous silica powders 1 and 2 on the adult progeny production of *O. surinamensis*. A similar procedure was followed as explained in the first and second tests. Each powder-exposure time combination was replicated five times. Progeny counts were recorded at 42 d by subtracting the initial count of ten parental adults from the overall counts.

6.3.5 Data analysis

The adult mortality response data, following exposure to different application rates of silica powder 1 and silica powder 2, were modeled using the non-linear equation $y = a + bx^{0.5}$ with Table Curve2D software (Jandel Scientific, San Rafael, California, USA). For adult progeny production data, a non-linear model $y = a + be^{-x}$ was fitted, representing the function of application rates of silica powders 1 and 2. Additionally, a non-linear model $y^{0.5} = a + bx^{0.5}$ was employed for adult mortality, and a non-linear model $y^{-1} = a + bx^{1.5}$ for adult progeny production data as a function of exposure to silica powders 1 and 2. Model comparisons were conducted among exposure periods or between powders, with the *F*-test utilized to identify any individual

model deviating from the pooled data (Draper and Smith, 1998). All analyses were performed using SAS 9.4 software (SAS Institute, 2012), and graphs were generated using SigmaPlot 12.5 software (Systat, Software, Inc., San Jose, California, USA).

6.4 Results

6.4.1 Mortality responses of *O. surinamensis* adults to silica powders

The mortality (mean $\pm$ SE) of *O. surinamensis* adults in untreated controls varied from 6 ± 4 to $16 \pm 6.8\%$. The corrected mortality (mean $\pm$ SE) of *O. surinamensis* adults exposed for 12 to 48 h to 0.1 to 4.0 g/m² of silica powder 1 varied from 0 to 100%. Complete mortality of *O. surinamensis* adults was not achieved at any application rates of silica powder 1 exposed for 12 h. Minimum application rates required for 100% mortality of *O. surinamensis* exposed for 24, 36, and 48 h to silica powder 1 were 3.0, 3.5 and 2.5 g/m², respectively. The corrected mortality (mean $\pm$ SE) of *O. surinamensis* adults exposed for 12 to 48 h to 0.1 to 4.0 g/m² of silica powder 2 varied from 10 ± 7.7 to 100%. The minimum application rates required for complete mortality of *O. surinamensis* when adults were exposed for 12, 24, 36, and 48 h to silica powder 2 were 4.0, 3.0, 1.5, and 1.0 g/m², respectively (Table 6.1). The non-linear model fitted for mortality data of *O. surinamensis* exposed to varying rates of silica powder 1 and 2 exhibited a non-linear trend. The models fitted to adult mortality data elucidated the correlation between *O. surinamensis* mortality and the application rates of silica powder 1 ($r^2_{\text{range}} = 0.84 - 0.94$) (Fig. 6.1) and silica powder 2 ($r^2_{\text{range}} = 0.82 - 0.96$) (Fig. 6.2). The model comparison showed that the mortality response of *O. surinamensis* adults was not significantly difference for silica powder 1 at 36 and 48 h ($F = 1.09$, $df = 2, 24$; $P = 0.3535$). Significant differences ($P < 0.05$) were observed in all remaining pairwise comparisons (Table 6.2).

The mortality (mean $\pm$ SE) of *O. surinamensis* adults exposed to 3.5 g/m² of silica powder 1 varied from 14 $\pm$ 5.1 to 100%. Complete mortality of *O. surinamensis* adults was achieved after exposure for 20-24 h to 3.5 g/m² of silica powder 1. The mortality (mean $\pm$ SE) of *O. surinamensis* adults exposed to 3.5 g/m² of silica powder 2 varied from 18 $\pm$ 5.8 to 100%. Complete mortality of *O. surinamensis* adults was achieved after exposure for 18-24 h to 3.5 g/m² of silica powder 2. The non-linear model fitted for mortality data of *O. surinamensis* exposed for 24 h to 3.5 g/m² of silica powder 1 and 2 exhibited a non-linear trend. The models fitted to adult mortality data elucidated the correlation between adult mortality and exposure times to silica powder 1 ($r^2 = 0.96$) and silica powder 2 ($r^2 = 0.95$) (Fig 6.3). Comparison of non-linear models fitted for silica powder 1 was significantly different from silica powder 2 ($F = 7.04$; $df = 2, 24$; $P = 0.0039$), indicating superior efficacy of silica powder 2 over silica powder 1.

6.4.2 Progeny production

The number (mean $\pm$ SE) of adult progeny produced in untreated controls varied from 87.2 $\pm$ 13.8 to 111.0 $\pm$ 7.6%. The number (mean $\pm$ SE) of adult progeny produced when *O. surinamensis* adults were exposed for 12 to 48 h to 0.1 to 4.0 g/m² of silica powder 1 varied from 0 to 76.6 $\pm$ 4.2. Complete suppression of adult progeny production was not observed when *O. surinamensis* adults were exposed to 0.1 to 4 g/m² of silica powder 1 exposed for 12 h. Minimum application rates required for complete reduction of adult progeny production of *O. surinamensis* exposed for 24, 36, and 48 h to silica powder 1 were 3.0, 3.5 and 2.0 g/m², respectively. The number (mean $\pm$ SE) of adult progeny produced when adults of *O. surinamensis* were exposed for 12 to 48 h to 0.1 to 4.0 g/m² of silica powder 2 varied from 0 to 47.0 $\pm$ 6.1. The minimum application rates required for complete reduction of adult progeny production *O. surinamensis*

when adults were exposed for 12, 24, 36, and 48 h to silica powder 2 were 3.5, 3.0, 1.5, and 1.0 g/m², respectively (Table 6.3). The non-linear model fitted for progeny production data of *O. surinamensis* exposed to varying rates of silica powder 1 and 2 exhibited a non-linear trend. The models fitted to adult progeny production data elucidated a correlation between adult progeny production data and the application rates of silica powder 1 ($r^2_{\text{range}} = 0.98 - 0.99$) (Fig. 6.4) and silica powder 2 ($r^2_{\text{range}} = 0.93 - 0.99$) (Fig. 6.5). The model comparison showed that the adult progeny production data of *O. surinamensis* adults was not significantly different for silica powder 2 at 24 and 36 h ($F = 3.07$, $df = 2, 24$; $P = 0.0648$). Significant differences ($P < 0.05$) were observed in all remaining pairwise comparisons (Table 6.4).

The number (mean $\pm$ SE) of adult progeny produced in untreated controls varied from 82.4 ± 7.6 to 90.0 ± 11.1 . The number (mean $\pm$ SE) of adult progeny produced in when adults of *O. surinamensis* were exposed to 3.5 g/m² of silica powder 1 varied from 0 to 43.4 ± 6.7 . Complete suppression of adult progeny production was achieved when adults were exposed for 20-24 h to 3.5 g/m² of silica powder 1. The number (mean $\pm$ SE) of adult progeny produced in when adults of *O. surinamensis* were exposed to 3.5 g/m² of silica powder 2 varied from 0 to 34.8 ± 5.1 . Complete suppression of adult progeny production was achieved when adults were exposed for 18-24 h to 3.5 g/m² of silica powder 2. The non-linear model fitted for mortality data of *O. surinamensis* exposed for 24 h to 3.5 g/m² of silica powder 1 and 2 exhibited a non-linear trend. The models fitted to adult progeny production data elucidated the correlation between adult progeny production and exposure times to silica powder 1 ($r^2 = 0.99$) and silica powder 2 ($r^2 = 0.98$) (Fig 6.6). Comparison of non-linear models fitted for silica powder 1 was significantly different from silica powder 2 ($F = 9.87$; $df = 2, 24$; $P = 0.0007$), indicating superior efficacy of silica powder 2 over silica powder 1.

6.5 Discussion

Diatomaceous earth formulations are effective against a wide range of stored product insects (Subramanyam and Roesli, 2000; Cook and Armitage, 2002; Collins, 2003; Kavallieratos et al., 2007; Athanaisou et al., 2009; Athanaisou et al., 2011; Işıkber et al., 2016; Erturk et al., 2017; Erturk et al., 2020; Balliota and Athanaisou, 2023, Manivannan and Subramanyam, 2023a,b). The effectiveness of these formulations against stored products insects is dependent upon the mobility of the insect species (Malia et al., 2016) and the application rate (Le Patourel et al., 1989). In addition, insects such as the *R. dominica* and *O. surinamensis* with more body hairs can pick up more particles easily compared to smooth bodied insects such as the *T. confusum* (Quales, 1992). *O. surinamensis* and *C. ferrugineus* have been reported to be most susceptible to diatomaceous earth formulations in structural treatments compared to *T. castaneum* (Liu, 2005). Moreover, *O. surinamensis* are relatively smaller insect species and can easily succumb to dehydration effects caused by diatomaceous earth activity (Korunic, 1998).

The study findings reveal that silica powder 2 demonstrated higher efficacy against *O. surinamensis* at lower application rates with a minimal exposure time of 12 h. The application rates needed for complete mortality of *O. surinamensis* adults decreased as exposure times increased. After 36 h of exposure to silica powder 1, the minimum application rates for complete mortality were higher compared to 24 h, indicating adult survival at the highest test application rate of 4.0 g/m². However, the observed mortality at the highest tested application rate of 4.0 g/m² was 94 ± 6%, signifying that very few adults survived at this rate. Exposure for 12 h to 4.0 g/m² of silica powder 2 effectively mitigated *O. surinamensis* adults. Li (2018) reported that 3 g/m² of synthetic amorphous silica (SAS) exposed for 36 h was effective in achieving complete mortality of *O. surinamensis* during the structural treatment of empty floor bins. He also

observed that exposure of five species of stored product insects, including *R. dominica*, the maize weevil, *Sitophilus zeamais* Motschulsky, *T. castaneum*, and the flat grain beetle, *Cryptolestes ferrugineus*, and *O. surinamensis* to surfaces treated with 3 g/m² of food-grade inert dust exhibited complete mortality of adults of all species after 72 h. In previous experiments with these silica powders against *R. dominica*, 24-h exposure to concrete arenas treated with 1.5 and 0.25 g/m² of silica powder 1 and 2, respectively, resulted in complete mortality of adults (Manivannan and Subramanyam, 2023a). Silica powder 1 induced complete mortality of *T. castaneum* exposure for 36 h to 1.5 g/m², while silica powder 2 achieved these effects after 48 h of exposure to a 0.75 g/m² application rate (Manivannan and Subramanyam, 2023b). These variable results highlight the species-specific insecticidal activity of the two amorphous silica powders, 1 and 2, against stored product insects.

The decrease in adult progeny production exhibited a non-linear pattern with increasing application rates of silica powders 1 and 2. Adults exposed to silica powder 2 produced a notably lower number of progeny compared to those exposed to silica powder 1. Complete reduction of adult progeny occurred when *O. surinamensis* adults were subjected to 3.5 g/m² of silica powder 2 for 12 h. In contrast, complete mortality was not achieved at any tested application rate of silica powder 1, indicating that *O. surinamensis* adults were more susceptible to silica powder 2 than to silica powder 1. Earlier studies with these two silica powders on other stored product insect species showed that the minimum application rates required for complete suppression of adult progeny production was achieved after exposure for 24 h to 1.5 and 0.25 g/m² of silica powder 1 and 2, respectively, against *R. dominica* (Manivannan and Subramanyam, 2023a), and after exposure of *T. castaneum* adults for 36 and 48 h to 1.5 g/m² of silica powder 1 and 0.75 g/m² of silica powder 2, respectively (Manivannan and Subramanyam, 2023b).

In addition, treatment of *O. surinamensis* to inert dusts have shown to decrease the locomotion speed and escaping behavior of the treated adults (Wang, 2008). As a more agile species, they possess the capability to evade treated surfaces with chemical insecticides. However, the decreased speed of adults after exposure to inert dusts can increase their vulnerability, leading to death by desiccation. The efficacy of diatomaceous earth formulations including silica-based powders are based on their particle size, oil adsorption capability, and pH value (< 8.5) (Korunić, 1998; Korunić, 2013; Liška et al., 2017). Hence, the insecticidal activity of the silica powders against *O. surinamensis* were linked with their physical properties and elemental composition. The silicon dioxide contents in both the powders were more than 94% in its total composition (Manivannan and Subramanyam, 2023a), suggesting that both the powders are expected to exhibit better responses on *O. surinamensis* adults. Another important property of the powder that can be linked to their efficacy is the oil adsorption capability. Filipović et al. (2010) indicated an increase in oil adsorption capability of the silica powders with an increase in their silicon dioxide contents. Considering the silicon dioxide contents of both the powders (silica powder 1 with 94% and silica powder 2 with 97.3%) no notable difference was observed. However, the oil adsorption capability of silica powder 2 was 386% compared to silica powder 1 with 241% (Manivannan and Subramanyam, 2023a). This suggests that the oil adsorption capability of silica powders may not be solely influenced by their silicon dioxide content. In addition, the pH value of the powders have also reported to influence the insecticidal efficacy of the powders (Korunić, 1998). Silica powder 2 had a pH value of 7.6 which is lower than the desirable value < 8.5 to exhibit better responses on the adults. The insecticidal activity of powders is known to be influenced by particle size (Alexander et al., 1944; Athanaisou et al., 2004; Athanaisou et al., 2016; Kavalliaertos et al., 2010; Rumbos et al., 2016). Although silica

powder 1 had significantly smaller particle diameter than silica powder 2, these differences did not impact the effectiveness of these powders on *O. surinamensis*.

The findings of this study reveal that both powders exhibit effectiveness at application rates of 3.0 g/m² or higher, when exposed for 24 h, leading to complete mortality and inhibition of adult progeny production in *O. surinamensis*. Notably, silica powder 2 consistently demonstrated superior efficacy compared to silica powder 1. These results suggest that both silica powders have significant potential for treating empty floor bins before storing new grains, effectively reducing *O. surinamensis* populations. However, further investigations into their long-term stability under diverse temperature and humidity conditions are warranted.

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Table 6.1. Minimum application rates (g/m²) required for 100% mortality of *O. surinamensis* adults exposed for different time periods to two amorphous silica powders treated concrete arenas.

Silica powder	Application rate (g/m ²)			
	12 h	24 h	36 h	48 h
Silica 1	— ^a	3.0	3.5	2.5
Silica 2	4.0	3.0	1.5	1.0

—^a Complete mortality was not achieved at any application rate.

Table 6.2. Pairwise comparison of non-linear models fit to mortality data of *O. surinamensis* adults exposed for different time periods to silica powders 1 and 2.

Powder	Exposure times compared	<i>F</i> - value	df	<i>P</i> -value
Silica 1	12 h vs 24 h	11.24	2, 24	0.0004*
	12 h vs 36 h	12.81	2, 24	0.0002*
	12 h vs 48 h	25.05	2, 24	< 0.0001*
	24 h vs 36 h	5.17	2, 24	0.0135*
	24 h vs 48 h	6.38	2, 24	0.0060*
	36 h vs 48 h	1.09	2, 24	0.3535
Silica 2	12 h vs 24 h	13.06	2, 24	0.0002*
	12 h vs 36 h	4.90	2, 24	0.0164*
	12 h vs 48 h	8.90	2, 24	0.0020*
	24 h vs 36 h	10.50	2, 24	0.0005*
	24 h vs 48 h	1.50	2, 24	0.2445*
	36 h vs 48 h	5.30	2, 24	0.0124*

* Significant ($P < 0.05$)

Table 6.3. Minimum application rates (g/m²) required for complete reduction of adult progeny production of *O. surinamensis* exposed for different time periods to two amorphous silica powders treated concrete arenas.

Silica powder	Application rate (g/m ²)			
	12 h	24 h	36 h	48 h
Silica 1	— ^a	3.0	3.5	2.0
Silica 2	3.5	3.0	1.5	1.0

—^a Complete reduction in adult progeny not achieved at any application rate.

Table 6.4. Pairwise comparison of non-linear models fit to 42 days adult progeny production data of *O. surinamensis* as a function of application rate when exposed to silica powders 1 and 2 for different time periods.

Powder	Exposure times compared	<i>F</i> - value	df	<i>P</i> -value
Silica 1	12 h vs 24 h	120.31	2, 24	<0.0001*
	12 h vs 36 h	194.24	2, 24	<0.0001*
	12 h vs 48 h	286.27	2, 24	<0.0001*
	24 h vs 36 h	28.03	2, 24	<0.0001*
	24 h vs 48 h	96.59	2, 24	<0.0001*
	36 h vs 48 h	33.72	2, 24	<0.0001*
Silica 2	12 h vs 24 h	0.83	2, 24	0.4478
	12 h vs 36 h	12.81	2, 24	0.0002*
	12 h vs 48 h	17.71	2, 24	0.0001*
	24 h vs 36 h	3.07	2, 24	0.0648
	24 h vs 48 h	4.04	2, 24	0.0307*
	36 h vs 48 h	11.43	2, 24	0.0003*

* Significant ($P < 0.05$)

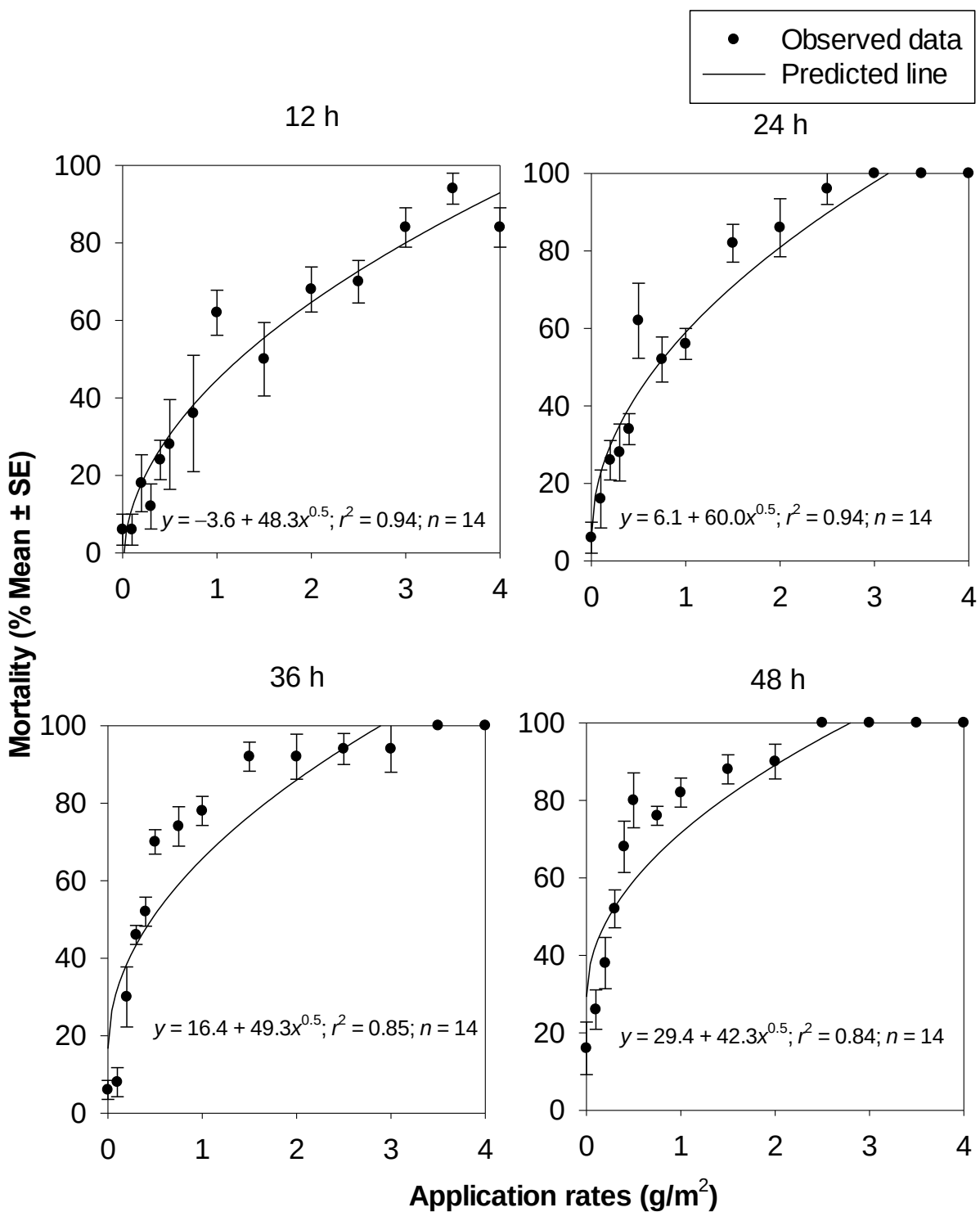


Figure 6.1. Percentage mortality of *O. surinamensis* adults at 14 d after exposure to concrete arenas treated with silica powder 1 for different application rates and exposure times.

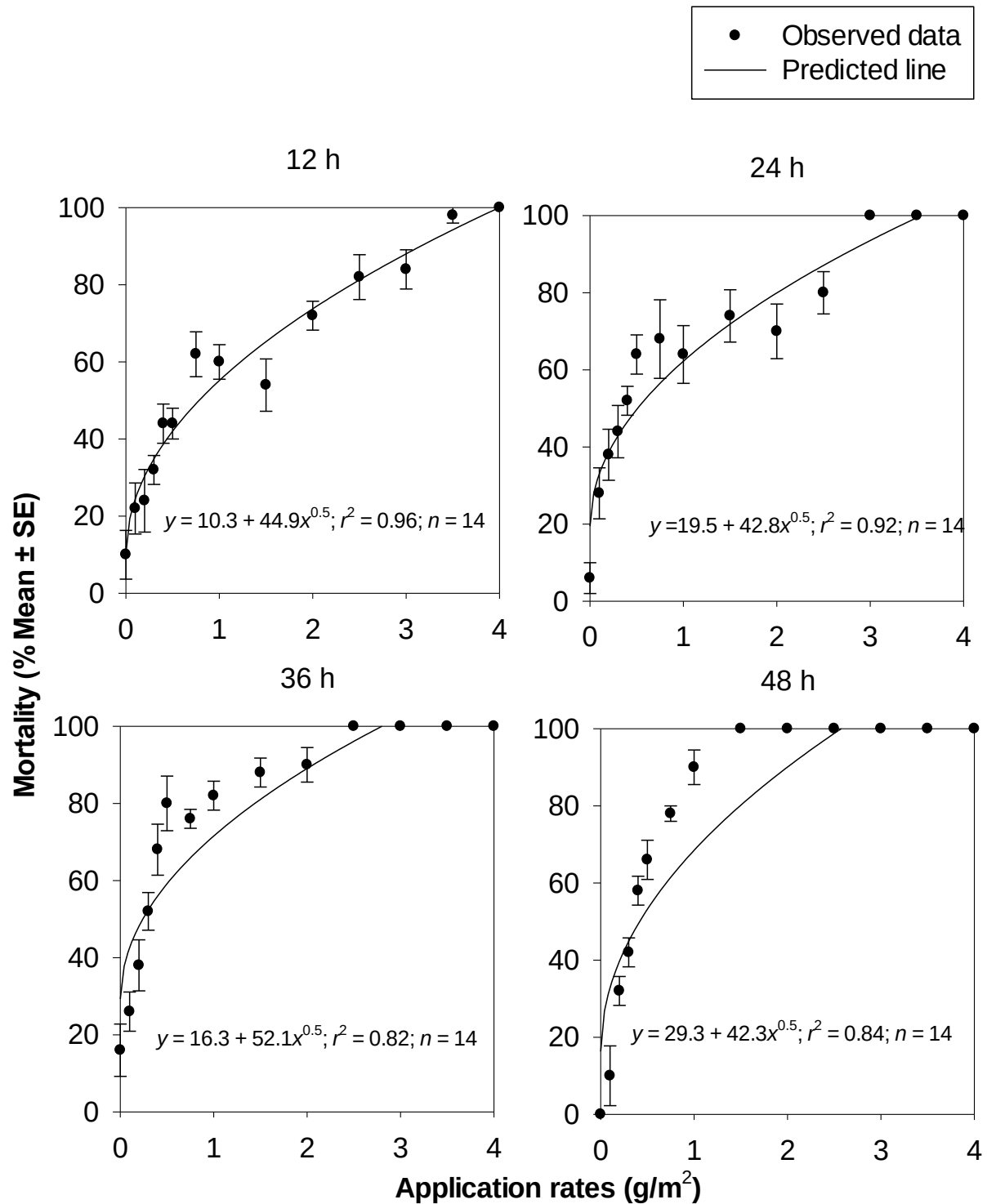


Figure 6.2. Percentage mortality of *O. surinamensis* adults at 14 d after exposure to concrete arenas treated with silica powder 2 for different application rates and exposure times.

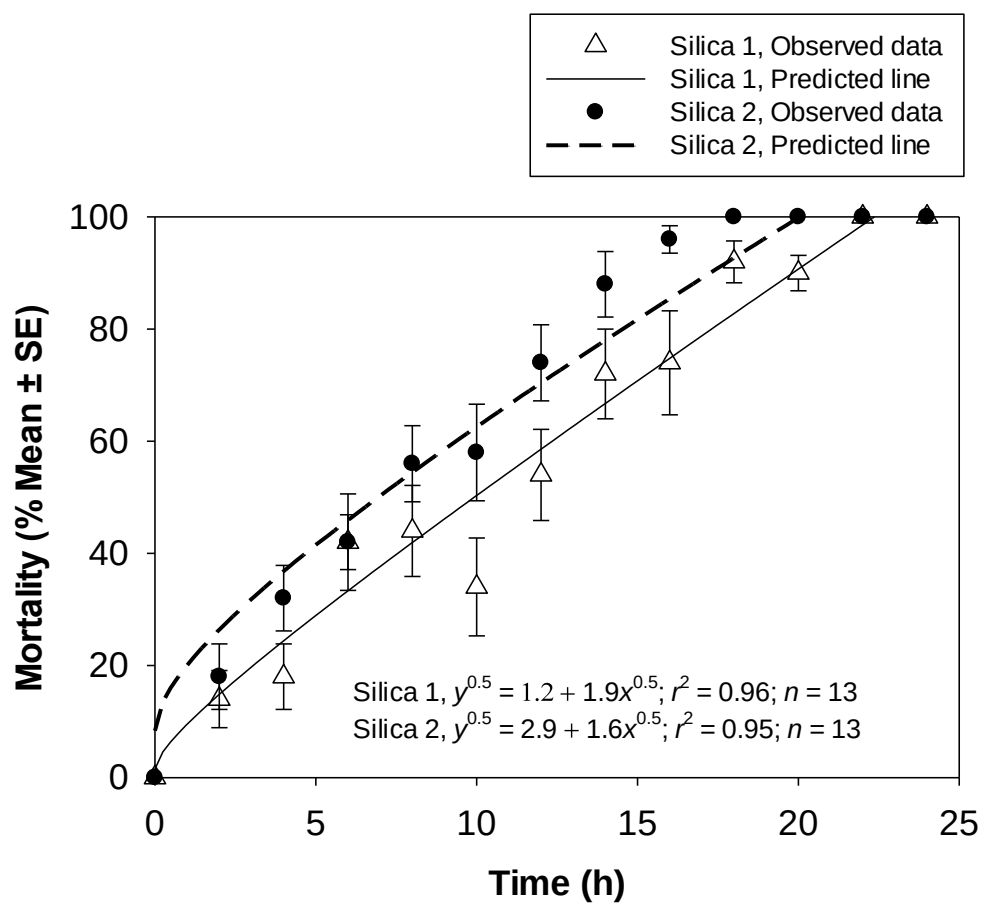


Figure 6.3. Percentage mortality of *O. surinamensis* adults at 14 d after exposure to concrete arenas treated with 3.5 g/m² of silica powder 1 and 2 for various exposure times.

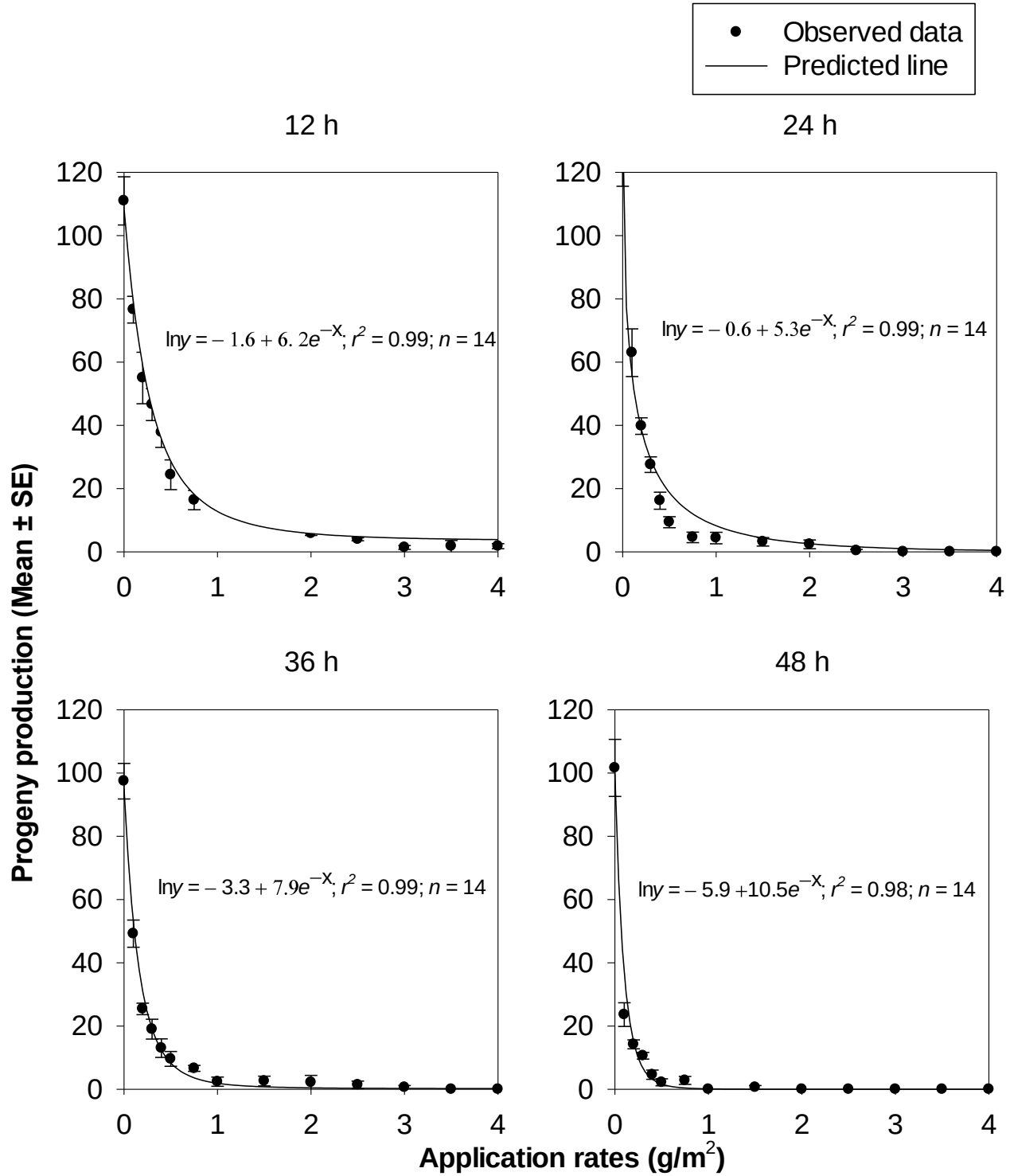


Figure 6.4. Adult progeny production from *O.surinamensis* adults at 42 d after exposure to concrete arenas treated with silica powder 1 for different application rates and exposure times.

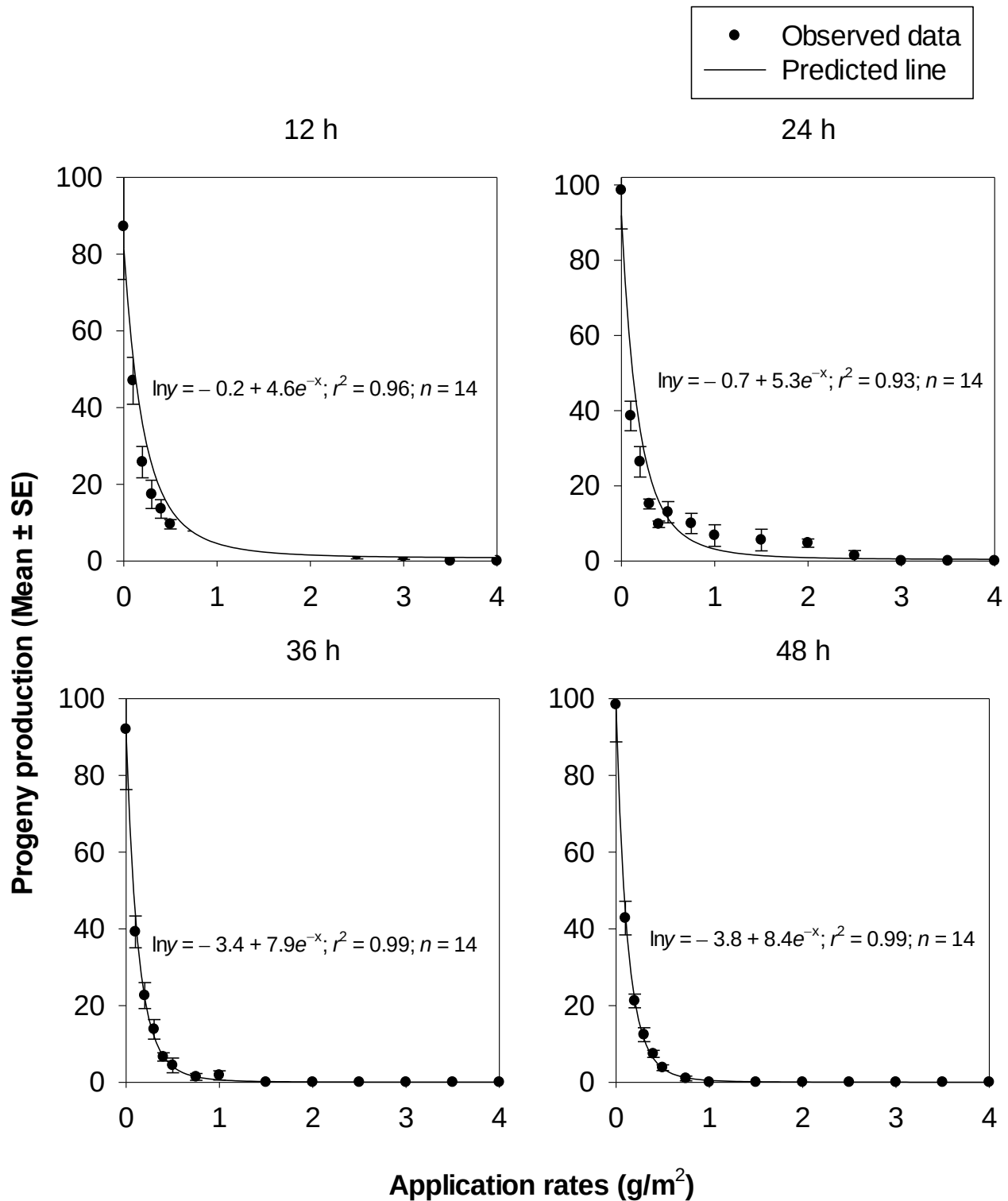


Figure 6.5. Adult progeny production from *O. surinamensis* adults at 42 d after exposure to concrete arenas treated with silica powder 2 for different application rates and exposure times.

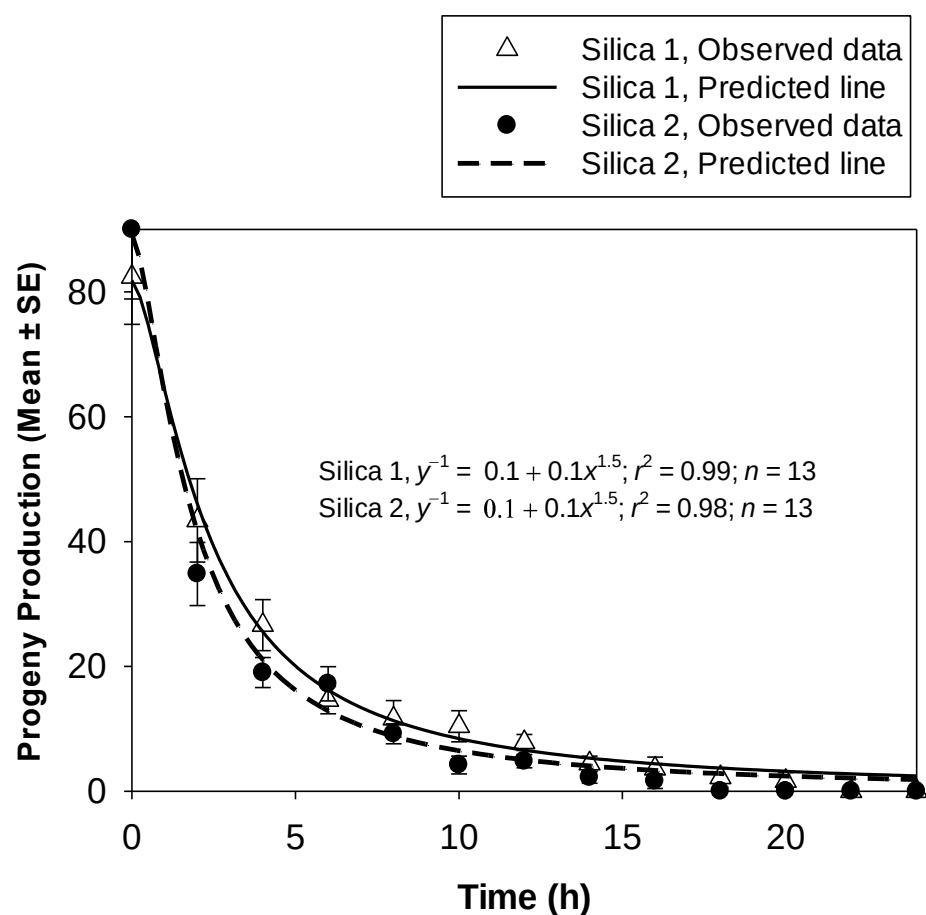


Figure 6.6. Adult progeny production from *O. surinamensis* adults at 42 d after exposure to concrete arenas treated with 3.5 g/m² of silica powder 1 and silica powder 2 for various exposure times.

Chapter 7 - Efficacy of two amorphous silica powders applied to soft red winter wheat against the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

7.1 Abstract

The increased demand for residue-free grains and development of insect resistance to insecticides has led to a surge in the utilization of diatomaceous earths as grain protectants. In this study, the efficacy of two different amorphous silica powders applied to soft red winter wheat was tested against the lesser grain borer, *Rhyzopertha dominica* (F.). Twenty adults of *R. dominica* were exposed to 100 g of organic soft red winter wheat admixed with varying application rates (0 for the untreated control, 0.05, 0.1, 0.15, and 0.20 g/kg) of silica powder 1 and 2. Mortality assessments were made after 14 d of exposure, and data on adult progeny production, insect-damaged kernels, and grain weight loss at 42 d. Exposing *R. dominica* adults to wheat treated with 0.20 g/kg of silica powder 1 resulted in $94.5 \pm 5.5\%$ mortality and 1.3 ± 1.3 adult progeny production. Insect-damaged kernels and grain weight loss caused by *R. dominica* exposed to 0.20 g/kg of silica powder 1 were 0.19 ± 0.19 and $0.1 \pm 0.1\%$, respectively. Exposure of *R. dominica* adults to wheat admixed with 0.20 g/kg of silica powder 2 resulted in complete mortality, with no observed adult progeny production, insect-damaged kernels, or grain weight loss. Overall, silica powder 2 exhibited greater efficacy than silica powder 1, and this superior performance can be attributed to its higher oil absorption capacity and low pH (< 8.5) value.

Keywords: Amorphous silica powders; Soft red winter wheat; Insect-damage kernels; Grain weight loss; Efficacy assessment.

7.2 Introduction

Inert dusts such as the diatomaceous earths are eco-friendly alternatives to traditional chemical grain protectants (Collins and Cook, 2006; Athanassiou and Korunić, 2007). Diatomaceous earth formulations are derived from the fossils of phytoplanktons or diatoms (Korunić et al., 1998; Subramanyam and Roesli, 2000; Kavallieratos, et al., 2010; Losic and Kornunić, 2018). They exhibit low toxicity to mammals (Shawir et al., 1988; Korunić et al., 1998; Subramanyam and Roesli, 2000), offer long-term stability (Korunić, 1999; Subramanyam and Roesli, 2000; Losic and Korunić, 2018) and do not leave harmful residues on treated commodities (Collins and Cook, 2006; Shah and Khan, 2014; Korunić et al., 2017; Paponja et al., 2020). Furthermore, diatomaceous earths demonstrate efficacy against insect species that are resistant to chemical insecticides (Collins and Cook, 2006). Many studies have reported diatomaceous earths as effective grain protectants against a variety of stored product insects (Subramanyam, et al., 1994; Fields and Korunic, 2000; Saez and Mora, 2007; Athanassiou and Korunić, 2007; Vardeman et al., 2007; Kljajić et al., 2010; Sadeghi et al., 2012; Perišić et al., 2018; Astuti, et al., 2019; Paponja et al., 2020; Agrafioti et al., 2023). The suggested mechanisms of action of diatomaceous earths on insect pests encompass diverse modes of action including, dehydration-induced surface enlargement of the insect integument (Zacher and Kunicke, 1931), damage to the digestive tract (Smith, 1969), and the blocking of spiracles and trachea (Webb, 1945), and adsorption of epicuticular waxes, leading to death by desiccation (Ebeling, 1971; Korunic et al., 1998; Subramanyam and Roesli, 2000).

Diatomaceous earth formulations have been reported to exhibit variation in their insecticidal activity based on the differences in the application rate, exposure time, treatment conditions, type of grain, and insect species and their life stages (Vayias and Athanassiou, 2004;

Athanassiou et al., 2006; Kavallieratos et al., 2007; Zeni et al., 2021). For instance, Agrafioti et al. (2023) studied the efficacy of Celatom[®] MN-23, Silicid, and SilicoSec[®] applied to soft wheat at application rates of 0.1 to 1.0 g/kg against seven stored product insect species exposed for 3, 7, 14, and 21 d. Results from their study revealed variability in the efficacy of diatomaceous earth, influenced by both the insect species and specific diatomaceous earth formulation used. Notably, Silicid demonstrated high effectiveness against all tested insect species when applied at a rate of 1.0 g/kg when exposed for 21 d, resulting in 100% adult mortality. However, SilicoSec[®] was observed to be the least effective among powders against the lesser grain borer, *Rhyzopertha dominica* (F.), red flour beetle, *Tribolium castaneum* (Herbst), and confused flour beetle, *Tribolium confusum* Jacquelin du Val. It exhibited better efficacy on saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), when adults were exposed to wheat treated at an application rate of 1.0 g/kg for 21 d. In other study, Kljajić et al. (2010) evaluated the efficacy of Protect-It[™] applied to wheat at application rate of 0.15, 0.20 and 0.30 g/kg against the rice weevil, *Sitophilus oryzae* (L.), *R. dominica*, and *T. castaneum*, respectively. They noted that complete mortality of *S. oryzae* and *T. castaneum* adults could be attained at their respective application rates after a 14 d exposure. However, in the case of *R. dominica*, 100% mortality was not achieved even after exposing the adults to wheat treated with 0.20 g/kg of Protect-It[™] for 21 d. These findings underscore differences in species and rate specific activity of diatomaceous earth formulations against stored product insects.

The efficacy of diatomaceous earth powders against insect pests is known to be influenced by their physical properties (pH, particle size distribution, lipid adsorption capability, moisture content of the powders, silicon dioxide content, and the diameter of inner pores of particles) of the formulations (Korunić, 1997; Fields and Korunić, 2000). A recent study by

Manivannan and Subramanyam (2023a) demonstrated the influence of pH, silicon dioxide content, and lipid adsorption capabilities of two amorphous silica powders in exhibiting variable efficacy against *R. dominica* adults when exposed to silica powder treated concrete arenas. Amorphous silica dusts are considered more beneficial for preservation of stored products from insect pests, given their classification as "unlimited" active daily intake (ADI) (Anonymous, 1974). Additionally, they find use as anti-caking agents in various commodities at concentrations less than 1.5% w/w (Anonymous, 1986; Shawir et al., 1988). In this context, the insecticidal efficacy of the two candidate amorphous silica powders with different elemental composition and physical properties were evaluated by exposing adults of *R. dominica* to soft red winter wheat treated at different application rates for 14 d. Additionally, we also determined the impact of these two silica powders on adult progeny production, insect-damaged kernels, and grain weight loss caused by *R. dominica*. This evaluation was aimed to identify which silica powder was effective for the management of *R. dominica*.

7.3 Materials and Methods

7.3.1 Silica powders

Two amorphous silica powders, silica powder 1 and silica powder 2, provided by Imery's Chemicals, Lompoc, California, USA, were evaluated for their efficacy against *R. dominica*. These powders exhibited distinct elemental compositions and variations in size and shape characteristics and were provided in detail elsewhere (Manivannan and Subramanyam, 2023a). The silicon dioxide content in silica powder 1 and silica powder were 94.5 and 97.3%, respectively. Silica powder 1 was primarily characterized by particles with a D₉₀ (Diameter fraction where 90% of particles were within a certain size range) of 26.40 µm, while silica powder 2 had particles with a D₉₀ of 31.43 µm. The pH values were 9.6 for silica powder 1 and

7.2 for silica powder 2. The oil adsorption capability of silica powder 1 and silica powder 2 were 231 and 386%, respectively (Manivannan and Subramanyam, 2023a).

7.3.2 Insect rearing

Cultures of *R. dominica* were established by using adults isolated from insect strains established in the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA. Insect cultures were reared in 0.94 L glass mason jars with organic soft winter wheat (Great River Organic Milling Company, Fountain City, Wisconsin, USA). The jars were secured with lids containing 250 µm wire mesh screens and filter paper (9 cm diameter) lids. The culture jars were placed inside a temperature/humidity chamber at 28°C and 65% r.h. (Pervical Scientific, Inc., Model I-36 VL, Perry, Iowa, USA) with a light:dark photoperiod of 14:10 h.

7.3.3 Treatment of wheat with silica powders and insect exposure

Organic soft red winter wheat was held inside a deep freezer at -13°C for a week to kill any insects if present. After disinfestation, the grains were cleaned by sieving using a 2.38 mm diameter round-holed aluminum sieve (Seedburo Equipment Company, Des Plaines, Illinois, USA) to eliminate damaged kernels and dockage. From this lot, 100 g of cleaned wheat (13% moisture, wb) were transferred to individual 0.45 L glass jars. The glass jars were then secured with 250 µm wire mesh screens and filter paper (7 cm diameter) lids to facilitate air diffusion. The jars with wheat grains were individually treated to silica powder 1 and silica powder 2 concentrations of 0 (untreated control), 0.05, 0.10, 0.15, and 0.20 g/kg. The jars were manually shaken for one minute to ensure uniform mixing of the powders on wheat. Twenty unsexed adults (1-4-week-old) of *R. dominica* from the culture jars were introduced into each glass jar and was covered with wire mesh screens and filter paper lids. Each treatment combination

consisted of five replications. All the jars with wheat treated with silica powders were placed inside a temperature/humidity chamber at 28°C and 65% r.h. Mortality counts were taken after 14 d of exposure to silica powders 1 and 2 by sieving the wheat from each glass jar using 2.38 mm circular round-holed aluminum sieve to isolate the adults. The insects which were immobile when prodded with fine camel's hair brush were regarded as dead. Once mortality assessments were performed, all live and dead adults and wheat were returned to individual glass jars and held inside a temperature humidity chamber at 28°C and 65% r.h for an additional 28 d. At 42 d, adult progeny production was estimated by subtracting twenty parent adults from the overall count. The wheat from each powder-concentration combination replicate were passed through a Boerner® divider (Seedburo Equipment Company, Des Plaines, Illinois, USA) five times to obtain a working sample of ≈ 3.5 g to determine the number of insect-damaged kernels and undamaged kernels. The percentage of grain weight loss caused by *R. dominica* was estimated using the formula provided by Adams and Schulten (1978), Boxall (1986).

7.3.4 Data analysis

The mortality of *R. dominica* adults in untreated controls was $2.7 \pm 2.7\%$ for silica powder 1 and $5.3 \pm 3.5\%$ for silica powder 2. Therefore, mortality in treatments were calculated by correcting responses in the control treatments (Abott, 1925). The data on percentage mortality, insect-damaged kernels, and weight loss was transformed to angular values (Zar, 1984) for analysis using Statistical Analysis System 9.4 (SAS Institute, 2012). Adult progeny counts were transformed to $\log_{10}(x + 1)$ for statistical analysis (Zar, 1984). Two-way analysis of variance (ANOVA) was performed to determine the main effects of silica powders and application rates and their interaction effect. One-way analysis of variance (ANOVA) was carried out to determine their significant ($\alpha = 0.05$) differences between powders and application rates

among means. Means separation was done using Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test (SAS Institute, 2012).

7.4 Results

7.4.1 Responses of *R. dominica* adults exposed to two amorphous silica powders

Complete mortality of *R. dominica* was not attained at any application rate during a 14 d exposure to 0.05–0.20 g/kg of silica powder 1. The mean $\pm$ SE mortality of adults when exposed to silica powder 1 at 0.05–0.20 g/kg for 14 d ranged from 28.0 ± 6.0 to $94.5 \pm 5.5\%$ (Table 7.1). Mortality was 100% when adults were exposed to wheat mixed with 0.20 g/kg of silica powder 2 for 14 d. The mean $\pm$ SE mortality of *R. dominica* adults when exposed to silica powder 2 at 0.05–0.20 g/kg for 14 d ranged from 39.4 ± 6.1 to 100%. Two-way ANOVA showed significant differences in adult mortality responses between powders ($F = 9.47$; $df = 1, 16$; $P = 0.0072$) and among application rates ($F = 53.66$; $df = 4, 16$; $P < 0.0001$). The powder $\times$ application rate interaction ($F = 1.36$; $df = 4, 16$; $P = 0.2908$) was not significant. One-way ANOVA revealed significant differences in adult mortality response among application rates of silica powder 1 ($F = 18.44$; $df = 3, 8$; $P = 0.0006$) and silica powder 2 ($F = 39.10$; $df = 3, 8$; $P < 0.0001$). No significant difference ($P > 0.05$) in mortality response was observed between silica powder 1 and 2 at application rates of 0.05–0.20 g/kg, except at 0.15 g/kg which was significant (Table 7.1).

7.4.2 Adult progeny production

The mean $\pm$ SE adult progeny produced in controls at 42 d ranged from 98.0 ± 2.9 to 110.3 ± 7.8 . The mean $\pm$ SE number of adult progeny produced after exposure to 0.05–0.20 g/kg of silica powder 1 for 14 d ranged from 1.3 ± 1.3 to 63.7 ± 5.2 (Table 7.2). Adult progeny production was not observed to be completely suppressed at any application rate when adults were exposed to 0.05–0.20 g/kg of silica powder 1 for 14 d. The mean $\pm$ SE number of *R.*

dominica adult progeny produced after exposure to 0.05–0.20 g/kg of silica powder 2 ranged from 0 to 35.3 ± 3.0 . Adult progeny production was found to be completely inhibited when adults were exposed to 0.20 g/kg of silica powder 2 for 14 d. Two-way ANOVA showed significant differences in adult progeny production data between powders ($F = 51.12$; $df = 1, 20$; $P < 0.0001$) and among application rates ($F = 132.70$; $df = 4, 20$; $P < 0.0001$). The powder x application rate interaction ($F = 15.49$; $df = 4, 20$; $P < 0.0001$) was also significant. One-way ANOVA showed that the adult progeny production data among application rates of silica powder 1 ($F = 42.04$; $df = 4, 10$; $P < 0.0001$) and silica powder 2 ($F = 145.61$; $df = 4, 10$; $P < 0.0001$) were significantly different. The two-sample *t*-test showed significant differences ($P < 0.05$) in adult progeny production response between silica powders 1 and 2 at application rates of 0.05 - 0.15 g/kg (Table 7.2).

7.4.3 Insect-damaged kernels

The mean $\pm$ SE percent insect-damaged kernels in controls at 42 d ranged from 15.0 ± 1.2 to $16.3 \pm 2.2\%$. The mean $\pm$ SE percent insect-damaged kernels caused by *R. dominica* adults after exposure to 0.05–0.20 g/kg of silica powder 1 for 14 d ranged from 0 to $8.9 \pm 0.8\%$ (Table 7.3). Complete inhibition of insect-damaged kernels was not observed at any of the application rates of silica powder 1 after 14 d. The mean $\pm$ SE percent insect-damaged kernels caused by *R. dominica* adults after exposure to 0.05–0.20 g/kg of silica powder 2 after 14 d ranged from 0 to $4.2 \pm 0.6\%$. No insect-damaged kernels were observed when adults were exposed to 0.20 g/kg of silica powder 2 after 14 d. Two-way ANOVA showed significant differences in percentage insect-damaged kernels between powders ($F = 32.15$; $df = 1, 20$; $P < 0.0001$) and among application rates ($F = 147.27$; $df = 4, 20$; $P < 0.0001$). Powder x application rate interaction ($F = 5.22$; $df = 4, 20$; $P = 0.0048$) was also significant. One-way ANOVA showed significant

differences in percent insect-damaged kernels caused by *R. dominica* among application rates of silica powder 1 ($F = 79.99$; $df = 4, 10$; $P < 0.0001$) and silica powder 2 ($F = 73.68$; $df = 4, 10$; $P < 0.0001$). The two-sample *t*-tests showed significant differences ($P < 0.05$) in percent insect-damaged kernels between silica powder 1 and 2 at application rates of 0.05–0.15 g/kg (Table 7.3).

7.4.4 Weight loss

The mean $\pm$ SE percent weight loss in controls at 42 d ranged from 16.8 ± 1.7 to $19.2 \pm 3.0\%$. The mean $\pm$ SE percent weight loss caused by *R. dominica* adults after exposure to 0.05–0.20 g/kg of silica powder 1 for 14 d ranged from 0.1 ± 0.1 to 9.3 ± 1.0 (Table 7.4). Complete inhibition of grain weight loss was not observed at any of the application rates of silica powder 1 after 14 d. The mean $\pm$ SE percent weight loss caused by *R. dominica* adults after exposure to 0.05–0.20 g/kg of silica powder 2 after 14 d ranged from 0 to 3.0 ± 1.4 . No weight loss was observed when adults were exposed to 0.15 and 0.20 g/kg of silica powder 2 after 14 d. Two-way ANOVA showed significant differences in percentage grain weight loss between powders ($F = 15.79$; $df = 1, 16$; $P = 0.0009$) and among application rates ($F = 144.61$; $df = 4, 16$; $P < 0.0001$). Powder $\times$ application rate interaction ($F = 5.24$; $df = 4, 16$; $P = 0.0056$) was also significant. One-way ANOVA showed significant differences in percent weight loss caused by *R. dominica* among application rates of silica powder 1 ($F = 77.26$; $df = 4, 10$; $P < 0.0001$) and silica powder 2 ($F = 55.49$; $df = 4, 10$; $P < 0.0001$). The two-sample *t*-tests showed significant differences ($P < 0.05$) in percent grain weight loss between silica powder 1 and 2 at application rates of 0.05–0.15 g/kg (Table 7.4).

7.5 Discussion

Numerous researchers have investigated the efficacy diatomaceous earth formulations admixed with grains against *R. dominica* (Fields and Korunić, 2000; Athanassiou and Korunic, 2007; Chanbang et al., 2007; Vardeman et al., 2007; Kavallieratos et al., 2010; Kljajić et al., 2010; Sadeghi et al., 2012; Stadler et al., 2012; Perišić et al., 2018; Astuti et al., 2019; Paponja et al., 2020; Agrafioti et al., 2023). The insects that were exposed to the two amorphous silica powders displayed total brittleness, indicating the extent of water loss from their bodies caused by the action of the powders. This validation establishes that the primary mechanism leading to the mortality of insects exposed to silica powders 1 and 2 was the adsorption of epicuticular waxes, resulting in death through desiccation (Ebeling, 1971).

Typically, many diatomaceous earth products demonstrate effectiveness on stored product insects at higher application rates. The current recommended application rates of diatomaceous earth formulations when mixed with grains typically range between 0.5 to 3.5 g/kg (Korunić, 1997; Korunić et al., 1998). The treatment of grains at this rate can adversely affect the physical and mechanical properties of treated grains (Korunić, 1997; Korunić et al., 1998; Shah and Khan, 2014). We observed complete mortality of *R. dominica* adults when exposed to wheat grains treated at 0.20 g/kg of silica powder 2 for 14 d, which is lower than the present recommended rate. Additionally, we observed about 95% mortality of adults exposed to 0.20 g/kg of silica powder 1 over the same 14 d exposure period. In a study, Perišić et al. (2018) observed that *R. dominica* adults exposed to wheat mixed with 1.5 g/kg of two diatomaceous earths, DE-S1 and DE-S2 from Serbia and Protect-It™, for 14 d had mortalities of 92, 76.5 and 97%, respectively). However, this application rate is higher than those noticed in the present observation, the mortality rates of *R. dominica* was comparable with only silica powder 1 which showed 95%

mortality when adults were exposed at an application rate of 0.20 g/kg. While Kavallieratos et al. (2010) reported complete mortality of *R. dominica* when adults were exposed to wheat (Athos and Sifnos varieties) treated with 0.5 g/kg of Protector, SilicoSec[®], and Insecto[®] for 14 d. Complete suppression of adult progeny production was not observed at any of the application rates of silica powder 1. On the other hand, progeny production was completely inhibited when adults were exposed to wheat mixed with 0.20 g/kg of silica powder 2 for 14 d. This indicated that silica powder 2 was more effective compared to silica powder 1. A similar observation was reported by Kavallieratos et al. (2010) who reported complete suppression of adult progeny production of *R. dominica* when adults were exposed to wheat (Athos and Sifnos varieties) mixed with 0.25 g/kg of Protector, SilicoSec[®] or Insecto[®]. Notably, Perišić et al. (2018) did not report complete inhibition of adult progeny production, even when adults were exposed to wheat mixed with 1.5 g/kg of DE-S1, DE-S2, or Protect-It[™] exposed for 14 d. The effectiveness of diatomaceous earth powders against stored product insects relies on factors such as silicon dioxide content, pH, particle size distribution, and oil adsorption capability (Korunić, 1997; Fields and Korunić, 2000). Since the silica dioxide content of both the powders were > 94% (Manivannan and Subramanyam, 2023a), both the powders were anticipated to perform better at lower application rates, with silica powder 2 outperforming silica powder 1 with 97.3% silicon dioxide content. The diatomaceous earth powders used by Perišić et al. (2018) had silicon dioxide contents of 78.8 and 63.2% for DE-S1 and DE-S2, respectively (Andric et al., 2012) and 83.7% for Protect-It[™] (Arnaud et al., 2005). Kavallieratos et al. (2010) reported silicon dioxide contents in Protector, SilicoSec[®], and Insecto[®] to be 66.8% with 10% silica gel, 92%, and 86.7%, respectively (Vayias, et al., 2006). The varied results observed in the responses of *R. dominica* to the tested diatomaceous earth powders in our study and the aforementioned research indicate that

factors beyond silicon dioxide content play a pivotal role in influencing the effectiveness of diatomaceous earth powders on stored product insects. For instance, the favorable pH value of < 8.5 (Korunić, 1998) and the high oil adsorption capacity of silica powder 2 (386%) compared to a pH value of 9.7 and oil adsorption capability 281% could have been attributed to the enhanced activity of silica powder 2 against *R. dominica* (Manivannan and Subramanyam, 2023a). Though the particle size diameters (D_{10} , D_{50} and D_{90}) of silica powder 1 (Manivannan and Subramanyam 2023a) was significantly smaller than silica powder 2, this did not influence mortality responses of *R. dominica* adults. However, this was not true with our earlier test with silica powder 1 and silica powder 2 on *T. castaneum*, where smaller particle diameter of silica powder 1 exhibited better response and reduced progeny production compared to silica powder 2 with larger particle diameter (Manivannan and Subramanyam, 2023a).

Insect-damaged kernels were not completely inhibited when the adults of *R. dominica* were exposed to wheat mixed with 0.05 to 0.20 g/kg of silica powder 1 for 14 d. Similarly, grain weight loss was not completely reduced at any of the application rates of silica powder 1. On the other hand, complete inhibition of insect-damaged kernels and grain weight loss at 42 d was observed after exposing the adults of *R. dominica* to wheat mixed with 0.20 g/kg of silica powder 2 for 14 d. These observations indicated that silica powder 2 was more efficacious than silica powder 1 in reducing the insect-damaged kernels and grain weight loss caused by *R. dominica*. Complete reduction of insect-damaged grains was not achieved after 70 d even when *R. dominica* adults were exposed to 0.5 to 1.5 g/kg of DE-S1, DE-S2, or Protect-It™ after a 21 d exposure period (Perišić et al., 2018).

In conclusion, our study provides valuable insights into the differential efficacy of silica powders 1 and 2 in controlling *R. dominica*. Silica powder 2 consistently demonstrated superior

performance in terms of increased mortality and complete reduction of adult progeny production, insect-damaged kernels, and grain weight loss. The superior efficacy of silica powder 2 over silica powder 1 could be attributed to the high oil adsorption capability and low pH. These findings contribute to a systematic understanding of the potential of amorphous silica powders in pest management strategies, with implications for practical applications in the preservation of stored grains.

7.6 References

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Table 7.1. Effect of two amorphous silica powders on the mortality of *R. dominica* adults exposed for 14 d to wheat treated with different application rates.

Application	Mean $\pm$ SE corrected mortality (%) ^{a,b}			
rate (g/kg)	Silica 1	Silica 2	<i>t</i> – value	<i>P</i> – value
0.05	28.0 $\pm$ 6.0a	39.4 $\pm$ 6.1a	-1.24 (4) ^b	0.2819
0.1	50.7 $\pm$ 6.3ab	56.3 $\pm$ 3.7a	- 0.78 (4) ^b	0.4800
0.15	68.6 $\pm$ 3.6b	93.0 $\pm$ 3.7b	- 3.17 (4) ^b	0.0340*
0.20	94.5 $\pm$ 5.5c	100 $\pm$ 0b	-1.00 (2) ^c	0.4226

^aMeans followed by different letters are significantly different among application rates ($P < 0.05$, by REGWQ test).

^bVariances between silica powder 1 and 2 were equal ($F_{\text{range}} = 1.13 - 2.83$; $df = 2, 2$; $P_{\text{range}} = 0.229 - 0.9384$).

^cVariances between silica powder 1 and 2 were unequal ($F = \infty$; $df = 2, 2$; $P < 0.0001$).

* Significant ($P < 0.05$; two-sample *t*-test).

Table 7.2. Effect of two amorphous silica powders on adult progeny production of *R. dominica*.

Application	Mean $\pm$ SE adult progeny production ^a			
rate (g/kg)	Silica 1	Silica 2	<i>t</i> – value	<i>P</i> – value
0	98.0 $\pm$ 2.9a	110.3 $\pm$ 7.8a	- 1.46 (4) ^b	0. 2170
0.05	63.7 $\pm$ 5.2a	35.3 $\pm$ 3.0b	5.00 (4) ^b	0.0075*
0.1	42.3 $\pm$ 3.8a	18.7 $\pm$ 3.2b	4.61 (4) ^b	0.0100*
0.15	34.3 $\pm$ 2.4a	1.0 $\pm$ 0.6c	9.05 (4) ^b	0.0008*
0.20	1.3 $\pm$ 1.3b	0 $\pm$ 0c	0.20 (2) ^c	0.3729

^aMeans in a column followed by different letters are significantly different among application rates ($P < 0.05$, by REGWQ test).

^bVariances between silica powder 1 and 2 were equal ($F_{\text{range}} = 1.07 - 23.18$; $df = 2, 2$; $P_{\text{range}} = 0.0827 - 0.9651$).

^cVariances between silica powder 1 and 2 were equal ($F_{\text{range}} = \infty$; $df = 2, 2$; $P < 0.0001$).

* Significant ($P < 0.05$; two-sample *t*-test).

Table 7.3. Insect-damaged kernels caused by *R. dominica* at 42 d after exposure to wheat treated with two silica powders at different application rates.

Application	Mean $\pm$ SE insect-damaged kernels (%) ^a			
rate (g/kg)	Silica 1	Silica 2	<i>t</i> – value	<i>P</i> – value
0	15.0 $\pm$ 1.2a	16.3 $\pm$ 2.2a	- 0.52 (4) ^b	0. 6336
0.05	8.9 $\pm$ 0.8b	4.2 $\pm$ 0.6b	4.73 (4) ^b	0.0091*
0.1	5.4 $\pm$ 0.3c	1.8 $\pm$ 0.2b	9.64 (4) ^b	0.0007*
0.15	2.0 $\pm$ 0.4d	0.2 $\pm$ 0.2c	4.06 (4) ^b	0.0154*
0.20	0.19 $\pm$ 0.19e	0 $\pm$ 0c	1.00 (2) ^c	0.3739

^aMeans in a column followed by different letters are significantly different among application rates ($P < 0.05$, by REGWQ test).

^bVariances between silica powder 1 and 2 were equal ($F_{\text{range}} = 1.25 - 10.90$; $df = 2, 2$; $P_{\text{range}} = 0.4380 - 0.9580$).

^cVariances between silica powder 1 and 2 were equal ($F = \infty$; $df = 2, 2$; $P < 0.0001$).

* Significant ($P < 0.05$; two-sample *t*-test).

Table 7.4. Weight loss caused by *R. dominica* at 42 d after exposure to wheat grains treated with two silica powders at different application rates for 14 d.

Application	Mean $\pm$ SE grain weight loss (%) ^{a,b}			
rate (g/kg)	Silica 1	Silica 2	<i>t</i> – value	<i>P</i> – value
0	16.8 $\pm$ 1.7a	19.2 $\pm$ 3.0a	- 0.66 (4) ^c	0. 5462
0.05	9.3 $\pm$ 1.0b	3.0 $\pm$ 1.4b	4.45 (4) ^c	0.0211*
0.1	5.3 $\pm$ 0.4c	1.4 $\pm$ 0.4bc	6.57 (4) ^c	0.0028*
0.15	1.6 $\pm$ 0.4d	0.1 $\pm$ 0.1cd	4.01 (4) ^c	0.0160*
0.20	0.1 $\pm$ 0.1e	0 $\pm$ 0d	0.77 (2) ^c	0.4950

^a Means in a column followed by different letters are significantly different among application rates ($P < 0.05$, by REGWQ test).

^b Variances between silica powder 1 and 2 were equal ($F_{\text{range}} = 1.95 - 24.44$; $df = 2, 2$; $P_{\text{range}} = 0.2832 - 0.4966$).

^c Variances between silica powder 1 and 2 were equal ($F = \infty$; $df = 2, 2$; $P < 0.0001$).

* Significant ($P < 0.05$; two-sample *t*-test).

Chapter 8 - Efficacy of two amorphous silica powders applied to hard red spring wheat against the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)

8.1 Abstract

In this study, we evaluated the efficacy of two amorphous silica powders, silica powder 1 and silica powder 2, applied to hard red spring wheat against the rice weevil, *Sitophilus oryzae* (L.) were evaluated. Laboratory experiments were performed using a 2×5 factorial design by exposing 25 adults of *S. oryzae* to 100 g of wheat in glass jars admixed with five different application rates, 0 (untreated control), 0.05, 0.15, 0.25, and 0.30 g/kg of silica powders 1 and 2. Untreated and treated wheat in jars were held inside a growth chamber at 28°C and 65% r.h. with a light:dark photoperiod of 14:10 h. Each powder and application rate combination was replicated five times. Mortality assessments were made after 14 d, and the data on adult progeny production, insect-damaged kernels and grain weight loss were recorded at 42 d. Complete mortality of *S. oryzae* occurred after 14 d at an application rate of 0.25 g/kg of silica powder 2. No adult progeny production, insect-damaged kernels and grain weight loss were observed when adults were exposed to 0.30 g/kg of silica powder 1 and 0.25 g/kg of silica powder 2. Silica powder 2, characterized by high oil absorption capacity and low pH (< 8.5), proved effective against *S. oryzae* than silica powder 1. The findings underscore the potential of both powders, particularly silica powder 2, in effectively managing *S. oryzae* infestations in stored wheat.

Keywords: Amorphous silica; Hard red winter wheat; Mortality; Insect-damage kernels; Grain weight loss.

8.2 Introduction

In recent years, the search for alternatives to residual chemical insecticides has increased significantly due to concerns over their mammalian toxicity, risk of harmful residues in treated commodities, and development of insect resistance (Arthur, 1996; Athanassiou et al., 2005). Silica-based inert dusts, such as diatomaceous earths, are potential alternatives to chemical insecticides (McLaughlin, 1994; Golob, 1997). Diatomaceous earth formulations are advantageous over synthetic insecticides as they do not exhibit toxicity to mammals, are easy to remove from commodities prior to processing, efficacy against a variety of insect species (Golob, 1997; Korunić et al., 1998; Subramanyam and Roesli, 2000; Collins and Cook, 2006; Athanassiou et al., 2016), long-term stability (Subramanyam et al., 1994; Korunić, 1999; Subramanyam and Roesli, 2000; Losic and Korunić, 2018), and lack of harmful residues (Korunić et al., 2017). However, various modes of action of inert dusts on insect pests were proposed, including, dehydration induced surface enlargement of the insect integument (Zacher and Kunicke, 1930), damage to the digestive tract (Smith, 1969), and impediment of spiracles and trachea (Webb, 1946). The absorption of epicuticular waxes in insects leading to death by desiccation (Ebeling, 1971; Korunić et al., 1998; Subramanyam and Roesli, 2000) is considered the most accepted mechanism of action of diatomaceous earth formulations against insects.

New diatomaceous earth products are effective at lower application rates compared to older formulations (Korunić, 1998; Subramanyam et al., 1998; Manivannan and Subramanyam, 2023a,b; Manivannan et al., 2024). The efficacy of diatomaceous earth formulations used for insect pest management can vary based on the geographical origin, pH, diatom species, density, particle size distribution, and lipid absorption capability (Korunić, 1997; Fields and Korunić, 2000; Korunić et al., 2014). The insecticidal efficacy of diatomaceous earth formulations varies

depending on factors such as the application rate, exposure time, treatment conditions, grain type, and insect species and life stages (Vayias and Athanassiou, 2004; Athanassiou et al., 2006; Kavallieratos et al., 2007; Zeni et al., 2021).

Many diatomaceous earth formulations are lethal to insects only at application rates of 1 g/kg or higher (Fields and Korunić, 2000; Arthur, 2003; Athanassiou et al., 2005), and the recommended application rates of diatomaceous earth formulations typically range between 0.5 g/kg to 3.5 g/kg (Korunić, 1997). It was reported that complete mortality of rice weevil, *Sitophilus oryzae* (L.) adults could only be achieved when the adults were exposed for 21 d to 0.5 g/kg of Silicid and 1 g/kg of SilicoSec® (Agrafioti et al., 2023). Andrić et al. (2012) reported complete mortality of *S. oryzae* adults exposed for 21 d to 0.75 g/kg of two diatomaceous earth powders, DE S-1 and DE S-2, from Serbia. Their study also reported 98 and 94% inhibition of adult progeny production in *S. oryzae* when adults were exposed to 1.0 g/kg of DE S-1 and DE S-2. Application of diatomaceous earths at these rates can adversely affect the grain quality including, reduction in grain bulk density and grain flowability (Korunić et al., 1996; Liska et al., 2017), and decrease in grain moisture (Fields and Korunić, 2000). Hence, diatomaceous earth formulations that are effective at lower application rates can reduce the physio-mechanical changes linked to their application in preservation of grains.

In our prior study, two amorphous silica powders, 1 and 2, supplied by Imerys, Lompoc, California, USA (Imerys.com) were evaluated for their efficacy against the lesser grain borer, *Rhyzopertha dominica* (F.). We observed that complete mortality of adults can be achieved after exposing them for 14 d to wheat admixed with 0.20 g/kg of silica powder 2. Moreover, treatment at this application rate completely inhibited adult progeny production, insect-damaged kernels, and grain weight loss caused by *R. dominica* (Manivannan et al., 2024). Stored-product insects

exhibit variation in their susceptibility to diatomaceous earth formulations (Carlson and Ball, 1962, Desmarchelier and Dines, 1987, Korunić, 1998, Subramanyam et al., 1998). Hence, in this study, we evaluated the insecticidal efficacy of silica powders 1 and 2 against *S. oryzae*. Adults of *S. oryzae* were exposed for 14 d to hard red spring wheat treated at various application rates of silica powders 1 and 2 to determine mortality. We also studied the influence of these silica powders on adult progeny production, insect-damaged kernels, and grain weight loss caused by *S. oryzae*.

8.3 Materials and Methods

8.3.1 Two amorphous silica powders

Two amorphous silica powders, referred to as silica powder 1 and silica powder 2 with different elemental composition and particle diameters (Manivannan and Subramanyam, 2023a) were used in this study. Silica powder 1 had an oil absorption capability of 231%, while silica powder 2 had a higher oil absorption capability of 386%. The silicon dioxide contents in silica powders 1 and 2 were found to be 94.5 and 97.3%, respectively. Silica powder 1 was characterized by smaller particle size with a D_{90} of 26.40 μm (representing the diameter fraction where 90% of particles were within a specific size range), compared with silica powder 2, which had a D_{90} of 31.43 μm . The pH values were 9.6 for silica powder 1 and 7.2 for silica powder 2 (Manivannan and Subramanyam, 2023a).

8.3.2 Insect rearing

Laboratory insect cultures of *S. oryzae* were reared using adults isolated from stock cultures maintained at the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA, since 1999. Cultures of *S. oryzae* were reared in 0.94L glass mason jars containing organic hard red spring wheat (Great River Organic Milling Company, Fountain

City, Wisconsin, USA). The glass jars were fitted with metal lids that had wire mesh screens (250 µm) and 9 cm diameter filter papers. The insect culture jars were held inside a growth chamber at 28°C and 65% r.h. (Pervical Scientific, Inc., Model I-36 VL, Perry, Iowa, USA), with a light:dark photoperiod of 14:10 h.

8.3.3 Exposure of *S. oryzae* to silica treated grains

Organic hard red spring wheat was placed inside a deep freezer at -13°C to kill any live insects present. After a week, the grains were cleaned by sieving them using a 2.38 mm diameter round-holed aluminium sieve (Seedburo Equipment Company, Des Plaines, Illinois, USA) to separate the damaged kernels and dockage. From this lot, 100 g of wheat (13% moisture, wet basis) were transferred to individual 0.45 L glass jars. A 2 × 5 factorial design was used to evaluate the efficacy of the two silica powders at five application rates against *S. oryzae*. Each jar containing wheat grains was treated with silica powder 1 or silica powder 2 at five different application rates, 0 (untreated control), 0.05, 0.15, 0.25, and 0.30 g/kg. The glass jars were covered with metal lids that had wire mesh screens (250 µm) and 7 cm diameter filter paper to facilitate air diffusion. The jars were manually shaken for a minute to ensure uniform distribution of silica powders on wheat. Twenty-five unsexed adults (1-4-week-old) of *S. oryzae*, isolated from the laboratory culture jars were introduced into each glass jar and secured with lids. Five replications were maintained for each powder and application rate combination. All the jars were placed inside a growth chamber at 28°C and 65% r.h. Mortality assessments were performed after 14 d by sieving wheat from each glass jar using a 2.38 mm circular round-holed aluminium sieve to isolate the adults. The adults that were immobile when prodded with fine camel's hair brush were considered dead. Mortality was expressed as a percentage based on number of dead adults out of the total exposed to treatments. Once the mortality assessments were performed,

both live and dead insects were transferred back to the jars and were held inside a growth chamber at 28°C and 65% r.h for an additional 28 d. At 42 d, data on adult progeny produced were determined, and the 25 parental adults initially introduced into each jars were subtracted from the total counts. Wheat grains obtained from each powder and application rate combination was passed through a Boerner[®] divider (Seedburo Equipment Company, Des Plaines, Illinois, USA) five times to obtain a working sample of ≈ 3.5 g. From this working sample, the data on the number of insect-damaged kernels, undamaged kernels, and grain weight loss were determined. The percentage grain weight loss caused by *S. oryzae* was estimated using the formula provided by Adams and Schulten (1978) and Boxall (1986).

8.3.4 Data analysis

The mean $\pm$ SE mortality of *S. oryzae* adults in untreated controls was $9.6 \pm 3.7\%$ for silica powder 1 and $8.8 \pm 3.2\%$ for silica powder 2. Therefore, mortality in treatments were corrected for responses in the control treatments (Abott, 1925). Data on percentage corrected mortality, insect-damaged kernels, and grain weight loss were transformed to angular values (Zar, 1984) for analysis using the Statistical Analysis System 9.4 (SAS Institute, 2012). Adult progeny counts were transformed to $\log_{10}(x + 1)$ for statistical analysis (Zar, 1984). Two-way analysis of variance (ANOVA) was performed to determine the effects of silica powders and application rates and their interaction. One-way analysis of variance (ANOVA) was carried out to determine the significant ($\alpha = 0.05$) differences among application rates including control for each powder. Means among application rates were separated using Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test (SAS Institute, 2012). Two-sample *t*-tests were performed to determine the significant difference between the silica powders at each application rate only for control corrected mortality data.

8.4 Results

8.4.1 Mortality of *S. oryzae* adults exposed to two amorphous silica powders

Complete mortality of *S. oryzae* could not be achieved at 0.05 to 0.30 g/kg application rates of silica powder 1. The mean $\pm$ SE corrected mortality of *S. oryzae* adults exposed to 0.05 to 0.30 g/kg of silica powder 1 for 14 d ranged from 33.6 ± 7.0 to $96.5 \pm 2.6\%$ (Table 1). Complete mortality of *S. oryzae* was achieved when adults were exposed for 14 d to wheat treated with 0.25 g/kg of silica powder 2. The mean $\pm$ SE corrected mortality of *S. oryzae* adults exposed for 14 d to 0.05 to 0.30 g/kg of silica powder 2 ranged from 52.6 ± 12.6 to 100%. Two-way ANOVA showed that the corrected mortality responses of *S. oryzae* adults between silica powders ($F = 14.32$; $df = 1, 32$; $P = 0.0006$) and among application rates ($F = 34.57$; $df = 3, 32$; $P < 0.0001$) were significant. However, the powder $\times$ application rate interaction was not significant ($F = 0.64$; $df = 3, 32$; $P = 0.5923$), indicating that the trends in adult mortality between the powders were consistent across the application rates. One-way ANOVA showed significant differences in corrected mortality response among application rates of silica powder 1 ($F = 14.38$; $df = 3, 16$; $P < 0.0001$) and silica powder 2 ($F = 22.83$; $df = 3, 16$; $P < 0.0001$). The corrected mortality response at 0.30 g/kg was significantly different ($P < 0.05$) from other application rates of silica powder 1 (Table 8.1). No significant difference ($P > 0.05$) in mortality response was observed between 0.05 g/kg and other application rates of silica powder 1. At 0.25 g/kg application rate, the corrected mortality response was significantly different ($P < 0.05$) from 0.05 and 0.15 g/kg of silica powder 2. Two-sample *t*-tests showed significant differences ($P < 0.05$) in mortality responses between silica powder 1 and silica powder 2 exposed only to an application rate of 0.25 g/kg (Table 8.1).

8.4.2 Adult progeny production

The mean $\pm$ SE number of *S. oryzae* adult progeny produced in controls at 42 d ranged from 197.6 ± 11.5 to 225.3 ± 24.1 per jar. The mean $\pm$ SE number of adult progeny produced at 42 d after exposure to 0.05 to 0.30 g/kg of silica powder 1 ranged from 0 to 46.0 ± 6.3 per jar (Table 8.2). Complete suppression of adult progeny production was achieved when adults were exposed to 0.30 g/kg of silica powder 1. The mean $\pm$ SE number of *S. oryzae* adult progeny produced after exposure to 0.05 to 0.30 g/kg of silica powder 2 ranged from 0 to 35.30 ± 9.5 per jar. Adult progeny production was completely suppressed when adults were exposed to 0.25 to 0.30 g/kg of silica powder 2. Two-way ANOVA showed that the number of adult progeny counts among application rates was significantly different ($F = 56.85$; $df = 4, 40$; $P < 0.0001$). However, the data on number of adult progeny counts between the powders ($F = 1.78$; $df = 1, 40$; $P = 0.1902$) as well as the powder $\times$ application rate interaction were not significant ($F = 0.38$; $df = 4, 40$; $P = 0.8238$). One-way ANOVA showed that there were significant differences in the number of adult progeny counts among application rates of silica powder 1 ($F = 56.50$; $df = 4, 20$; $P < 0.0001$) and silica powder 2 ($F = 195.92$; $df = 4, 20$; $P < 0.0001$). The number of adult progeny counts in controls were significantly higher ($P < 0.05$) than treatments at 0.05 to 0.30 g/kg of silica powders 1 and 2. At 0.30 g/kg, the number of adult progeny counts were significantly different ($P < 0.05$) between control and other application rates of silica powder 1. Two-sample *t*-tests were not performed to show significant differences in the number of adult progeny counts between silica powders 1 and 2 at each application rate because two-way ANOVA showed no differences between powders.

8.4.3 Insect-damaged kernels

The mean $\pm$ SE insect-damaged kernels in controls at 42 d ranged from 13.4 ± 1.8 to $17.8 \pm 2.0\%$. The mean $\pm$ SE insect-damaged kernels caused by *S. oryzae* adults after exposure to 0.05 to 0.30 g/kg of silica powder 1 ranged from 0 to $6.4 \pm 1.6\%$ (Table 8.3). No insect-damaged kernels were observed when *S. oryzae* were exposed for 14 d to 0.30 g/kg of silica powder 1. The mean $\pm$ SE insect-damaged kernels caused by *S. oryzae* adults after exposure to 0.05 to 0.30 g/kg of silica powder 2 for 14 d ranged from 0 to $6.0 \pm 1.3\%$. No insect-damaged kernels were observed when *S. oryzae* adults were exposed for 14 d to 0.25 g/kg of silica powder 2. Two-way ANOVA showed that the percentage of insect-damaged kernels caused by *S. oryzae* among application rates were significantly different ($F = 49.77$; $df = 3, 32$; $P < 0.0001$). However, the percentage of insect-damaged kernels caused by *S. oryzae* between powders ($F = 0.48$; $df = 1, 32$; $P = 0.2330$) as well as the powder $\times$ application rate interaction was not significantly different ($F = 0.29$; $df = 3, 32$; $P = 0.5875$). One-way ANOVA showed that there were significant differences in percentage insect-damaged kernels caused by *S. oryzae* among application rates of silica powder 1 ($F = 35.10$; $df = 4, 20$; $P < 0.0001$; REGWQ test) and silica powder 2 ($F = 60.52$; $df = 4, 20$; $P < 0.0001$; REGWQ test). The percentage insect-damaged kernels caused by *S. oryzae* was significantly higher ($P < 0.05$) in controls compared to other treatments for silica powders 1 and 2. At 0.30 g/kg, the percentage insect-damaged kernels caused by *S. oryzae* was significantly different ($P < 0.05$) from other application rates of silica powder 1 and was not significantly different from an application rate of 0.25 g/kg. Similarly, the percentage insect-damaged kernels at 0.30 g/kg of silica powder 2 was not significantly different ($P > 0.05$) from application rates of 0.15 and 0.25 g/kg. Two-sample *t*-tests were not performed

to show significant differences in percentage insect-damaged kernels between powders because two-way ANOVA showed no differences between powders.

8.4.4 Grain weight loss

The mean $\pm$ SE grain weight loss caused by *S. oryzae* in controls at 42 d ranged from 21.3 ± 2.8 to $14.9 \pm 2.4\%$. The mean $\pm$ SE grain weight loss caused by *S. oryzae* at 42 d after exposure to 0.05 to 0.30 g/kg of silica powder 1 ranged from 0 to $6.2 \pm 1.8\%$ (Table 8.4). No grain weight loss was observed when adults of *S. oryzae* were exposed to 0.30 g/kg of silica powder 1. The mean $\pm$ SE weight loss caused by *S. oryzae* after exposure to 0.05 to 0.30 g/kg of silica powder 2 ranged from 0 to $5.4 \pm 1.5\%$. No weight loss was observed when adults were exposed to 0.25 and 0.30 g/kg of silica powder 2. Two-way ANOVA showed that the percentage grain weight loss caused by *S. oryzae* among application rates were significantly different ($F = 86.27$; $df = 1, 40$ $P < 0.0001$). However, the percentage grain weight loss caused by *S. oryzae* between powders ($F = 0.41$; $df = 1, 40$; $P = 0.5246$) as well as the powder $\times$ application rate interaction ($F = 2.28$; $df = 4, 40$; $P = 0.0785$) were not significantly different. One-way ANOVA showed that there were significant differences in percentage grain weight loss caused by *S. oryzae* among application rates of silica powder 1 ($F = 29.29$; $df = 4, 20$; $P < 0.0001$; REGWQ test) and silica powder 2 ($F = 63.29$; $df = 4, 20$; $P < 0.0001$). The percentage grain weight loss caused by *S. oryzae* was significantly higher ($P < 0.05$) in controls compared to other treatments for silica powders 1 and 2. The percentage grain weight loss caused by *S. oryzae* after exposure to 0.30 g/kg of silica powder 1 was significantly different ($P < 0.05$) from other application rates but was not significantly different from 0.25 g/kg. Similarly, the percentage grain weight loss caused by *S. oryzae* at 0.30 g/kg, was significantly different ($P < 0.05$) from control and 0.05 g/kg of silica powder 2. Two-sample *t*-tests to test significant differences between powders at

each application rate were not performed because two-way ANOVA showed no significant differences between powders.

8.5 Discussion

Numerous studies have documented the efficacy of diatomaceous earth formulations admixed with grains for the management of *S. oryzae* (McLaughlin, 1994; Arthur and Throne, 2003; Fields and Korunić, 2000; Kljajić, 2006; Kljajić et al., 2010; Andric et al., 2012; Zidan, 2013; Chiriloaie et al., 2013, 2014; Athanassiou et al., 2016; Korunić, 2016; Faliagka et al., 2020; Agrafioti et al., 2023). Diatomaceous earth formulations are typically effective at higher rates when admixed with grains (Korunić, 1997; Korunić et al., 1998). The use of such higher application rates can adversely affect the physical and mechanical properties of treated grains such as reducing their bulk density and flowability (Korunić, 1997; Korunić et al., 1998; Shah and Khan, 2014). Therefore, formulations effective at lower application rates can potentially minimize these undesirable effects on treated grains.

In the present research, silica powder 2 was found to be more effective than silica powder 1. Exposure of *S. oryzae* to 0.25 g/kg of silica powder 2 for 14 d resulted in complete mortality of adults. This application rate is lower than the current recommended rate of 0.5 to 3.5 g/kg reported for diatomaceous earth formulations (Korunić, 1997; Korunić et al., 1998). Kljajić et al. (2010) reported that Protect-It™ applied to wheat at 0.30 g/kg for 14 d exposure was effective in inducing complete mortality of *S. oryzae* adults. In the case of silica powder 1, we did not achieve complete mortality of *S. oryzae* even when adults were exposed for 14 d to the highest application rate of 0.30 g/kg.

The effectiveness of diatomaceous earth formulations on insects is dependent upon the physical characteristics, such as the pH, particle size distribution, lipid absorption capability,

moisture content, silicon dioxide content, and the diameter of inner pores of particles (Korunić, 1997; Fields and Korunić, 2000). For instance, Silicosec[®] (containing 92% silicon dioxide) and PyriSec (containing of 1.2% natural pyrethrum, 3.1% piperonyl butoxide, and 95.7% Silicosec[®]) demonstrated efficacy against *S. oryzae*, achieving 98% adult mortality when adults were exposed for 21 d at an application rate of 0.9 g/kg of wheat at 30 °C and 55% r.h. (Athanassiou et al., 2016). Exposure of *S. oryzae* adults for 14 d to a diatomaceous earth formulation from Egypt (containing 83.7% silicon dioxide content) at 1.6 g/kg of wheat gave complete mortality only after a 21 d exposure (Zidan, 2013). Both the above-mentioned studies (Zidan, 2013; Athanassiou et al., 2016), recommended higher rates and longer exposure periods compared to the present investigation where we observed that an exposure for 14 d to 0.25 g/kg of silica powder 2 was effective in achieving complete mortality of *S. oryzae*. The probable reason for this variation could be explained by differences in silicon dioxide content between their study and the present investigation. However, the two silica powders tested in the present study had silicon dioxide contents < 94% suggesting that both the powders are expected to exhibit better efficacy against *S. oryzae* adults.

Fields and Korunić (2000) evaluated the effect of temperature and moisture content of grains on the efficacy of six different diatomaceous earth formulations (Protect-It[™], Diaherb, Insecto[®], Japan 3, Dryacide[®], and Perma Guard D-10) admixed with wheat at 0.4 g/kg against *S. oryzae* adults after 5 d of exposure. Their study that all the formulations except Japan 3 and Perma Guard D-10 gave more than 90% mortality of *S. oryzae* adults when 12% moisture grains were treated at 30 °C. In the present study, we observed complete mortality of *S. oryzae* adults after 14 d of exposure to 0.25 g/kg of silica powder 2. Fields and Korunić (2000) examined the effectiveness of diatomaceous earth formulations at an application rate of 0.4 g/kg, which is

higher than the rate used in the present study (0.25 g/kg). Their study involved the exposure of *S. oryzae* adults to a shorter exposure period of 5 d, compared to the present investigation which involved exposing the adults of *S. oryzae* to silica powders 1 and 2 for 14 d. However, it is important to note that their study did not evaluate the effect of these formulations on the adult progeny production.

We observed that exposure of *S. oryzae* adults to 0.30 g/kg of silica powder 1 and 0.25 g/kg of silica powder 2 completely inhibited the adult progeny production. In our previous investigation with these two powders, we observed that 0.20 g/kg of silica powder 2 was effective against *R. dominica* to completely suppress the adult progeny production (Manivannan et al., 2024). This difference in application rates required for complete inhibition of adult progeny production between the present study and our previous study against *R. dominica* confirms the species-specific variation in susceptibility of stored product insects to these powders. In addition, studies have shown that the efficacy of diatomaceous earth powders can be influenced by the particle diameter (Korunić, 1997; Fields and Korunić, 2000; Manivannan and Subramanyam, 2023b). Silica powder 1 had significantly smaller particle diameter ($D_{90} = 26.4 \mu\text{m}$) compared to silica powder 2 ($D_{90} = 31.4 \mu\text{m}$), the particle diameter of the powders did not influence their efficacy against *S. oryzae*. In a recent study by Manivannan et al. (2024), it was highlighted that high oil absorption capability (386%) and a favorable low pH value (< 8.5) for silica powder 2 are contributing factors for enhanced mortality and complete suppression of adult progeny production in *R. dominica*. Consistent with this finding, the current observations underscore the superior efficacy of silica powder 2 in comparison to silica powder 1 on *S. oryzae*.

The findings of our study indicate that the exposure of *S. oryzae* adults to 0.30 g/kg of silica powder 1 and 0.25 g/kg of silica powder 2 can completely prevent insect-damaged kernels and grain weight loss. However, it is noteworthy that while 0.30 g/kg of silica powder 1 did not result in the complete mortality of *S. oryzae* adults, no offspring were produced, and the surviving insects did not cause any insect-damaged kernels or grain weight loss. In contrast, our previous investigation against *R. dominica* demonstrated that only silica powder 2 effectively suppressed adult progeny production, inhibited insect-damaged kernels, and grain weight loss at an application rate of 0.20 g/kg. It is important to highlight that our previous study did not explore application rates beyond 0.20 g/kg of silica powder 1 (Manivannan et al., 2024). These findings underscore the potential differential effectiveness of silica powders against different insect species.

The present study results clearly demonstrated that exposure for 14 d of *S. oryzae* adults to 0.25 g/kg of silica powder 2 was highly effective in inducing complete mortality, suppressing adult progeny production, and inhibiting insect-damaged kernels and grain weight loss caused by *S. oryzae*. This higher efficacy of silica powder 2 against *S. oryzae* can be attributed to its higher silicon dioxide content, high oil adsorption capacity, and low pH (< 8.5) values. These results emphasize the potential of both silica powders, particularly silica powder 2, as promising options for grain treatment against *S. oryzae* at lower application rates. However, further investigations into the impact of these silica powders on physio-mechanical properties are necessary to assess their feasibility for commercial applications.

8.6 References

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Table 8.1. Effect of two amorphous silica powders on the mortality of *S. oryzae* exposed to wheat treated at different application rates for 14 d.

Application	Mean $\pm$ SE corrected mortality (%) ^{a,b}			
rates (g/kg)	Silica 1	Silica 2	<i>t</i> -value (df)	<i>P</i> -value
0.05	33.6 $\pm$ 7.0ab	52.6 $\pm$ 12.0a	- 1.36 (8) ^c	0.2096
0.15	62.8 $\pm$ 11.8b	84.8 $\pm$ 5.1b	- 1.60 (8) ^c	0.1481
0.25	85.0 $\pm$ 4.6b	100 $\pm$ 0c	- 3.00 (4) ^d	0.0299*
0.30	96.5 $\pm$ 2.6c	100 $\pm$ 0c	- 1.37 (4) ^d	0.2420

^aControl mortality in tests with silica powders 1 and 2 were 9.6 $\pm$ 3.7 and 8.8 $\pm$ 3.2%, respectively.

^bMeans among application rates for each powder followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

^cVariances between silica powder 1 and 2 were equal ($F_{\text{range}} = 2.96 - 5.36$; df = 4, 4; $P_{\text{range}} = 0.1327 - 0.3177$).

^dVariances between silica powder 1 and 2 were unequal ($F = \infty$; df = 4, 4; $P < 0.0001$).

*Significant ($P < 0.05$; two-sample *t*-test).

Table 8.2. Effect of two amorphous silica powders on the progeny production of *S. oryzae* exposed to wheat treated at different application rates.

Application rates (g/kg)	Mean $\pm$ SE adult progeny ^a	
	Silica 1	Silica 2
0	225.5 $\pm$ 24.1a	197.6.0 $\pm$ 11.7a
0.05	46.0 $\pm$ 6.3b	35.0 $\pm$ 9.5b
0.15	10.2 $\pm$ 3.6c	5.6 $\pm$ 1.6c
0.25	3.4 $\pm$ 1.7c	0 $\pm$ 0d
0.30	0 $\pm$ 0d	0 $\pm$ 0d

^aMeans among application rates for each powder followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

Table 8.3. Insect-damaged kernels caused by *S. oryzae* at 42 d after exposure to wheat treated with two silica powders at different application rates.

Application rates (g/kg)	Mean $\pm$ SE insect-damaged kernels (%) ^a	
	Silica 1	Silica 2
0	13.4 $\pm$ 1.8a	17.8 $\pm$ 2.0a
0.05	6.4 $\pm$ 1.6b	6.0 $\pm$ 1.3b
0.15	2.2 $\pm$ 0.7c	1.3 $\pm$ 0.6c
0.25	0.8 $\pm$ 0.3cd	0 $\pm$ 0c
0.30	0 $\pm$ 0d	0 $\pm$ 0c

^aMeans among application rates for each powder followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

Table 8.4. Weight loss caused by *S. oryzae* at 42 d after exposure to wheat treated with two silica powders at different application rates.

Application rates (g/kg)	Mean $\pm$ SE grain weight loss (%) ^a	
	Silica 1	Silica 2
0	14.9 $\pm$ 2.4a	21.3 $\pm$ 2.8a
0.05	6.2 $\pm$ 1.8b	5.4 $\pm$ 1.5b
0.15	1.2 $\pm$ 0.8c	0.7 $\pm$ 0.4c
0.25	0.6 $\pm$ 0.3cd	0 $\pm$ 0c
0.30	0 $\pm$ 0d	0 $\pm$ 0c

^aMeans among application rates for each powder followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

Chapter 9 - Efficacy of two amorphous silica powders applied to soft red winter wheat against two externally developing stored product insects

9.1 Abstract

Two amorphous silica powders 1 and 2 were evaluated for their effectiveness to manage the saw-toothed grain beetle, *Oryzaephilus surinamensis* and the red flour beetle, *Tribolium castaneum*. Thirty adults (1- to 4-week-old) were exposed to wheat treated with different application rates (0, 0.05, 0.15, 0.25, 0.35, and 0.5 g/kg) of silica powder 1 and 2 for 14 d. Mortality assessments were carried out after 14 d and the progeny counts at 42 d. Silica powder 1 and 2 exhibited complete mortality of *O. surinamensis* at 0.35 g/kg rate after 14 d exposure. While 100% mortality of *T. castaneum* was achieved when adults were exposed to 0.50 g/kg of silica powder 1 and 0.35 g/kg of silica powder 2. No adult progeny production was observed when adults of *O. surinamensis* were exposed to 0.25 and 0.35 g/kg of silica powders 1 and 2, respectively. Similarly, complete suppression of adult progeny production was observed when *T. castaneum* were exposed for 14 d to 0.35 g/kg and 0.25 g/kg of silica powders 1 and 2, respectively. The results indicated that silica powder 2 was more effective to induce complete mortality and suppress adult progeny production of both the insect species.

Keywords: Amorphous silica powders, wheat, *O. surinamensis*, *T. castaneum*, Insecticidal activity.

9.2 Introduction

Post-harvest losses caused by stored product insects are estimated to be around 9% in the developed countries and 20% in the developing countries (Pimentel, 1991; Phillips and Throne, 2010). Management of insect pests in food grains predominantly involves fumigation (Rajendran and Sriranjini, 2008; Manivannan et al., 2016), use of grain protectants (Subramanyam et al., 2014; Hamel et al., 2020) and other Integrated Pest Management (IPM) approaches. However, the development of insect resistance to fumigants such as phosphine (Rajendran, 1999; Collins et al., 2002; Benhalima et al., 2004; Daglish et al., 2014; Nguyen et al., 2015; Aulicky et al., 2019) and grain protectants (Daglish, 1998; Athanassiou et al., 2014; Daglish and Nayak, 2018) had led to search for alternatives to chemical insecticides. Inert dusts such as the diatomaceous earth formulations are considered as promising substitutes for chemical insecticides, and they have demonstrated ability to enhance the effectiveness of other pest management strategies, including aeration, fumigation, and heat treatments (Winks and Russel, 1994; Fields et al., 1997). Moreover, they have been recommended for use in resistance management strategies (Balliota and Athanassiou, 2023).

Many researchers have reported diatomaceous earth formulations to be effective on stored insect insects when admixed with grains (Athanassiou et al., 2003, 2008; Demissie et al., 2008; Nukenine et al., 2010; Liška et al., 2017; Saeed et al., 2022; Manivannan et al., 2023). Older diatomaceous earth formulations significantly impacted the physical properties of grains and decreased the mechanical efficiency of grain handling equipments (Korunić, 1997). Newer products are more effective than the older products (Subramanyam and Roesli, 2000; Manivannan and Subramanyam, 2023a,b). The insecticidal activity of diatomaceous earths on insects is related to the particle size (Vayias and Stephou, 2009; Rumbos et al., 2016); pH and

flowability, geographical location, and diatom species (Korunić, 1997; Fields and Korunić, 2000). Most of the diatomaceous earth formulations are effective at higher rates, and the recommended dosage is within 0.5 to 3.5 g/kg (Korunić, 1997).

In studies assessing the effectiveness of diatomaceous earth formulations on different stored product insect species, the red flour beetle, *Tribolium castaneum* (Herbst), has been identified as the most tolerant species (Maceljski and Korunić, 1972; Desmarchelier and Dine, 1987; Vayias and Athanassou, 2004; Korunić, 2013; Manivannan and Subramanyam, 2023a). Conversely, flat-bodied insects like *Cryptolestes* spp. and the saw-toothed grain beetle, *O. surinamensis* are regarded as susceptible species (Korunić, 1998; Arthur, 2001). For instance, Balliota and Athanassou (2020) reported that *O. surinamensis* exhibited notable susceptibility, with 100% mortality achieved at 1 g/kg of DE 6 exposed for 7 d, while 0.5 g/kg of DE 6 resulted in complete mortality of adults after 14 and 21 d, respectively. Ziaee et al. (2007) evaluated the efficacy of SilicoSec® against *T. castaneum* by exposing adults to application rates of 0.7, 1.0, 1.3, and 1.7 g/kg exposed for 2, 7, and 14 d. However, they observed more than 90% mortality after exposing adults for 14 d to 0.7 g/kg, complete mortality was not reported for any treatment combination. Exposing *T. castaneum* adults to wheat treated at highest application rate of 0.4 g/kg of DiaFil 610, Protect-It, and SilicoSec gave maximum mortalities of 64.6, 78.3 and 71.7%, respectively after 7 d exposure period (Wakil et al., 2023).

Diatomaceous earth products with tapped densities < 300 g/L, consisting of more than 80% silicon dioxide contents, a pH values < 8.5 and effective at lower application rates are regarded to be more effective (Korunić, 1997) and should be available for treatment in grain processing environments. Recent studies have shown that silica powder 2 with silicon dioxide content of 97.3% and desirable pH values of 7.2 (< 8.5) exhibited remarkable efficacies against

the lesser grain borer, *Rhyzopertha dominica* (F.) in both concrete surfaces and when admixed with wheat (Manivannan and Subramanyam, 2023b; Manivannan et al., 2023). In this study, we evaluated the insecticidal activity of two amorphous silica powders 1 and 2 supplied by Imerys, Lompoc, California, USA on two externally developing insect pests, *T. castaneum* and *O. surinamensis* by exposing them to different application rates of silica powder 1 and 2 on the mortality and adult progeny production exposed for 14 d.

9.3 Materials and Methods

9.3.1 Silica powders

The study utilized two distinct amorphous silica powders, denoted as silica powder 1 and silica powder 2, each possessing unique elemental compositions and particle diameters (Manivannan and Subramanyam, 2023a). These powders, supplied by Imerys, Lompoc, California, USA varied in their physical characteristics and elemental composition. Silica powder 1 exhibited an oil adsorption capacity of 231%, while silica powder 2 had a higher oil adsorption capacity of 386%. The silicon dioxide contents were measured at 94.5% for silica powder 1 and 97.3% for silica powder 2. Additionally, silica powder 1 had smaller particle size (D_{90} of 26.40 μm) compared to silica powder 2 (D_{90} of 31.43 μm), and different pH values, 9.6 for silica powder 1 and 7.2 for silica powder 2 (Manivannan and Subramanyam, 2023a).

9.3.2 Test insects

Insect cultures of *O. surinamensis* and *T. castaneum* were reared using adults isolated from stock cultures maintained at the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA. Cultures were reared in 0.94 L glass mason jars containing rolled whole oats for *O. surinamensis* (Heartland Mills) and organic whole wheat flour supplemented with 5% yeast for *T. castaneum* (Great River Organic Milling Company, Fountain

City, Wisconsin, USA). Each glass jar was equipped with metal lids containing wire mesh screens (250 µm) and 9 cm diameter filter paper lids. The insect culture jars were held inside a growth chamber at 28°C and 65% r.h. (Pervical Scientific, Inc., Model I-36 VL, Perry, Iowa, USA), maintaining a light:dark photoperiod of 14:10 h.

9.3.3 Insect exposure to silica powder treated wheat

Cleaned organic soft winter wheat (12.5% wet basis) was used for the experiments. A 100g of wheat was filled in individual glass mason jars of 0.45 L capacity and were treated with silica powder 1 or silica powder 2 at six different application rates, 0 (untreated control), 0.05, 0.15, 0.25, 0.35 and 0.50 g/kg. Each glass jar was equipped with metal lids containing wire mesh screens (250 µm) and 7 cm diameter filter paper lids. Once the required amount of powder was added to the jars, the jars were shaken well for a min to allow uniform coating of powder with wheat. Thirty unsexed adults (aged 1-4 weeks) of *O. surinamensis* or *T. castaneum* isolated from the culture jars, were placed into individual glass mason jars and sealed with lids and were then placed within a growth chamber set at 28°C and 65% relative humidity. Each powder-application rate combination was replicated five times. After 14 d, mortality was determined by sifting wheat from each jar through a 2.38 mm circular round-holed aluminum sieve, isolating the adults. Insects that remained immobile when prodded with a fine camel's hairbrush were regarded as dead. Following the mortality assessments, both live and dead insects were returned to the jars and held inside a growth chamber at 28°C and 65% r. h. for an additional 28 days. On 42 d, the adult progeny production counts were recorded by subtracting thirty parental adults from the total count.

9.3.4 Data analysis

A non-linear model, $y = a + bx^{0.5}$ were fitted for the adult mortality responses of *T. castaneum* and *O. surinamensis* as a function of application rates of silica powder 1 and 2 (including controls). A non-linear model, $\ln y = a + be^{-x}$ were fitted for the adult progeny production data of *T. castaneum* and *O. surinamensis* as a function of application rates of silica powder 1 and 2 (including controls). The non-linear models were compared between silica powders and between the insect species. Any two models compared were regarded to be significantly different ($P < 0.05$) from each other if the *F*- test showed that the individual models to deviate from the pooled model (Draper and Smith, 1998). All analyses were carried out using SAS 9.4 software (SAS Institute, 2012), and the graphs were made using SigmaPlot 12.5 software (Systat, Software, Inc., San Jose, California, USA).

9.4 Results

9.4.1 Mortality responses of *T. castaneum* and *O. surinamensis*

Three-way ANOVA showed that the corrected mortality responses due to the effects of powder ($F = 43.88$; $df = 1, 80$; $P < 0.0001$), application rate ($F = 253.89$; $df = 4, 80$; $P < 0.0001$), and species ($F = 79.36$; $df = 1, 80$; $P < 0.0001$) were significant. The application rate x species ($F = 13.67$; $df = 4, 80$; $P < 0.0001$), and powder x application rate interactions ($F = 7.27$; $df = 4, 80$; $P < 0.0001$) were also significant. While the powder x species ($F = 1.66$; $df = 1, 80$; $P = 0.2014$), and powder x application rate x species interactions were not significant ($F = 2.19$; $df = 4, 80$; $P = 0.0779$).

The mean $\pm$ SE mortality of *O. surinamensis* adults in untreated controls ranged from 11.3 ± 2.0 to $14.0 \pm 2.5\%$. The increase in mortality of adults exposed to increasing application rates of silica powder 1 and 2 followed a non-linear trend. The mean $\pm$ SE corrected mortality of

O. surinamensis adults exposed for 14 d to 0.05 to 0.50 g/kg of silica powder 1 ranged from 22.9 ± 1.9 to 100%. The mean $\pm$ SE corrected mortality of *O. surinamensis* adults exposed for 14 d to 0.05 to 0.50 g/kg of silica powder 2 ranged from 39.5 ± 6.8 to 100%. Complete mortality of *O. surinamensis* was obtained at 0.35 g/kg of silica powders 1 and 2. The fitted non-linear model, $y = a + bx^{0.5}$ explained the relationship between the mortality response data of *O. surinamensis* adults to different application rates of silica powder 1 ($r^2 = 0.95$) and silica powder 2 ($r^2 = 0.86$) (Fig 9.1). Comparison of non-linear models between silica powder 1 and silica powder 2 showed no significant differences ($F = 0.66$; $df = 2, 8$; $P = 0.5421$).

The mean $\pm$ SE mortality of *T. castaneum* adults in untreated controls ranged from 0 to $2.3 \pm 1.3\%$. The mean $\pm$ SE corrected mortality of *T. castaneum* adults exposed for 14 d to 0.05 to 0.50 g/kg of silica powder 1 ranged from 13.3 ± 3.2 to 100%. The mean $\pm$ SE corrected mortality of *T. castaneum* adults exposed for 14 d to 0.05 to 0.50 g/kg of silica powder 2 ranged from 26.5 ± 3.2 to 100%. Complete mortality of *T. castaneum* was obtained after exposure for 14 d to 0.50 g/kg of silica powder 1 and 0.35 g/kg of silica powder 2. The fitted non-linear model, $y = a + bx^{0.5}$ explained the relationship between the mortality response data of *T. castaneum* adults to different application rates of silica powder 1 ($r^2 = 0.95$) and silica powder 2 ($r^2 = 0.96$) (Fig. 9.2). Comparison of non-linear models of adult mortality responses between silica powder 1 and silica powder 2 showed no significant differences ($F = 1.43$; $df = 2, 8$; $P = 0.2940$). The model comparison of adult mortality responses between species for silica powder 1 ($F = 3.94$; $df = 2, 8$; $P = 0.0644$) and silica powder 2 ($F = 2.42$; $df = 2, 8$; $P = 0.1503$) were not significant (Table 9.1).

9.4.2 Adult progeny production

Three-way ANOVA showed that the adult progeny production data due to powder ($F = 76.05$; $df = 1, 80$; $P < 0.0001$), application rate ($F = 321.63$; $df = 4, 80$; $P < 0.0001$), and species ($F = 28.60$; $df = 1, 80$; $P < 0.0001$) were significant. The application rate x species ($F = 10.66$; $df = 4, 80$; $P < 0.0001$), powder x species ($F = 7.60$; $df = 4, 80$; $P = 0.0072$), and powder x application rate ($F = 15.82$; $df = 4, 80$; $P < 0.0001$) interactions were also significant. While the powder x application rate x species interaction was not significant ($F = 1.79$; $df = 4, 80$; $P = 0.1387$), indicating that the trends in adult progeny production data between the powders and species were consistent across the application rates.

The mean $\pm$ SE number of *O. surinamensis* adult progeny produced in untreated controls ranged from 203.6 ± 12.6 to 210.2 ± 26.7 . The decrease in adult progeny production exposed to increasing application rates of silica powder 1 and 2 followed a non-linear trend. The mean $\pm$ SE number of *O. surinamensis* adult progeny produced when adults were exposed for 14 d to 0.05 to 0.50 g/kg of silica powder 1 ranged from 0 to 71.6 ± 11.2 . The mean $\pm$ SE number of *O. surinamensis* adult progeny produced when adults were exposed for 14 d to 0.05 to 0.50 g/kg of silica powder 2 ranged from 0 to 23.2 ± 2.9 . Complete suppression of *O. surinamensis* adult progeny production was obtained at 0.35 g/kg of silica powder 1 and at 0.25 g/kg of silica powder 2. The fitted non-linear model, $\ln y = a + be^{-x}$ explained the relationship between the *O. surinamensis* adult progeny production data and different application rates of silica powder 1 ($r^2 = 0.99$) and silica powder 2 ($r^2 = 0.99$) (Fig 9.3). Comparison of non-linear models for adult progeny production data showed significant differences between silica powder 1 and silica powder 2 ($F = 53.85$; $df = 2, 8$; $P < 0.0001$).

The mean $\pm$ SE number of *T. castaneum* adult progeny produced in untreated controls ranged from 32.8 ± 4.1 to 33.0 ± 2.9 . The decrease in adult progeny production exposed to increasing application rates of silica powder 1 and 2 followed a non-linear trend. The mean $\pm$ SE number of *T. castaneum* adult progeny produced when adults were exposed for 14 d to 0.05 to 0.50 g/kg of silica powder 1 ranged from 0 to 16.6 ± 2.7 . The mean $\pm$ SE number of *T. castaneum* adult progeny produced when adults were exposed for 14 d to 0.05 to 0.50 g/kg of silica powder 2 ranged from 0 to 8.4 ± 2.2 . Complete suppression of *T. castaneum* adult progeny production was obtained at 0.35 g/kg of silica powder 1 and at 0.25 g/kg of silica powder 2. The fitted non-linear model, $\ln y = a + be^{-x}$ explained the relationship between the *T. castaneum* adult progeny production data and different application rates of silica powder 1 ($r^2 = 0.97$) and silica powder 2 ($r^2 = 0.99$) (Fig 9.4). Comparison of non-linear models for adult progeny production data showed significant differences between silica powder 1 and silica powder 2 ($F = 12.59$; $df = 2, 8$; $P = 0.0034$). The model comparison for adult progeny production data between species for silica powder 1 ($F = 520.61$; $df = 2, 8$; $P < 0.0001$) and silica powder 2 ($F = 6704.62$; $df = 2, 8$; $P < 0.0001$) were significant.

9.5 Discussion

Diatomaceous earth formulations that are effective at lower application rates are crucial to avoid the adverse effects of higher application rates on the physical properties of grains. The efficacy of diatomaceous earths against stored product insects is related to their physical properties such as the tapped density, particle size, silicon dioxide content, pH, and oil adsorption capability (Korunić, 1997; Fields and Korunić, 2000; Vayias et al., 2009; Rumbos et al., 2016; Losic and Korunić, 2018). Diatomaceous earth formulations sourced from different geographical locations exhibit diverse properties and insecticidal activity. In the present study,

both the tested amorphous silica powders 1 and 2 exhibited effectiveness in controlling the *T. castaneum* and *O. surinamensis*. Silica powder 2 was more effective on both the insect species *O. surinamensis* and *T. castaneum* compared to silica powder 1.

We noticed that complete mortality of *O. surinamensis* was achieved at a much lower rate compared to *T. castaneum*. At 0.35 g/kg complete mortality of *O. surinamensis* adults was achieved after exposing adults for 14 d to silica powders 1 and 2. Likewise, Arthur (2001) reported the susceptibility of *O. surinamensis* to diatomaceous earth formulation Protect-It™. He observed that exposure for 3 days to 0.3 g/kg of Protect-It™ at temperatures of 27 and 32°C, and relative humidity levels of 40%, 57%, and 75% achieved 95% of the insects. It was noticed that 100% mortality of *T. castaneum* was obtained when adults were exposed for 14 d to an application rate of 0.35 g/kg of silica powder 2. While 0.5 g/kg of silica powder 1 was required to achieved 100% mortality after a similar exposure period. These results with silica powder 2 against *T. castaneum* are in agreement with Kljajić et al. (2010) who reported that exposure of *T. castaneum* adults to wheat treated at 0.30 g/kg of Protect-It™ resulted in 77-87% mortality after 7 d and complete mortality after 14 d. While 100% mortality of *T. castaneum* was reported when adults were exposed to 0.5 g/kg of diatomaceous earth formulation for 14 d (Junior et al., 2006). While 98% mortality and complete suppression adult progeny production was observed when adults of *T. castaneum* were exposed to milled rice treated at 2 g/kg of FossilShield™ for 14 d (Mewis and Ulrich, 2001).

Agrafioti et al. (2023) investigated the efficacy of three diatomaceous earth formulations including Silicid, Celatom® MN-23, and SilicoSec® against seven stored product species, *T. confusum*, *T. castaneum*, *S. oryzae*, the granary weevil, *S. granarius* (L), *R. dominica*, *O. surinamensis*, and the lesser mealworm, *Alphitobius diaperinus* (Panzer). They exposed adults

to three formulations at application rates ranging from 0.1 to 1 g/kg for 3, 7, 14, and 21 d. Their findings revealed that the formulations were more effective against secondary pests compared to primary pests. Specifically, Silicid induced complete mortality of *T. castaneum* after 14 d of exposure to a 1.0 g/kg application rate. Celatom[®] MN-23 resulted in over 90% mortality when *T. castaneum* adults were exposed for 21 d to 1 g/kg, whereas SilicoSec[®] exhibited the lowest efficacy. Conversely, *O. surinamensis* was identified as the most susceptible species, with over 90% mortality after exposure for 14 d to 1.0 g/kg rate of all three formulations. The application rates reported for the complete mortality of both the species in the present study are lower than those reported by Agrafioti et al. (2023) in their studies with Silicid, Celatom[®] MN-23, and SilicoSec[®].

We observed that the non-linear models fitted for the adult mortality data of *T. castaneum* and *O. surinamensis* satisfactorily explained the relationship between adult mortality response and the application rates of silica powder 1 and 2 with r^2 values ranging from 0.86 to 0.96. Furthermore, comparison of the non-linear models between silica powder 1 and 2 revealed no significant differences, indicating similar trends in mortality response for both powders against *O. surinamensis* and *T. castaneum*. Similarly, comparison of non-linear models of comparison of the non-linear models of *O. surinamenis* and *T. castaneum* for silica powder 1 and 2 revealed no significant differences, indicating similar trends in mortality response for both the species.

It was noticed that more numbers of adult progeny were produced in untreated controls in tests with *O. surinamensis*. While a low number of progenies were produced from wheat infested with *T. castaneum* adults. Adult progeny production was completely suppressed in test with *O. surinamensis* and *T. castaneum* when adults were exposed to 0.35 g/kg of silica powder 1. While complete suppression was observed in both insect species at 0.25 g/kg of silica powder 2,

indicating the superior efficacy of silica powder 2 than silica powder 1. Wakil et al. (2023) did not observe complete reduction of adult progeny production when *T. castaneum* adults were exposed to 0.1 to 0.4 g/kg of DiaFil 610, Protect-It and SilicoSec for 14 d. And the adult progeny counts were 11.8, 5.8 and 7.5, for DiaFil 610, Protect-It and SilicoSec, respectively after 62 d of adult removal. Furthermore, comparison of non-linear models of adult progeny responses showed that the models between powders and between species for silica powder 1 and silica powder 2 were significantly different.

Smaller particles are reported to be more effective than the larger particles, while smaller particles can influence the bulk density of the grains (Korunić, 1997; Korunić et al, 2016). However, the particles diameter of silica powder 1 used in the present study was smaller than silica powder 2, the particle diameter did not influence the adult responses to these powders. In contrast, we observed in our earlier research that silica powder 1 was more effective against *T. castaneum* in concrete surfaces compared to silica powder 2 (Manivannan and Subramanyam, 2023a). However, it is important to note that insects were exposed for 12 to 48 h confining them to concrete surfaces, while in this study the adults were exposed for 14 d with the presence of food, allowing enough chances for *T. castaneum* adults to avoid contact with powders. The superior efficacy of silica powder 2 over silica powder 1 can be linked to the high oil adsorption capability and the desirable pH values of 7.2 for silica powder 2 to compared to silica powder 1.

The present study results provide convincing evidence that both the *O. surinamensis* and *T. castaneum* can be mitigated at application rates of 0.5 g/kg of both the powders exposed for 14 d. While silica powder 2 exhibited slightly better efficacy at lower application rates compared to silica powder 1 in inducing adult mortality and suppressing adult progeny production. Further, evaluation of these powders treated to different commodities under different temperature and

humidity conditions is required. In addition, the effect of these powders on the physical properties of grains and their influence on the mechanical efficiency of grain handling equipment are necessary to evaluate their commercial feasibility in grain storage systems.

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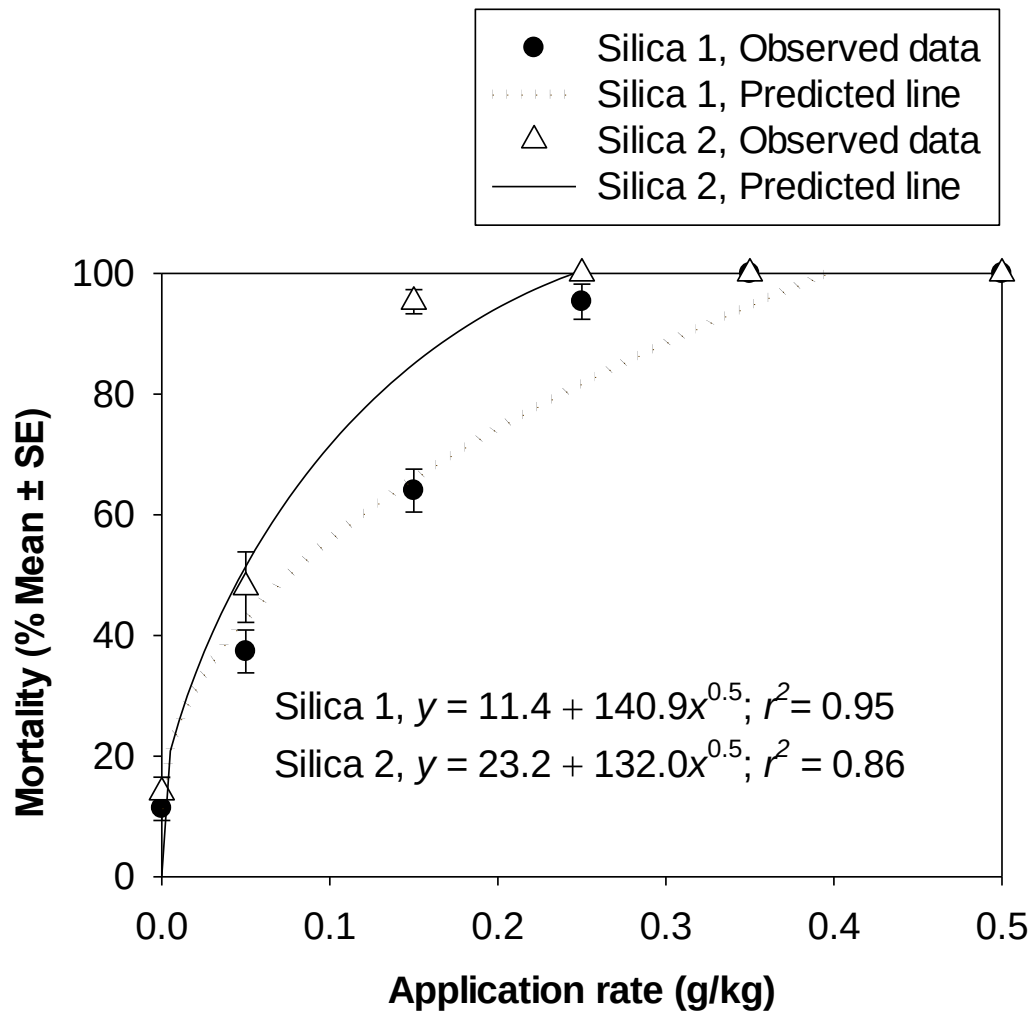


Figure 9.1. Percentage mortality of *O. surinamensis* adults at 14 d after exposure to concrete arenas treated to silica powders 1 and 2 at different application rates and exposure periods.

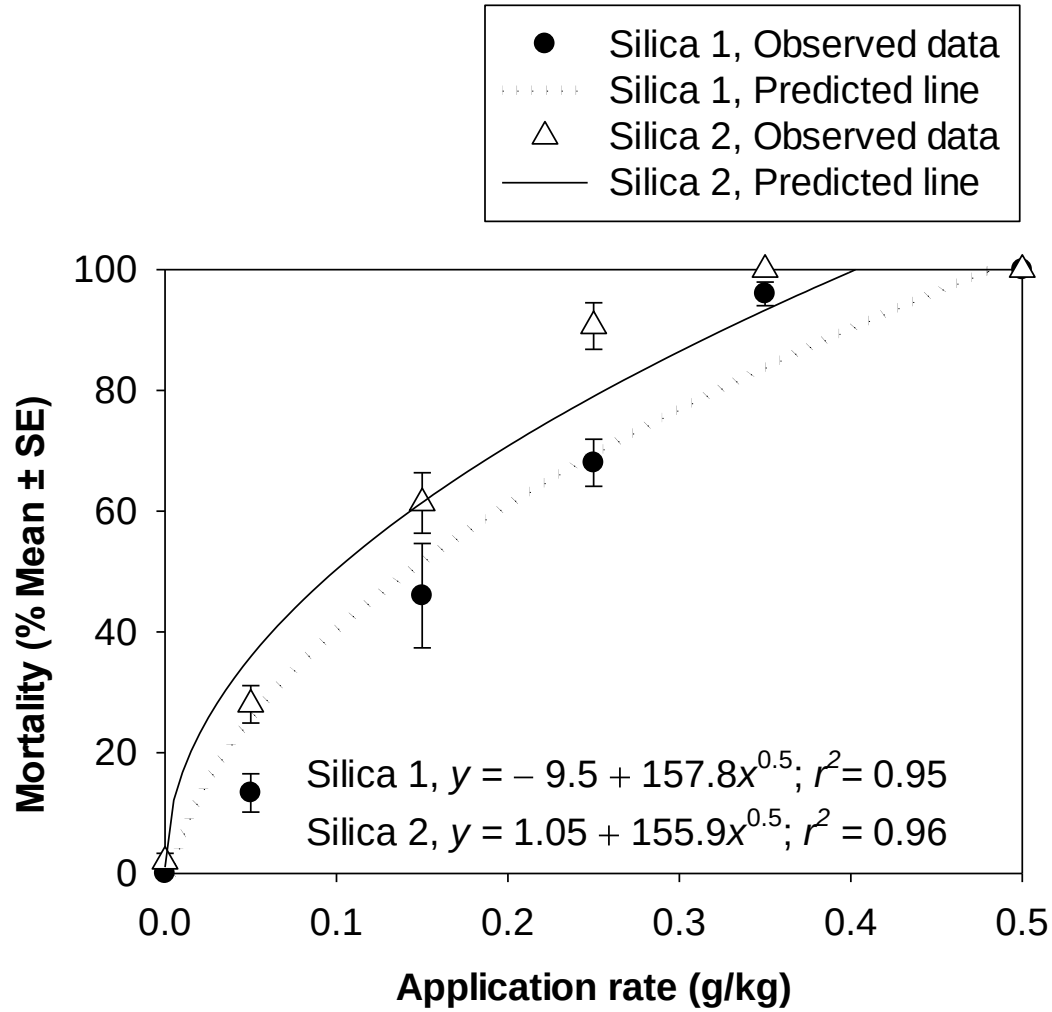


Figure 9.2. Percentage mortality of *T. castaneum* adults at 14 d after exposure to concrete arenas treated to silica powders 1 and 2 at different application rates and exposure periods.

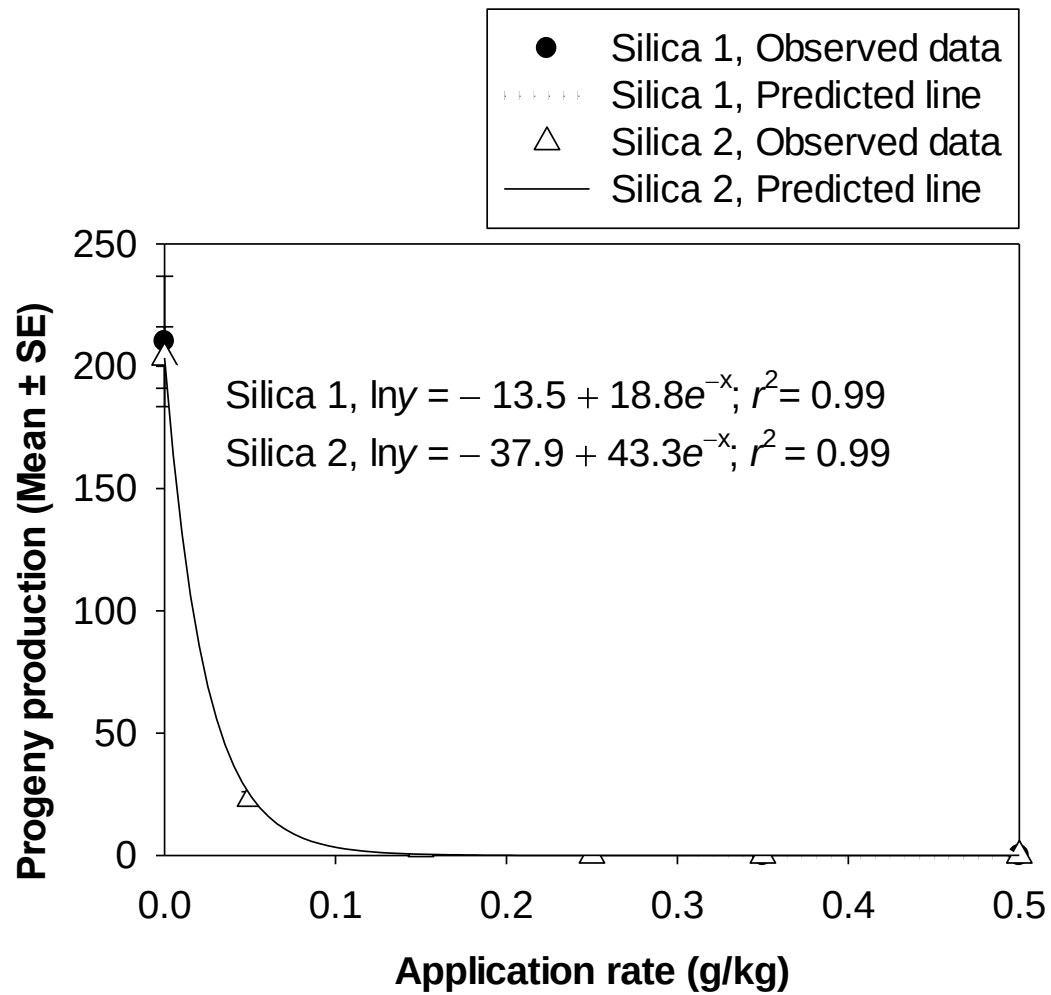


Figure 9.3. Adult progeny production from *O. surinamensis* adults at 42 d after exposure to concrete arenas treated to silica powders 1 and 2 at different application rates and exposure periods.

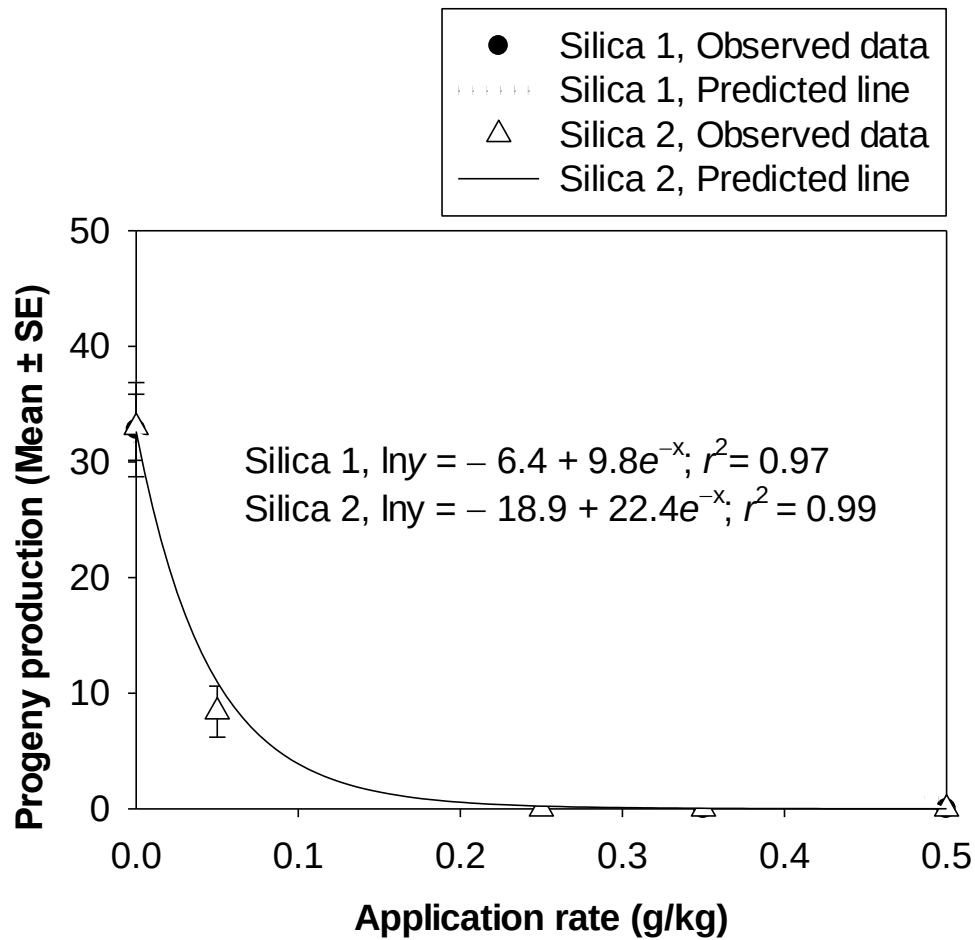


Figure 9.4. Adult progeny production from *T. castaneum* adults at 42 d after exposure to concrete arenas treated to silica powders 1 and 2 at different application rates and exposure periods.

Table 9.1. Pairwise comparison of non-linear model fitted for adult mortality responses and adult progeny production between species.

Comparison	<i>F</i> - value	df	<i>P</i> - value
Mortality			
Silica 1 - OS vs TC	3.94	2, 8	0.0644
Silica 2 - OS vs TC	2.42	2, 8	0.1503
Progeny production			
Silica 1 - OS vs TC	520.61	2, 8	<0.0001*
Silica 2 - OS vs TC	6704.62	2, 8	<0.0001*

OS -*Oryzaephilus surinamensis*, TC – *Tribolium castaneum*

*Significant at $P < 0.05$

Chapter 10 - Effect of two silica powders 1 and 2 on the physio-mechanical properties of hard red spring wheat over time

10.1 Abstract

Diatomaceous earths are gaining popularity owing to increasing consumer awareness over consumption of chemical free foods. However, research pertaining to use of diatomaceous earth powders for stored product protections has seen an increase, the widespread use of diatomaceous earths as grain protectants is still limited due to its adverse effects on physical properties of grain. In the present study, we determined the effect of two amorphous silica powders 1 and 2 procured from Imery's, Lompoc, California, USA treated to wheat on the physical properties and flowability of grains. Wheat grains mixed with 0.35 g/kg of silica powder 1 or silica powder 2 were exposed for 0, 1 and 3 months and determined for grain quality. The result showed that the bulk density of wheat significantly decreased, and the tapped density of wheat increased with increasing exposure to silica powder 1 and 2. The angle of repose, Carr index and Hausner Ratio was high for silica powder treated grains making them transition from good to passable flowability. The FT4 Powder Rheometer analysis showed that silica powder treated wheat required higher Basic Flow Energy, aeration ratio and were less stable compared to control wheat. Overall, the results suggest that silica powders 1 and 2 have potential as grain protectants with minimal impact on grain quality and flowability.

Keywords: Amorphous silica powders, Bulk density, Angle of repose, Carr index, Flowability.

10.2 Introduction

Management of stored product insect pests primarily involves the use of chemical insecticides. However, prolonged dependence on these chemicals has led to control failures, attributed to the emergence of resistance among various species of stored product insects (Subramanyam, 1995; Kljajić, 2014; Nayak et al., 2015). Moreover, in response to increasing consumer demand for chemical-free foods, there has been a surge in the search for viable alternatives to chemical insecticides. Inert dusts, such as diatomaceous earth sourced from fossilized marine algae, which are predominantly composed of amorphous silica, are increasingly being explored as promising substitutes (Subramanyam and Roesli, 2000; Korunić, 2013; Korunić, 1998; Losic and Korunić, 2018; Kılıç, 2022). Diatomaceous earth formulations adhere to insect bodies and cause damage to their epicuticular waxes through absorption, resulting in death by desiccation (Ebeling, 1971; Subramanyam and Roesli, 2000; Korunić, 2016). They have garnered attention for their safety, long-lasting protection (Athanassiou et al., 2005; Vayias et al., 2006), no impact on grain quality, and cost-effectiveness (Korunić et al., 1996a, 1996b; Fields and Korunić, 2000; Korunić, 2016). Ideally, the application of diatomaceous earth formulations in storage environments includes, admixing with grains, structural treatment of walls and bin floors and surface treatment of grain bulks (Korunić, 2013). Majority of diatomaceous earth formulations demonstrate effectiveness at higher application rates, typically ranging from 0.5 g/kg to 3.5 g/kg (Korunić, 1997; Fields and Korunić, 2000; Arthur, 2003; Athanassiou et al., 2005). However, the widespread adoption of these formulations as grain protectants is constrained by the adverse effects, they may induce at higher application rates on the physical and mechanical properties of grains such as the reduction in grain bulk density and flowability and imparting dust residues (Jackson and Webley, 1994; Subramanyam et al., 1994; Golob, 1997).

Grain bulk density plays a pivotal role in grading and assessing the economic value of wheat. Therefore, it is crucial for diatomaceous earth formulations intended for use as grain protectants to minimal impact on the grain quality including bulk density and flowability. Several studies have demonstrated that various diatomaceous earth formulations, applied at different rates, can significantly reduce the bulk densities of treated wheat. Bodroža-Solarov et al. (2012) reported a decrease in bulk density when wheat was treated with zeolite and diatomaceous earth formulations. Even exceptionally low concentrations (0.01 and 0.025 g/kg) of Celite 209 and DiaFil 610 were found to significantly reduce wheat bulk density (Korunic et al., 1996). Treatment with 0.05 g/kg of Dryacide® decreased bulk density and increased the angle of repose for wheat (Korunic et al., 1996). Since diatomaceous earth formulations treated to grains gets adhered to kernel surfaces, this can increase friction between grains, resulting in elevated angles of repose and decreased bulk densities (Korunic et al., 1998). In addition, the milling industry is hesitant to accept diatomaceous earth-treated grains due to concerns about potential damage to machinery (Korunić, 1997, 1998; Subramanyam et al., 1994).

Amorphous silica powders offer significant advantages as grain protectants in stored product protection, particularly because they are already used in food applications as anti-caking agents at concentrations below 1.5% w/w (Anonymous, 1986; Shawir et al., 1988). Moreover, they are considered safe for unlimited daily intake (Anonymous, 1974). These powders demonstrate effectiveness against stored product insects even at lower application rates (Manivannan and Subramanyam, 2023a,b; Manivannan et al., 2023). Additionally, it is crucial for grains treated with diatomaceous earth formulations, such as amorphous silica powders, to exhibit optimal flowability. Good flowability ensures that wheat kernels can exit bins or silos with ease, characterized by minimal particle-to-particle friction and low friction between particles and the

inner wall surfaces of storage containers (Yao et al., 2022). Hence, formulations of inert dust that are effective at lower dosages are essential to mitigate the potential impact of diatomaceous earth formulations on grain quality and food processing environments (Korunic, 1994; Manivannan et al., 2023).

In a previous study, we observed that two new amorphous silica powders, specifically silica powder 2, were effective against lesser grain borer, *Rhyzopertha dominica* (F.) adults at lower application rates of 0.20 g/kg when mixed with wheat (Manivannan et al., 2023). Additionally, 0.30 g/kg of silica powder 1 and 2 achieved complete control of the rice weevil, *Sitophilus oryzae* when mixed with grains for 14 d. However, these two silica powders 1 and 2 were effective, their impact on the physical and mechanical properties of treated wheat has not been thoroughly explored. Therefore, the present study aimed to assess the impact of silica powders 1 and 2, mixed with wheat at 0.35 g/kg and exposed for 0, 1, and 3 months, on the physical and mechanical properties of treated wheat.

10.3 Materials and Methods

10.3.1 Treatment of wheat with silica powders

Two amorphous silica powders 1 and 2 and sourced from Imerys, Lompoc, California, USA were used in this research. Cleaned organic hard red spring winter wheat (Great River Organic Milling Company, Fountain City, Wisconsin, USA) weighing 350 g (13% moisture content, wet basis) were transferred into individual 0.94 L glass jars. Each glass jar was equipped with a 250 µm wire mesh screen and a lid made of filter paper with a diameter of 9 cm to allow for air diffusion. The jars containing the wheat grains were then treated individually with 0 (untreated control) and at 0.35 g/kg of silica powder 1 and silica powder 2. Three sets of samples were prepared for analysis at different time intervals (0-month, 1 month, and 3 months) to assess

the impact of silica powders 1 and 2 on the physical properties of the grains. Each combination of powder-exposure period was replicated three times. After adding the powders to the jars, wheat was manually mixed for 60 seconds for homogeneous coating of the powders. Subsequently, all the jars were placed inside a growth chamber (Pervical Scientific, Inc., Model I-36 VL, located in Perry, Iowa, USA) at 28°C and 65 r.h. until physical property analysis was performed.

10.3.2 Determination of physical properties of wheat kernels

Grain characteristics were measured using the conventional method. The methods used for physical property determination was similar to those mentioned by Yao (2022) with modifications. Fifty wheat kernels were randomly selected from each silica powder treated and untreated jar, and the dimensions such as length (L), width (W), and thickness (T) were determined using a digital Vernier caliper. The kernel size plays a crucial role during the handling, processing, and storage (Masoumi and Tabil, 2003), it was crucial to determine the size of silica-treated kernels and compare them with untreated kernels. The estimated average dimensions of the kernels were used to calculate the size descriptors of the grain and compared with the untreated control. Similarly, each parameter was replicated thrice and expressed as mean $\pm$ SE.

A. Arithmetic mean diameter (Da)

The arithmetic mean diameter (Da), which is the average of the diameter of twenty kernels chosen at random, was estimated using the formula,

$Da = \frac{L+W+T}{3}$ where L, W, and T represent the length, width, and thickness of the kernels, respectively (Mpotokwane et al., 2008).

B. Geometric mean diameter (Dg)

The geometric mean diameter (Dg) of the kernel was calculated using the following relationship $Dg = (L * W * T)^{\frac{1}{3}}$ where L, W, and T are the length, width, and thickness of the kernels, respectively (Davis and El-Okene, 2009).

C. Surface area (S)

The surface area (S) of the wheat kernels were determined by the following formula, $S = \pi Dg^2$ where Dg is the geometric mean diameter of the kernel (Kallemullah and Gunasekar, 2002).

D. Aspect ratio (Ra)

The aspect ratio (Ra) of the wheat kernels were determined by the given formula, $Ra = \left(\frac{W}{L}\right) \times 100$, where W and L are the width and length of the kernels, respectively (Maduako and Faborode, 1990).

E. Volume (V)

The unit volume of the individual kernel was determined using the following formula, $V = LWT/6$, where L, W, and T are the length, width, and thickness of the kernels, respectively.

F. Bulk density (ρ_b)

Bulk density of wheat was determined using a standard Winchester cup arrangement. The grains were filled in a pint cup and leveled using a measurement scale and the weight of the sample was determined. The volume of the pint cup and weight of the grains accommodated within the pint cup were used to determine the bulk density of the grain.

$$\text{Bulk Density} = \frac{\text{Sample weight}}{\text{Volume}}$$

G. Tapped density (Td)

The tapped density is the ratio of the sample weight to the volume of the grains after tapping for a known number of times. The tap density was determined by using an Autotap density analyzer (Quantachrome Instruments, Boynton Beach, FL, USA). For this purpose, a known volume of grains was filled inside a graduated cylinder. The cylinder with grains was tapped 750 times at the rate of 260 taps per minute. The ratio of sample weight to the tapped volume of the grains was used to determine the tapped density.

10.3.3. Determination of flow property of wheat

A. Carr index (CI) and Hausner Ratio (HR)

The Carr index and Hausner ratio of wheat was estimated from the bulk and tapped density values.

$$CI = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

$$HR = \frac{\text{Tapped density}}{\text{Bulk density}} \times 100$$

B. Angle of repose (θ)

A standard Winchester cup arrangement was used to measure the angle of repose of the wheat and maize samples. The known amount of sample was initially put onto the hopper, and a horizontal surface, usually a construction material (iron, concrete), was placed 10 cm below the opening of the hopper. The material was allowed to fall freely from the hopper to form a heap. The height (H) and diameter (D) of the heap/cone were measured, and the angle of repose was determined using the equation below,

$$\theta = \tan^{-1} \left[\frac{2H}{D} \right]$$

10.3.4. Determination of flow properties using FT4 Powder Rheometer

The FT4 Powder Rheometer was used to determine the dynamic and bulk flow properties of grains mixed with silica powders. The functioning of the FT4 Powder Rheometer and its use to determine the flow characteristics were earlier described by Lindberg et al. (2004); Freeman (2007); Leturai et al. (2014). The grain flow properties were determined by displacing wheat kernels in a controlled fashion.

A. Basic Flowability Energy (BFE) and Stability Index (SI)

It is the energy required to establish a uniform flow for a given volume of wheat. Both BFE and stability index (SI) represent the flow properties of granular material under free surface conditions. The flow energy was estimated from the anticlockwise motion of the blade of 23.5 cm diameter through the wheat grains with a movement from top to bottom of the vessel. About 11 cycles were performed to estimate the BFE, of which, the first 7 cycles involved a blade tip speed of 100 mm/s and the remaining at 70, 40, and 10 mm/s speed, respectively, to determine the effect of segregation of silica powders on the bulk wheat during flow. The BFE and SI were estimated based on the following formulas:

Basic Flow Energy = Flow energy at test 7

$$SI = \frac{\text{Flow energy at test 7}}{\text{Flow Energy at test 1}}$$

B. Variable flow rate (VFR)

The variable flow rate measurement included four conditioning and four testing cycles (Yao, 2018). The testing cycles were conducted in a sequence of decreasing flow rates: 100, 70, 40, and 10 mm/s blade tip speed. The flow rate for silica powder treated wheat will be calculated, providing insight into the change in flow energy requirement when the flow rate is reduced by a factor of 10.

$$VFR = \frac{\text{Energy at test 4}}{\text{Energy at test 1}}$$

C. Aeration ratio (AR)

The bulk wheat would be porous and have air filled in intergranular spaces. However, silica powder-treated grains would be tightly packed. Hence, the air may influence the bulk flow properties of treated grains. Aeration ratio represents the extent by which the basic flowability energy decreased due to aeration at an air velocity of $n \text{ mms}^{-1}$. The aeration ratio was determined by increasing the air flow rate from 0 to 10 mms^{-1} at an increment of 2 mms^{-1} .

10.3.5. Data analysis

All the analysis was performed in triplicates. The physical property data of wheat grains treated to silica powders 1 and 2 were subjected to One-way ANOVA to determine the significance difference between treatments. Mean comparisons were performed using Ryan-Einot-Gabriel-Welsch multiple range (REGWQ) test. All the data analysis was performed using SAS version 9.4 (SAS Institute, 2012).

10.4 Results

10.4.1 Physical property of wheat grains treated to silica powders 1 and 2.

The arithmetic mean diameter $\pm$ SE of kernel sizes in control groups ranged from 4.08 ± 0.12 to 4.24 ± 0.11 mm. For kernels treated with silica powder 1, the arithmetic mean diameter $\pm$ SE ranged from 3.85 ± 0.06 to 3.98 ± 0.02 mm, while for silica powder 2, it ranged from 3.98 ± 0.02 to 4.08 ± 0.12 mm. Two-way ANOVA revealed that arithmetic mean diameter of kernels among treatments were significantly different ($F = 6.17$; $df = 2, 18$; $P = 0.0091$). While the arithmetic mean diameter of kernels among exposure periods ($F = 2.18$; $df = 2, 18$; $P = 0.1418$) and treatment $\times$ exposure period interaction ($F = 1.71$; $df = 4, 18$; $P = 0.1925$) were not significant. One-way ANOVA showed significant variation in kernel diameter among treatments ($F = 2.94$; $df = 8, 18$; $P = 0.0273$), with only the control after 1 month differing significantly from other treatments (Table 10.1).

The geometric mean diameter in control kernels ranged from 1.36 ± 0.04 to 1.42 ± 0.04 mm, while silica powder 1-treated kernels ranged from 1.28 ± 0.02 to 1.36 ± 0.01 mm, and silica powder 2-treated kernels ranged from 1.32 to 1.36 ± 0.01 mm. Two-way ANOVA showed that geometric mean diameter among treatments were significant ($F = 6.04$; $df = 8, 18$; $P = 0.0098$). While geometric mean diameter of kernels among exposure periods ($F = 2.49$; $df = 2, 18$; $P =$

0.1109) and treatment x exposure period interaction ($F = 1.80$; $df = 4, 18$; $P = 0.1726$) were not significant. One-way ANOVA also revealed significant differences in geometric mean diameter among treatments ($F = 3.03$; $df = 8, 18$; $P = 0.0240$), with only the control after 1 month differing significantly from others (Table 10.1).

The surface area of control kernels ranged from 5.83 ± 0.35 to 6.29 ± 0.33 , while silica powder 1 treated kernels ranged from 5.49 ± 0.01 to 5.84 ± 0.07 , and silica powder 2 treated kernels ranged from 5.17 ± 0.17 to 5.78 ± 0.10 . Two-way ANOVA indicated that surface of kernels among treatments were significant ($F = 6.04$; $df = 8, 18$; $P = 0.0098$). While surface area of kernels among exposure periods ($F = 2.07$; $df = 2, 18$; $P = 0.1556$) and treatment x exposure period interaction ($F = 1.58$; $df = 4, 18$; $P = 0.2237$) were not significant. One-way ANOVA indicated significant differences among treatments ($F = 2.82$; $df = 8, 18$; $P = 0.0324$), with only the control after 1 month differing significantly from others.

Two-way ANOVA indicated that aspect ratio of kernels among treatments ($F = 3.32$; $df = 2, 18$; $P = 0.0594$), among exposure periods ($F = 1.71$; $df = 2, 18$; $P = 0.2092$), and the treatment x exposure period interaction ($F = 0.49$; $df = 4, 18$; $P = 0.7416$) were all not significant. Two-way ANOVA indicated that volume of kernels among exposure periods ($F = 3.70$; $df = 2, 18$; $P = 0.0451$) was significant, but the volume of kernels among exposure periods ($F = 2.12$; $df = 2, 18$; $P = 0.1488$) and the treatment x exposure period interaction ($F = 2.03$; $df = 4, 18$; $P = 0.1338$) were not significant (Table 10.1).

10.4.2. Physical and flow properties of wheat treated with silica powders 1 and 2

A. Bulk density

The mean $\pm$ SE bulk density of wheat in controls ranged from 790.96 ± 1.70 to 791.57 ± 1.85 kg/m³. The mean $\pm$ SE bulk density of wheat treated with silica powder 1 ranged from

691.08 $\pm$ 1.29 to 716.43 $\pm$ 2.99 kg/m³. The mean $\pm$ SE bulk density of wheat treated with silica powder 2 ranged from 702.85 $\pm$ 2.39 to 736.20 $\pm$ 5.75 kg/m³. Two-way ANOVA showed that the bulk density of kernels among treatments ($F = 515.72$; $df = 2, 18$; $P < 0.0001$), among exposure periods ($F = 29.06$; $df = 2, 18$; $P < 0.0001$), and the treatment x exposure period interaction were all significant ($F = 6.33$; $df = 2, 18$; $P = 0.0023$). One-way ANOVA showed significant differences in bulk density of wheat among treatments ($F = 3.03$; $df = 8, 18$; $P = 0.0240$); REGWQ test (Table 10.2). The bulk density of wheat showed a significant decrease after exposure to silica powders 1 and 2, regardless of the exposure period. This decrease in bulk density was observed to be correlated with the duration of exposure to the powders. Specifically, it was noted that wheat bulk density decreased by 12% after three months of exposure to 0.35 g/kg of silica powder 1 and by 11% after three months of exposure to 0.35 g/kg of silica powder 2, compared to control samples.

B. Tapped density

The mean $\pm$ SE tapped density of wheat in controls ranged from 820.38 $\pm$ 0.31 to 822.37 $\pm$ 0.56. The mean $\pm$ SE tapped density of wheat treated with silica powder 1 ranged from 843.37 $\pm$ 4.50 to 896.49 $\pm$ 3.40. The mean $\pm$ SE tapped density of wheat treated with silica powder 2 ranged from 841.14 $\pm$ 3.19 to 897.83 $\pm$ 6.19. Two-way ANOVA showed that the tapped density of kernels among treatments ($F = 64.93$; $df = 2, 18$; $P < 0.0001$), among exposure periods ($F = 30.86$; $df = 2, 18$; $P < 0.0001$), and the treatment x exposure period interaction were all significant ($F = 8.50$; $df = 2, 18$; $P = 0.0005$). One-way ANOVA showed significant differences in tapped density of wheat among treatments ($F = 28.20$; $df = 8, 18$; $P < 0.0001$); by REGWQ test. The tapped density of wheat kernels exhibited a significant increase ($P < 0.05$) following exposure to 0.35 g/kg of silica powders 1 and 2 in comparison to control samples. Notably, after

one month, the tapped density of control samples differed significantly from that of wheat treated with silica powder 1 and silica powder 2. A similar trend was observed after three months of exposure to 0.35 g/kg of silica powder 1 and 2 (Table 10.2).

C. Carr index

The Carr's index values among treatments ($F = 512.10$; $df = 2, 18$; $P < 0.0001$), among exposure periods ($F = 90.25$; $df = 2, 18$; $P < 0.0001$), and the treatment x exposure period interaction were all significant ($F = 22.03$; $df = 2, 18$; $P < 0.0001$); by Two-way ANOVA. One-way ANOVA showed significant differences in Carr's index of wheat kernels among treatments ($F = 188.86$; $df = 8, 18$; $P < 0.0001$); by REGWQ test. There was a significant difference in Carr's index values between wheat treated with silica powder 1 and 2 compared to the control, regardless of the exposure period. Carr's index values were significantly higher were noted for wheat treated with silica powder 1 compared to those treated with silica powder 2 at 0 month. Furthermore, a significantly higher Carr's index value ($P < 0.05$) was recorded for grains treated with 0.35 g/kg of silica powder 1 and silica powder 2 after a 3-month exposure, in contrast to those treated for 0 and 1 month, respectively. These findings suggest a reduction in powder flowability with prolonged exposure to silica powders 1 and 2 based on the Carr's index values > 21.

D. Hausner ratio

The Hausner's ratio values among treatments ($F = 633.53$; $df = 2, 18$; $P < 0.0001$), among exposure periods ($F = 81.61$; $df = 2, 18$; $P < 0.0001$), and the treatment x exposure period interaction were all significant ($F = 20.15$; $df = 2, 18$; $P < 0.0001$); by Two-way ANOVA. One-way ANOVA showed significant differences in Hausner ratio values of wheat among treatments ($F = 161.60$; $df = 8, 18$; $P < 0.0001$); by REGWQ test. There was a significant difference in the

Hausner ratio observed between the control group and other treatment groups, regardless of the exposure duration. Significantly higher ($P < 0.05$) values were noted when wheat was subjected to silica powder 1 and 2 for 3 months compared to those treated for 0 month. The significantly higher Hausner ratio observed for treatments after 3 months indicated a reduction in wheat flowability following exposure to silica powder 1 (1.30 ± 0.05) and 2 (1.28 ± 0.01), respectively.

E. Angle of repose

The angle of repose of wheat among treatments ($F = 55.69$; $df = 2, 18$; $P < 0.0001$), among exposure periods ($F = 7.49$; $df = 2, 18$; $P = 0.0043$), and the treatment x exposure period interaction were all significant ($F = 3.49$; $df = 2, 18$; $P = 0.0281$); by Two-way ANOVA. One-way ANOVA showed significant differences in angle of repose of wheat among treatments ($F = 17.54$; $df = 8, 18$; $P < 0.0001$); by REGWQ test. There was a significant difference in the angle of repose observed between the control group and other treatment groups, regardless of the exposure duration. Angle of repose was significantly higher ($P < 0.05$) when wheat was subjected to silica powder 1 and 2 for 3 months compared to those treated for 0 month. A significantly higher angle of repose values was observed for silica powder 1 treatment after 3 months ($\theta = 41.07$) compared to other treatment and control groups.

10.4.3. Flow property analysis of wheat by FT4 Powder Rheometer

The BFE for untreated control groups ranged from 805.03 ± 1.71 to 808.41 ± 1.23 , while silica powder 1 treated wheat ranged from 862.47 ± 14.91 to 914.06 ± 4.38 , and silica powder 2 treated kernels ranged from 829.52 ± 2.81 to 937.88 ± 16.70 . Two-way ANOVA showed that the angle of repose of wheat among treatments ($F = 105.27$; $df = 2, 18$; $P < 0.0001$), among exposure periods ($F = 33.78$; $df = 2, 18$; $P < 0.0001$), and the treatment x exposure period interaction were all significant ($F = 12.21$; $df = 2, 18$; $P < 0.0001$). One-way ANOVA showed

significant differences in BFE required for displacement of wheat kernels among treatments ($F=188.86$; $df=8, 18$; $P < 0.0001$); by REGWQ test. A maximum flow energy of 914.06 ± 4.38 and 937.88 ± 16.70 was required for the displacement of grains treated with silica powders 1 and 2 for 3 months. The BFE energy for control samples were consistently lower than silica powder 1 and 2 treated wheat, regardless of the exposure period (Fig 10.1). The BFE for silica powder 2 after 3 months, was significantly different from other treatments. The stability index values among treatments were significantly different ($F=17.74$; $df=8, 18$; $P < 0.0001$); by REGWQ test. However, the values for all the treatments including the treatment with silica powder 1 and 2 were within 0.9 to 1.1 indicating good stability, except after 3 months of exposure to silica powder 1 and 2 which were > 1.1 (Fig 10.2).

The aeration ratio among treatments were significantly different ($F=99.37$; $df=8, 18$; $P < 0.0001$); by REGWQ test. The aeration ratio for silica powder treated wheat was consistently higher than control groups, regardless of the exposure period (Fig 10.3). The aeration values for silica powder 1 and 2 treated grains were greater than 2 indicating that the wheat samples were sensitive to air flow. Silica powder 2 treated grains after exposure for 3 months had significantly higher compared to other treatment combinations. However, One-way ANOVA showed significant differences in flow rate index among treatments ($F=188.86$; $df=2, 18$; $P < 0.0001$), a significant difference was observed only between silica powder 2 treated grains exposed for 3 months and other treatments (Fig 10.4).

10.5 Discussion

Silica-based inert powders are considered as promising alternatives to chemical grain protectants. However, their adoption as grain protectants is constrained due to the adverse effects inert dusts can have on treated grains. Numerous studies have evaluated the insecticidal activity of various diatomaceous earth formulations from different geographic locations when mixed with grains (Arthur, 2002; Athanassiou et al., 2003; Athanassiou et al., 2008; Demissie et al., 2008; Nukenine et al., 2010; Jean et al., 2015; Fazlabad et al., 2017; Liška et al., 2017; Balliota and Athanassou, 2020; Agrafioti et al., 2023; Manivannan et al., 2023). However, while many studies have reported the efficacy of diatomaceous earth formulations at lower application rates, few have assessed their impact on grain quality.

The application rate of 0.35 g/kg of silica powder 1 and 2 used in the study for treating wheat is lower than the recommended application rates of 0.5 to 3.5 g/kg for diatomaceous earth formulations (Korunić, 1997; Fields and Korunić, 2000; Arthur, 2003; Athanassiou et al., 2005). Our observations revealed that treating wheat at this application rate and exposing it for 0, 1, and 3 months did not have a significant influence on the physical properties of treated wheat compared to untreated controls. While the arithmetic mean diameter, geometric mean diameter, and surface area of grains in control samples exhibited slight variations from treated samples, the differences were significant only for control samples analyzed after 1 month comparison to the treatments. Similarly, the aspect ratio values for wheat kernels treated with silica powder 1 and 2 did not exhibit significant variation. We did not sort the grains based on size for physical property analysis, as it would reflect field-level implications. Consequently, we observed wide variation in kernel characteristics even in the control samples. This variability in kernel size

could explain the lack of significant differences observed between silica powder-treated wheat and untreated controls in terms of kernel characteristics.

It was observed that the bulk density of wheat in silica powder 1 and silica powder 2 treated wheat significantly reduced at all exposure periods compared to untreated control. The exposure of wheat to silica powder 1 and silica powder 2 at 0.35 g/kg for 3 months reduced the bulk densities of wheat by 12 and 11%, respectively. We also observed that the maximum reduction of bulk density was observed in wheat treated with silica powder 1 compared to silica powder 2. And there was a significant variation in bulk density values among exposure periods, indicating that prolonged exposure of wheat to silica powders can reduce their bulk densities considerably. The higher reduction in bulk density of silica powder 1 compared to silica powder 2 can be attributed to the smaller particle diameter ($D_{90} = 26.30 \mu\text{m}$) of silica powder 1 compared to a D_{90} values of $31.43 \mu\text{m}$ for silica powder 2 (Manivannan and Subramanyam, 2023).

It was observed that treatment of wheat, barley, oats, rye or corn to 0.5 g/kg of diatomaceous earth reduced their bulk densities by 60 kg/m^3 (Korunic et al., 1998). Korunic and Mackay (2000) reported that mixing hard red spring wheat with 0.5 and 0.75 g/kg of Protect-It reduced the bulk density by 49 and 52 kg/m^3 , respectively. The significant reduction in the bulk density values in wheat treated to silica powder 1 and 2 could be attributed to the duration of exposure to the grains. In addition, none of the studies determined the bulk density of wheat after 3 months of exposure to diatomaceous earth like the present study. Differences in bulk density reduction among diatomaceous earth sources can vary by as much as fourfold (Korunic et al., 1998). Moreover, the most effective insecticidal diatomaceous earths also tend to cause the greatest reduction in bulk densities (Korunic, 1997). So, the significant decrease in the bulk density of wheat treated to silica powder 1 and 2 can be linked to their higher insecticidal activity

to stored product insects. We observed that the tapped density of wheat increased significantly in silica powder 1 and silica powder 2 after 1 month and 3 months of exposure. Notably there was more than 8% increase in tapped density in wheat treated with silica powder 1 and 2, respectively after 3 months. Yao et al. (2022) observed increased tapped density with decreasing bulk density of wheat treated to 2.0 g/kg of zeolite powder at 35% moisture content.

The angle of repose for both the silica powder 1 and 2 treated wheat were significantly increased compared to controls. Notably, wheat treated to silica powder 1 for 3 months had a significantly highest increase in angle of repose compared to other treatments. Based on the angle of repose and flowability classification given by Carr (1965), both the silica powder 1 and 2 treated wheat evolved from good into having fair flowability (θ values between 36-40°). While wheat treated with silica powder 1 for 3 months ($\theta = 41^\circ$) evolved from good to passable. Treatment of wheat with 2.0 g/kg of zeolite at 35% moisture content gave an angle of repose (θ) value of 47° evolving from good into poor (Yao et al., 2022).

The Carr Index (CI) and Hausner Ratio (HR) are commonly employed as single index parameters to indirectly evaluate the flowability of a specific powder or granular material. We observed that the Carr index values were below 4 for controls, while silica powder 1 and 2 treated wheat exposed for 3 months had Carr index values between 21 to 22 making the wheat evolve from excellent to passable. Similarly, the Hausner Ratio for wheat treated with silica powder 1 and 2 were significantly higher than controls at all exposure periods. After 3 months, the Hausner Ratio for wheat treated with silica powder 1 and 2 evolved from excellent (1.00 to 1.11) to passable (1.26 to 1.34). This increase in both the Carr Index (CI) and the Hausner Ratio (HR) suggests the presence of a cohesive effects of silica powders specifically after 3 months which exhibits reluctance to flow easily or demonstrates resistance to flow.

Basic Flow Energy is the stabilized energy required to displace a bulk particulate during testing. We observed that the BFE increased with increasing exposures and was significantly higher for both silica powders treated wheat compared to control. Maximum energy was required for the flow of wheat at longer exposure periods. This indicated that the wheat treated with silica powders had high compressibility and low transmission of particle-particle forces making the treated wheat to not flow easily. The Stability Index (SI) values were significantly higher for silica powder 1 and 2 treated wheat after 3 months. Since the SI values for silica powder 1 and 2 treated with after 3 months were > 1.1 indicating that the particles were not stable. The probable reason for deviation from stability could be attributed to the segregation of silica powder and wheat during the tests. Since the powders are hygroscopic, they tend to absorb moisture while testing leading to an increase in particle size and creation of surface frictional forces. A similar observation was reported by Yao (2018) in his research involving stability test analysis with wheat treated to 1 g/kg of zeolite. The aeration ratio values closer to 1 indicates that the samples are not sensitive to aeration. The aeration ratio values for all the silica powder treated wheat were > 2.1 indicating that the materials were not sensitive to air. This indicated air flow was required for the flow of silica powder-treated grains regardless of the exposure.

The present study results indicated that the wheat treated with 0.35 g/kg of silica powder 1 and 2 exhibited decreased bulk density and increased tapped density values, related to the duration of exposure to these dusts. The angle of repose, Carr's index and Hausner Ratio values were higher for wheat treated with silica powder 1 and 2 and exposed for 3 months indicating the flowability of wheat would significantly decrease when tested at these rates the wheat making the transition to passable flowability. Flow property analysis by FT4 Powder Rheometer revealed requirement of higher flow energy and aeration ratio for silica powder-treated wheat, indicating

increased sensitivity to air flow. Overall, the results suggest that silica powders 1 and 2 have potential as grain protectants with minimal impact on grain quality and flowability. However, further field research is needed to assess their effects under varying temperature and humidity conditions for optimal implementation in storage facilities.

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Table 10.1. Kernels characteristics from wheat treated with 0.35 g/kg of silica powders 1 and 2 exposed for different periods.

Treatments	Kernel characteristics (Mean $\pm$ SE) ^a				
	Arithmetic	Geometric	Surface area	Aspect ratio	Volume
	mean diameter	mean diameter	(m ²)		
Control - 0 month	4.08 $\pm$ 0.12ab	1.36 $\pm$ 0.04ab	5.83 $\pm$ 0.35ab	46.37 $\pm$ 1.29	9.04 $\pm$ 0.83
Control - 1 month	4.24 $\pm$ 0.11a	1.42 $\pm$ 0.04a	6.29 $\pm$ 0.33a	49.77 $\pm$ 3.40	10.53 $\pm$ 0.85
Control - 3 months	4.11 $\pm$ 0.03ab	1.37 $\pm$ 0.01ab	5.88 $\pm$ 0.09ab	45.44 $\pm$ 3.65	9.19 $\pm$ 0.52
Silica 1 - 0 month	4.07 $\pm$ 0.03ab	1.36 $\pm$ 0.01ab	5.84 $\pm$ 0.07ab	50.36 $\pm$ 0.28	9.32 $\pm$ 0.30
Silica 1 - 1 month	3.96 $\pm$ 0.08ab	1.32 $\pm$ 0.03ab	5.53 $\pm$ 0.06ab	51.70 $\pm$ 2.09	8.35 $\pm$ 0.55
Silica 1 - 3 months	3.85 $\pm$ 0.06ab	1.28 $\pm$ 0.02ab	5.49 $\pm$ 0.01ab	46.46 $\pm$ 2.84	7.68 $\pm$ 0.47
Silica 2 - 0 month	3.98 $\pm$ 0.02ab	1.36 $\pm$ 0.01ab	5.78 $\pm$ 0.10ab	52.18 $\pm$ 1.53	9.56 $\pm$ 0.20
Silica 2 - 1 month	3.97 $\pm$ 0.00ab	1.33 $\pm$ 0.01ab	5.46 $\pm$ 0.21ab	51.69 $\pm$ 0.83	8.68 $\pm$ 0.18
Silica 2 - 3 months	4.08 $\pm$ 0.12ab	1.32 $\pm$ 0.00ab	5.17 $\pm$ 0.17ab	51.44 $\pm$ 0.71	8.64 $\pm$ 0.04

^aMeans in a column followed by different letters are significantly different among treatments ($P < 0.05$, by REGWQ test).

Table 10.2. Physical and flow properties of wheat treated with 0.35 g/kg of silica powders 1 and 2 exposed for different periods.

Treatment	Properties (Mean $\pm$ SE) ^a				
	Bulk density	Tapped density	Carr Index (CI)	Hausner ratio (HR)	Angle of repose (AoR)
Control - 0 month	791.57 $\pm$ 1.85a	821.11 $\pm$ 0.52d	3.60 $\pm$ 0.28e	1.04 $\pm$ 0.01d	32.90 $\pm$ 0.07c
Control - 1 month	796.76 $\pm$ 0.41a	822.37 $\pm$ 0.56d	3.12 $\pm$ 0.04e	1.03 $\pm$ 0.01d	33.03 $\pm$ 0.03c
Control - 3 months	790.96 $\pm$ 1.70a	820.38 $\pm$ 0.31d	3.58 $\pm$ 0.22e	1.04 $\pm$ 0.04d	32.99 $\pm$ 0.06c
Silica 1 - 0 month	716.43 $\pm$ 2.99cd	843.37 $\pm$ 4.50bcd	15.05 $\pm$ 0.31c	1.18 $\pm$ 0.01b	36.80 $\pm$ 0.58b
Silica 1 - 1 month	715.78 $\pm$ 4.53cd	868.22 $\pm$ 5.80b	17.56 $\pm$ 0.08b	1.21 $\pm$ 0.03b	36.99 $\pm$ 0.28b
Silica 1 - 3 months	691.08 $\pm$ 1.29e	896.49 $\pm$ 3.40a	22.91 $\pm$ 0.26a	1.30 $\pm$ 0.05a	41.07 $\pm$ 1.56a
Silica 2 - 0 month	736.20 $\pm$ 5.75b	841.14 $\pm$ 3.19cd	12.48 $\pm$ 0.53d	1.14 $\pm$ 0.01c	36.78 $\pm$ 0.75b
Silica 2 - 1 month	725.55 $\pm$ 5.79bc	856.47 $\pm$ 13.42bc	15.25 $\pm$ 1.29c	1.18 $\pm$ 0.02b	37.27 $\pm$ 0.71b
Silica 2 - 3 months	702.85 $\pm$ 2.39de	897.83 $\pm$ 6.19a	21.71 $\pm$ 0.78a	1.28 $\pm$ 0.01a	38.21 $\pm$ 0.17b

^aMeans in a column followed by different letters are significantly different among treatments ($P < 0.05$, by REGWQ test).

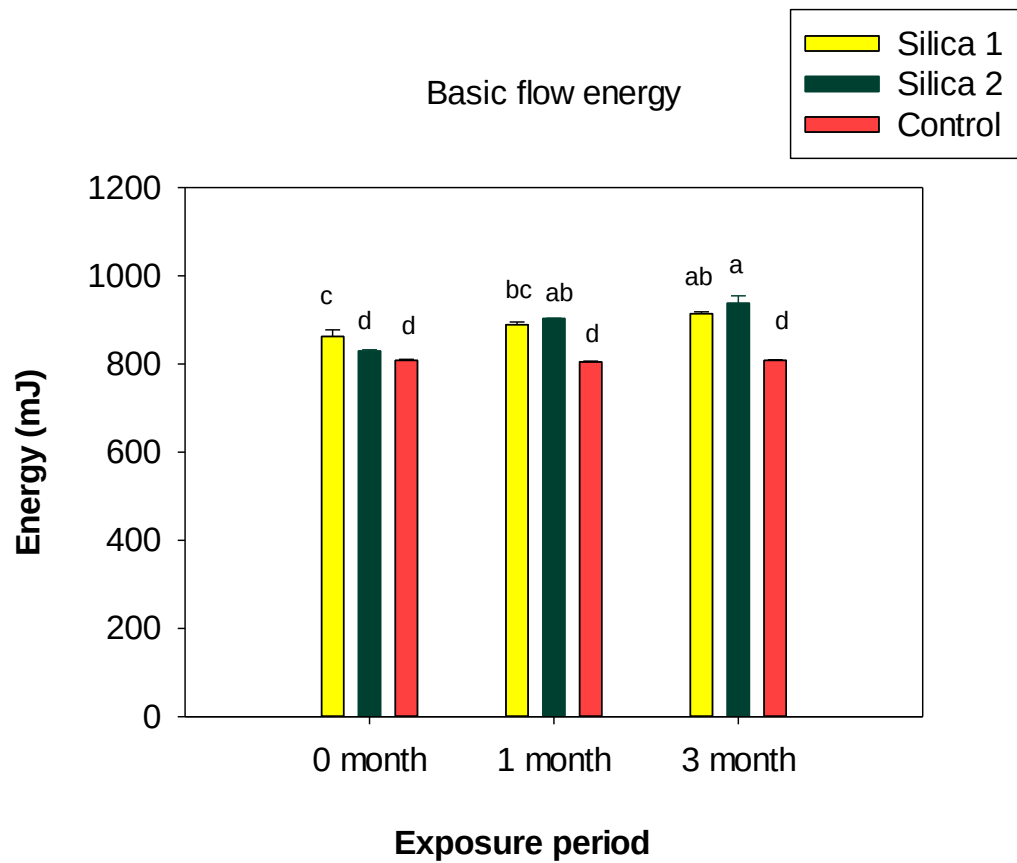


Figure 10.1. Basic flow energy of wheat treated to 0.35 g/kg of silica powder 1 and 2 for 0, 1 and 3 months.

Means in a column followed by different letters are significantly different among treatments ($P < 0.05$, by REGWQ test).

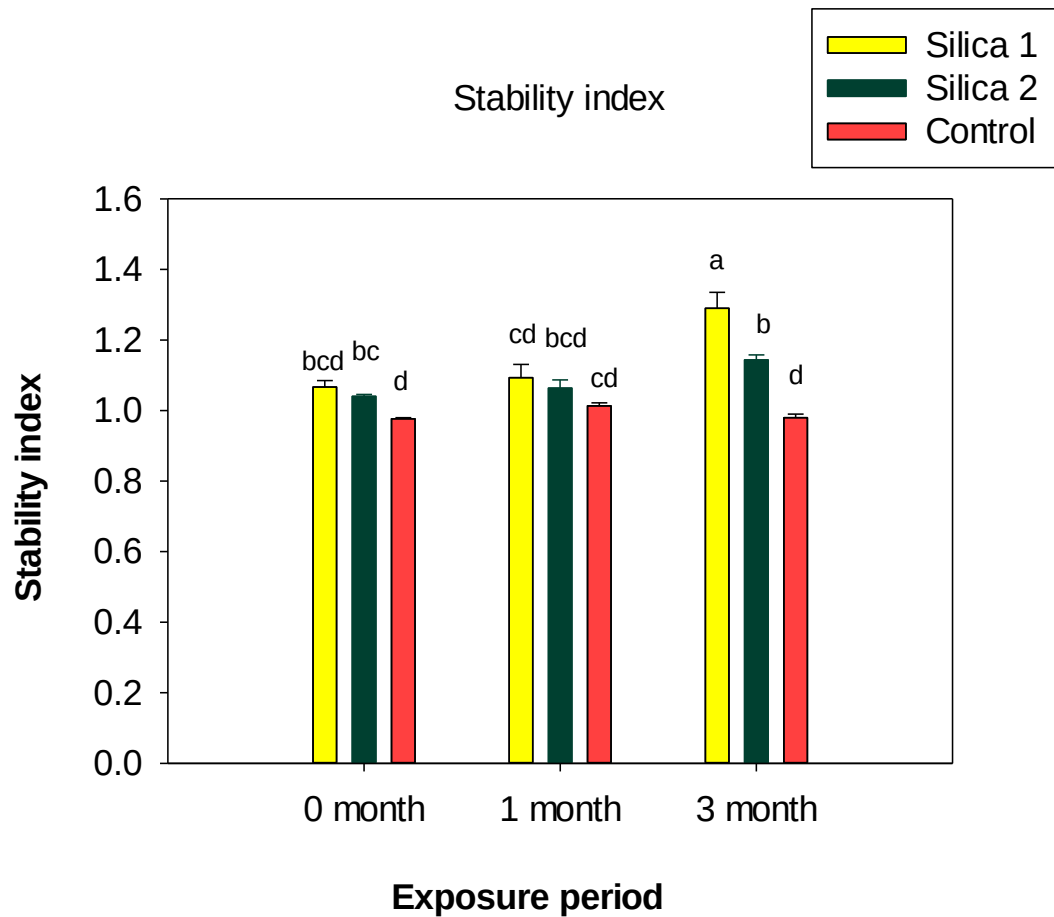


Figure 10.2. Stability index of wheat treated to 0.35 g/kg of silica powder 1 and 2 for 0, 1 and 3 months.

Means in a column followed by different letters are significantly different among treatments ($P < 0.05$, by REGWQ test).

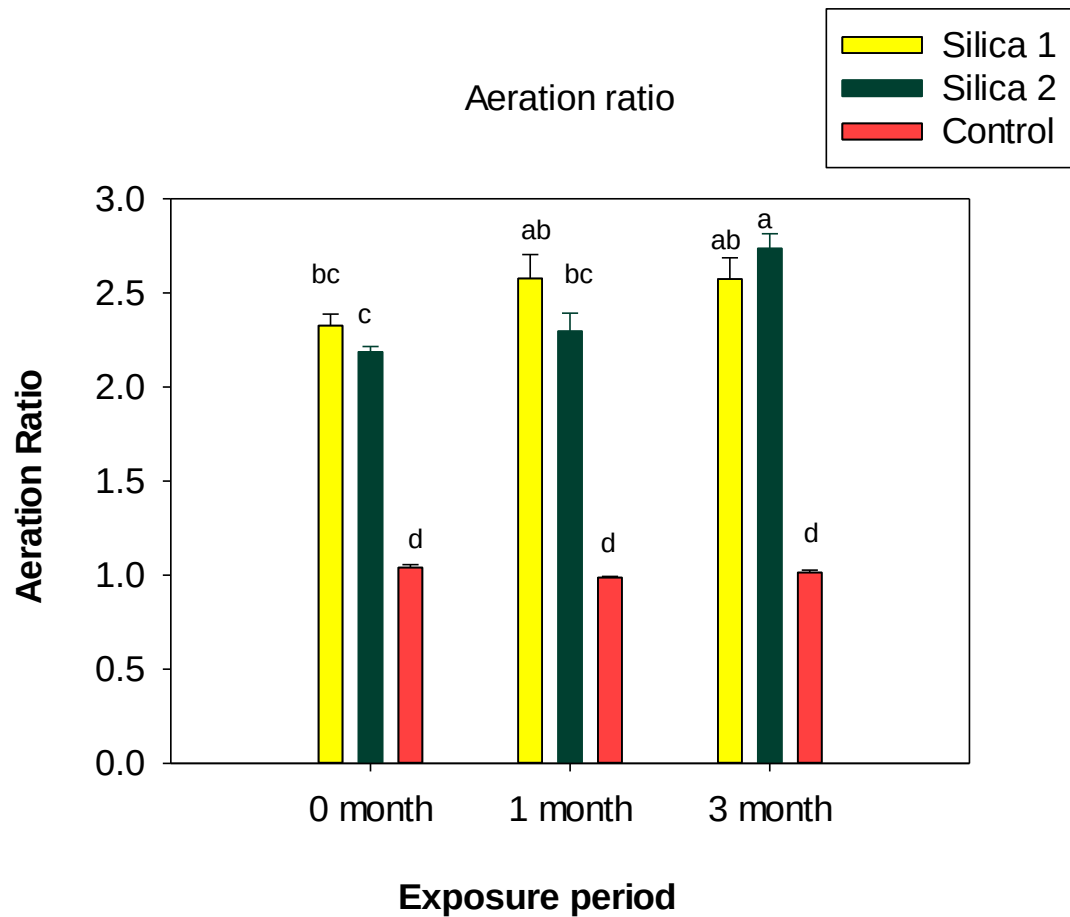


Figure 10.3. Aeration ratio of wheat treated to 0.35 g/kg of silica powder 1 and 2 for 0, 1 and 3 months.

Means in a column followed by different letters are significantly different among treatments ($P < 0.05$, by REGWQ test).

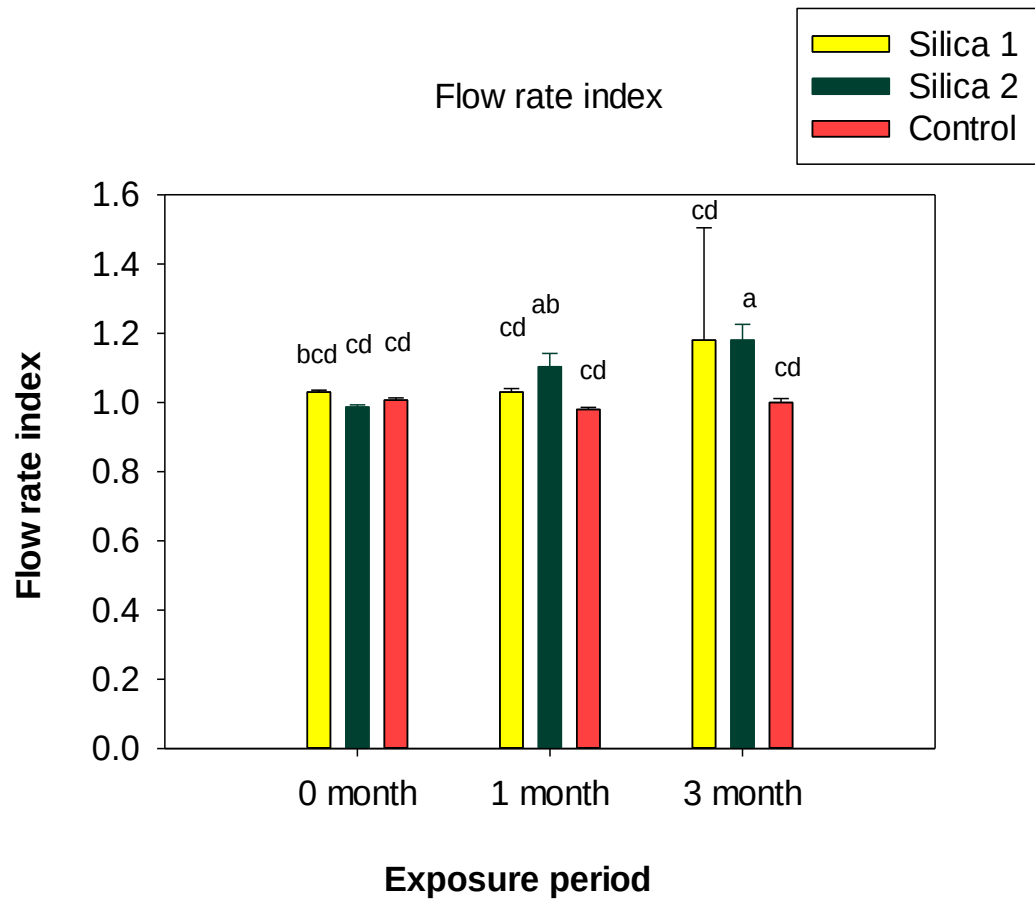


Figure 10.4. Flow rate index of wheat treated to 0.35 g/kg of silica powder 1 and 2 for 0, 1 and 3 months.

Means in a column followed by different letters are significantly different among treatments ($P < 0.05$, by REGWQ test).

Chapter 11 - Efficacy of two amorphous silica powders at different relative humidities, and evaluate Gravista-D plus silica powder combination against the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)

11.1 Abstract

Diatomaceous earth formulations have emerged as promising candidates due to their natural, environmentally friendly nature and broad-spectrum effectiveness against insects. This study evaluated the efficacy of two amorphous silica powders procured from Imerys, Lompoc, California, USA, against the rice weevil, *Sitophilus oryzae* (L.), exposed to different application rates, 0 to 0.35 g/kg treated at different relative humidities. Additionally, the study investigated the effectiveness of these silica powders in combination with Gravista-D, a registered grain protectant at two application rates of 0.18 g/kg and 0.09 g/kg. Results show that both silica powders induced significant adult mortality and suppressed adult progeny production, insect-damaged kernels and grain weight loss caused by *S. oryzae* at lower relative humidities of $30 \pm 2\%$ r.h and at $50 \pm 5\%$ r.h than at higher relative humidity. The combination of silica powders with Gravista-D gave more than 98% at the recommended application rate. While at 0.09 g/kg of Gravista-D, the insecticidal efficacy of sub-lethal doses of silica powder 1 and 2 resulted in complete mortality and suppression of progeny production, insect-damaged kernels and grain weight loss caused by *S. oryzae*.

Keywords: Amorphous silica powders, relative humidity, Gravista-D, Insect-damaged kernels, grain weight loss, Efficacy assessment.

11.2 Introduction

Traditional synthetic chemical pesticides are frequently utilized to address insect infestations in bulk grain. Nonetheless, the increasing concerns over the pesticide residues in food grains and the emergence of chemical resistance among stored product insects have increased the demand for search for viable alternatives (Subramanyam, 1995). Diatomaceous earth formulations offer a promising solution, serving as natural inert materials that are environmentally friendly and effective against a broad spectrum of insects. They possess long-term stability and do not leave behind any hazardous residues (Korunic, 2016). Diatomaceous earth's effect on insects is attributed to the adsorption of epicuticular lipids upon direct contact, ultimately resulting in death due to desiccation (Ebeling, 1971; Subramanyam and Roesli, 2000). The insecticidal activity of diatomaceous earth relies on various factors, including the application rate, duration of exposure, treatment environment, grain type, and the specific life stages of the targeted insect species (Athanassiou et al., 2006; Kavallieratos et al., 2007; Zeni et al., 2021). It is well reported that the efficacy of the diatomaceous earth formulations can be affected by changes in the relative humidity. Arthur (2000) evaluated the effect of temperature and humidity on the mortality of red flour beetle, *Tribolium castaneum* (Herbst) exposed for 8-72 h to 0.5 g/m² of Protect-It. He observed that exposing adults of *T. castaneum* at 32 °C for 48 h gave 100, 98 and 40% mortality at 40, 57, and 75% r.h. respectively. Vayias and Athanassiou (2004) investigated the susceptibility of larvae of the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), to diatomaceous earths (DEs). Their study revealed that the effectiveness of commercial DEs diminished with an increase in relative humidity (RH), transitioning from 55% to 65%. In contrary, few studies have shown that the effectiveness of DEs was relatively unaffected by elevated relative humidity (RH), indicating

that specific types of DEs may exhibit minimal interaction with moisture (Zeni et al., 2021). The diatomaceous earth formulations are effective at higher application rates. Hence the recommended rates vary between 0.5 g/kg to 3.5 g/kg (Korunić, 1997) for diatomaceous earths used for stored product protection. Diatomaceous earth is not widely preferred for mixing with grains considering the challenges associated with their use. Diatomaceous earth treatment with grains can impact on grain flowability, reduction in bulk density and grading value, variations in insect species' susceptibility to DEs, notable influences of grain moisture and temperature on DE efficacy, and the substantial variation in DE effectiveness against insects across different commodities (Korunic, 2016).

Amorphous silica powders have greater potential to be used for stored product protection considering they are generally recognized as safe (GRAS) and have unlimited daily uptake (Anonymous, 1974). Previous studies have shown that the two amorphous silica powders 1 and 2 exhibit promising insecticidal activity at lower application rates on concrete surfaces (Manivannan and Subramanyam, 2023a) and on grains (Manivannan et al., 2023) against the lesser grain borer, *Rhyzopertha dominica* Fabricius. However, these formulations were found to be effective at lower application rates on stored product insects, the most effective insecticidal diatomaceous earths also tend to cause the greatest reduction in bulk densities in treated commodities (Korunic, 1997). Therefore, integrating diatomaceous earth formulations with other Integrated Pest Management strategies can help minimize dust application rates for grain treatment. For instance, the efficacy of inert dust formulations such as the Dryacide[®], Pennac-P[®], Natural Guard[®], Concern[®], Insecto[®], and Protect-It[®] were augmented when combination with heat treatments at 34 and 50 °C against *T. castaneum* (Dowdy, 1999). Similarly, Diafil 610 was used in combination with a biofungicide such as *Metarhizium anisopliae* against many stored

product insect species including, *R. dominica*, *T. castaneum*, the rusty grain beetle, *Cryptolestes ferrugineus*, (Stephens, 1831) and the book louse, *Liposcelis paeta* Pearman (Ashraf et al., 2017). The DE Celite was evaluated for its efficacy against the maize weevil, *Sitophilus zeamais* Motschulsky, *R. dominica* and *T. castaneum* when combined with deltamethrin (Korunic and Rozman, 2010). These studies highlight the importance of diatomaceous earth treatments in combination with other methods for effective control of stored product insects. In this study, we evaluated the efficacy of two amorphous silica powders 1 and 2 procured from Imerys, Lompoc, California, USA against the rice weevil, *Sitophilus oryzae* (L.) exposed at three different relative humidities. We further determined the efficacy of these two silica powders 1 and 2 in combination with Gravista-D powder at two different application rates against *S. oryzae*.

11.3 Materials and Methods

Two amorphous silica powders, referred to as silica powder 1 and silica powder 2 with distinct elemental compositions and particle diameters (Manivannan and Subramanyam, 2023a) supplied by Imerys, Lompoc, California, USA were used in this study. Gravista-D powder (S-methoprene – 0.64%, deltamethrin – 0.26% and piperonyl butoxide – 7.47% and other ingredients- 91.63%) was procured from Central Garden and Pet Company, Schaumburg, Illinois, USA.

11.3.1 Insect rearing

Laboratory insect cultures of *S. oryzae* were reared using adults isolated from stock cultures maintained at the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA, since 1999. Cultures of *S. oryzae* were reared in 0.94 L glass mason jars containing organic hard red spring wheat (Great River Organic Milling Company, Fountain City, Wisconsin, USA). The glass jars were fitted with metal lids that had wire mesh screens

(250 µm) and 9 cm diameter filter paper lids. The insect culture jars were held inside a controlled temperature/humidity chamber at 28°C and 65% r.h. (Pervical Scientific, Inc., Model I-36 VL, Perry, Iowa, USA), with a light:dark photoperiod of 14:10 h.

11.3.2 Exposure of *S. oryzae* to silica powder treated grains.

A. Effect of humidity on the insecticidal efficacy of silica powders

Organic hard red spring wheat was placed inside a deep freezer at -13°C to kill any live insects if present. After a week, the grains were cleaned by sieving using a 2.38 mm diameter round-holed aluminum sieve (Seedburo Equipment Company, Des Plaines, Illinois, USA) to separate the damaged kernels and dockage. From this lot, 100 g of cleaned wheat (13% moisture, wet basis) were transferred to individual 0.45 L glass jars. Each glass jar containing wheat grains was treated with silica powder 1 or silica powder 2 at with three different sets each including four different application rates, 0 (untreated control), 0.15, 0.25, and 0.35 g/kg. The glass jars were covered with metal lids that had wire mesh screens (250 µm) and 7 cm diameter filter paper to facilitate air diffusion. Each glass jar was shaken manually by hand for one minute to ensure uniform distribution of silica powders on the wheat surface. Thirty-five unsexed adults (1-4-week-old) of *S. oryzae*, isolated from the culture jars, were introduced into each glass jar and secured with lids. Likewise separate sets of jars were maintained for each of the three relative humidities, 30, 50 and 75 % r.h. Three replications were maintained for each powder-application rate- relative humidity combination. The jars were placed inside three individual chambers at constant temperature of 28°C and with relative humidities of 30, 50, and 75% r.h. Mortality counts were taken after 14 d by sieving wheat from each glass jar using a 2.38 mm circular round-holed aluminum sieve to isolate the adults. The insects which were immobile when prodded with fine camel's hairbrush were considered dead. After the mortality assessments were

performed, both the live and dead insects were transferred back to the jars and were held inside a growth chamber at 28°C and 65% r.h for an additional 28 d. At 42 d, the data on adult progeny produced was counted and the twenty-five parental adults were subtracted from the total counts. The wheat from each powder-application rate- humidity combination was passed through a Boerner[®] divider (Seedburo Equipment Company, Des Plaines, Illinois, USA) five times to obtain a working sample of ≈ 3.5 g. From this working sample, the data on the number of insect-damaged kernels and undamaged kernels were determined. The percentage of grain weight loss caused by *S. oryzae* was estimated using the formula provided by Adams and Schulten (1978) and Boxall (1986).

B. Effect of Gravista plus silica powder combination on S.oryzae.

The procedure followed for the tests to evaluate the Gravista-D plus silica powder combination against *S. oryzae* was similar to those mentioned in section 2.2.1. However, for the combination studies, Gravista was tested alone at two different rates, 0.183 g/kg and 0.092 g/kg and in combination with silica powder 1 or silica powder 2 at application rates of 0 (control), 0.05, 0.15, and 0.25 g/kg. Unlike the procedure mentioned above in section 2.2.1, all the jars were held inside a growth chamber at 28°C and 65% r.h. and observed for mortality after 14 d and progeny counts, insect-damaged kernels and grain weigh loss at 42 d.

11.3.3 Data analysis

The data on percentage mortality, insect-damaged kernels, and grain weight loss were transformed to angular values (Zar, 1984) for analysis using the Statistical Analysis System 9.4 (SAS Institute, 2012). Adult progeny counts were transformed to $\log_{10}(x + 1)$ for statistical analysis (Zar, 1984). Three-way analysis of variance (ANOVA) was performed to determine the effects of silica powders, application rates, relative humidities and their interaction effects. One-

way analysis of variance (ANOVA) was carried out to determine the significant ($\alpha = 0.05$) differences among application rates including control for each powder. Means among application rates were separated using Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test (SAS Institute, 2012).

11.4. Results

11.4.1. Responses of *S. oryzae* to two amorphous silica powders at three relative humidities

A. Adult mortality

The mean $\pm$ SE corrected mortality of *S. oryzae* adults exposed to three different humidities in untreated controls ranged from 0 to $3.8 \pm 3.8\%$ (Table 11.1). The results showed that complete mortality of *S. oryzae* could be achieved when the adults were exposed to 0.35 g/kg of silica powder 1 and silica powder 2 at 28 °C and 30 ± 2 and $50 \pm 5\%$ r.h. The mean $\pm$ SE mortality of *S. oryzae* adults exposed to 0.15 to 0.35 g/kg of silica powder 1 for 14 d at $75 \pm 4\%$ r.h ranged from 42.9 ± 12.9 to 100% (Table 1). The mean $\pm$ SE mortality of *S. oryzae* adults exposed to 0.15 to 0.35 g/kg of silica powder 2 for 14 d at $75 \pm 4\%$ r.h ranged from 70.5 ± 2.5 to 100% (Table 11.1). Three-way ANOVA showed that the adult mortality responses of *S. oryzae* between silica powders ($F = 8.09$; $df = 1, 48$; $P = 0.0065$) and among application rates ($F = 800.93$; $df = 3, 48$; $P < 0.0001$) and among relative humidities ($F = 29.88$; $df = 2, 48$; $P < 0.0001$), as well as the powder x application rate ($F = 4.24$; $df = 3, 48$; $P = 0.0097$) and application rate x relative humidity interaction ($F = 1.66$; $df = 6, 48$; $P = 0.1889$) were all significant. However, the powder x relative humidity ($F = 0.55$; $df = 3, 48$; $P = 0.5817$) and powder x application rate x relative humidity interaction ($F = 1.59$; $df = 6, 48$; $P = 0.1707$) interactions were not significant. One-way ANOVA showed that there were significant

differences in mortality response among application rates of silica powder 1 treated at different relative humidities ($F_{\text{range}} = 38.68 - 138.45$; $df = 3, 8$; $P < 0.0001$; REGWQ test) and silica powder 2 ($F_{\text{range}} = 138.18 - 752.29$; $df = 3, 8$; $P < 0.0001$; REGWQ test). However, there was a significant difference in mortality response between control and other application rates of silica powder 1 and 2 at 28 °C and 30 ± 3 or 50 ± 5% r.h, no significant difference was observed between application rates of silica powder 1 and 2.

B. Adult progeny production

The mean ± SE number of *S. oryzae* adult progeny produced in controls at 42 d ranged from 272.3 ± 49.1 to 569.0 ± 82.9 per jar (100 g). The results showed that complete suppression of adult progeny production of *S. oryzae* could be achieved when the adults were exposed to 0.35 g/kg of silica powder 1 and silica powder 2 at 28 °C and at 30 ± 2 and 50 ± 5% r.h. The mean ± SE number of adult progeny produced when *S. oryzae* adults were exposed to 0.15 to 0.35 g/kg of silica powder 1 at 42 d at 75 ± 4% r.h ranged from 3.0 ± 3.0 to 17.3 ± 2.3 (Table 11.2). The mean ± SE number of *S. oryzae* adult progeny produced when adults were exposed to 0.15 to 0.35 g/kg of silica powder 2 at 75 ± 4% r.h at 42 d, ranged from 0 to 23.3 ± 2. Complete suppression of adult progeny production was achieved only when adults were exposed to 0.35 g/kg of silica powder 2 at 28 °C and at 75 ± 4% r.h. Three-way ANOVA showed that the adult progeny production of *S. oryzae* between silica powders ($F = 27.26$; $df = 1, 48$; $P < 0.0001$), among application rates ($F = 279.14$; $df = 3, 48$; $P < 0.0001$) as well as the powder x application rate interaction ($F = 8.77$; $df = 3, 48$; $P < 0.001$) were all significant. However, the adult progeny production data among relative humidities ($F = 2.03$; $df = 2, 48$; $P = 0.1429$), application rate x relative humidity ($F = 0.47$; $df = 6, 48$; $P = 0.8238$), powder x relative humidity ($F = 0.99$; $df = 2, 48$; $P = 0.3378$) and powder x application rate x relative humidity interaction ($F = 2.28$; $df = 6,$

48; $P = 0.0512$) interactions were not significant. One-way ANOVA showed that there were significant differences in adult progeny production data among application rates of silica powder 1 treated at different relative humidities ($F_{\text{range}} = 32.41 - 257.12$; $df = 3, 8$; $P < 0.0001$; REGWQ test) and silica powder 2 ($F_{\text{range}} = 26.67 - 468.98$; $df = 3, 8$; $P < 0.0003$; REGWQ test). There was a significant difference in adult progeny production data between control and other application rates of silica powder 1 and 2 at all relative humidities. At 0.35 g/kg, a significant difference in adult progeny production data was observed between 0.15 and 0.25 g/kg of silica powder 1 and 2 at all relative humidities.

C. *Insect-damaged kernels*

The mean $\pm$ SE percentage insect-damaged kernels caused by *S. oryzae* adults in controls at 42 d ranged from 9.3 ± 0.9 to $15.7 \pm 4.0\%$. The results showed that complete inhibition of insect -damaged kernels could be achieved when the adults were exposed to 0.35 g/kg of silica powder 1 and silica powder 2 at 28 °C and at 30 ± 2 and $50 \pm 5\%$ r.h. Complete inhibition of insect-damaged kernels was achieved only when adults were exposed to 0.35 g/kg of silica powder 2 at 28 °C and at $75 \pm 4\%$ r.h (Table 11.3). Three-way ANOVA showed that the adult progeny production of *S. oryzae* between silica powders ($F = 19.91$; $df = 1, 48$; $P < 0.0001$), among application rates ($F = 104.20$; $df = 3, 48$; $P < 0.0001$) as well as the powder x application rate interaction ($F = 3.52$; $df = 3, 48$; $P = 0.0241$), application rate x relative humidity ($F = 3.72$; $df = 6, 48$; $P = 0.0077$), and powder x application rate x relative humidity interaction ($F = 3.06$; $df = 6, 48$; $P = 0.0396$) interactions were all significant. However, the adult progeny production data among relative humidities ($F = 2.16$; $df = 2, 48$; $P = 0.1295$) and powder x relative humidity ($F = 0.50$; $df = 2, 48$; $P = 0.6106$) were not significant. One-way ANOVA showed that there were significant differences in insect-damaged kernels data among application rates of silica powder 1

treated at different relative humidities ($F_{\text{range}} = 10.11 - 46.85$; $df = 3, 8$; $P < 0.0058$; REGWQ test) and silica powder 2 ($F_{\text{range}} = 3.40 - 40.62$; $df = 3, 8$; $P < 0.0003$; REGWQ test). There was a significant difference in insect-damaged kernels between control and other application rates of silica powder 1 and 2 at all relative humidities. At 0.35 g/kg, a significant difference in insect-damaged kernels was observed between 0.15 and 0.25 g/kg of silica powder 1 and 2 at all relative humidities.

D. Grain Weight loss

The mean $\pm$ SE percentage insect-damaged kernels caused by *S. oryzae* adults in controls at 42 d ranged from 9.3 ± 0.9 to $15.7 \pm 4.0\%$. The results showed that complete inhibition of insect -damaged kernels could be achieved when the adults were exposed to 0.35 g/kg of silica powder 1 and silica powder 2 at 28 °C and at 30 ± 2 and $50 \pm 5\%$ r.h. Complete inhibition of insect-damaged kernels was achieved only when adults were exposed to 0.35 g/kg of silica powder 2 at 28 °C and at $75 \pm 4\%$ r.h (Table 11.4). Three-way ANOVA showed that the grain weight loss caused by *S. oryzae* between silica powders ($F = 11.48$; $df = 1, 48$; $P = 0.0014$), among application rates ($F = 129.33$; $df = 3, 48$; $P < 0.0001$) as well as the application rate x relative humidity ($F = 3.94$; $df = 6, 48$; $P = 0.0028$), and powder x application rate x relative humidity interaction ($F = 2.73$; $df = 6, 48$; $P = 0.0230$) interactions were all significant. However, the grain weight loss data among relative humidities ($F = 1.71$; $df = 2, 48$; $P = 0.1923$), powder x relative humidity ($F = 1.46$; $df = 6, 48$; $P = 0.2430$), and powder x application rate ($F = 1.66$; $df = 3, 48$; $P = 0.1889$) were not significant. One-way ANOVA showed that there were significant differences in grain weight loss data among application rates of silica powder 1 treated at different relative humidities ($F_{\text{range}} = 22.22 - 28.25$; $df = 3, 8$; $P < 0.0004$; REGWQ test) and silica powder 2 ($F_{\text{range}} = 19.18 - 24.62$; $df = 3, 8$; $P < 0.0005$; REGWQ test). There was

a significant difference in grain weight loss data between control and other application rates of silica powder 1 and 2 at all relative humidities. At 0.35 g/kg, a significant difference in insect-damaged kernels was observed between 0.15 and 0.25 g/kg of silica powder 1 and 2 at all relative humidities.

11.4.2 Responses of *S. oryzae* adults exposed Gravista-D plus silica powder combination

A. Adult mortality

The mean $\pm$ SE mortality of *S. oryzae* adults in the control group was $12.38 \pm 1.9\%$. Treatment with 0.18 g/kg of Gravista-D, either alone or in combination with silica powders, resulted in close to 100% mortality. The mean $\pm$ SE corrected mortality of *S. oryzae* adults exposed to silica powder 1 at rates of 0.05 to 0.20 g/kg for 14 d ranged from 29.3 ± 3.9 to $82.6 \pm 2.2\%$ (Table 11.5). When silica powder 1 was combined with Gravista-D at 0.18 g/kg, the corrected mortality ranged from 98.9 ± 1.1 to 100%. Similarly, when silica powder 1 was combined with Gravista-D at 0.09 g/kg, the corrected mortality ranged from 84.8 ± 5.8 to $97.8 \pm 1.1\%$. One-way ANOVA indicated significant differences in corrected mortality response among treatments for silica powder 1 ($F = 50.96$; $df = 11, 22$; $P < 0.0001$; REGWQ test). Notably, at 0.10 g/kg of silica powder 1 alone, the corrected mortality response differed significantly from other treatments. A significantly lower mortality was observed between 0.09 g/kg and 0.18 g/kg of Gravista-D, whether alone or in combination with silica powder 1 (Table 1). For silica powder 2, the mean $\pm$ SE corrected mortality of *S. oryzae* adults exposed to rates of 0.05 to 0.20 g/kg for 14 days ranged from 84.8 ± 5.8 to 100% (Table 2). Complete mortality was achieved at all application rates of silica powder 2 and when combined with 0.18 g/kg of Gravista-D. When silica powder 2 was combined with 0.09 g/kg of Gravista-D, the corrected mortality ranged from

55.4 $\pm$ 6.6 to 96.7 $\pm$ 1.9%. Significant differences in corrected mortality response among treatments for silica powder 2 were observed through one-way ANOVA ($F = 6.06$; $df = 11, 22$; $P = 0.0002$; REGWQ test). A significant difference in corrected mortality response was noted between silica powder 2 alone at 0.05 g/kg and silica powder 2 combined with Gravista-D at 0.18 g/kg. Additionally, a significantly lower mortality was observed between 0.09 g/kg of Gravista-D and the combination of 0.18 g/kg of Gravista-D with silica powder 2 at application rates of 0.05 to 0.20 g/kg (Table 11.6).

B. Adult progeny production

The mean $\pm$ SE number of adult progeny produced in the control groups was 381.7 $\pm$ 11.6. Treatment with 0.18 g/kg of Gravista-D, either alone or in combination with silica powder, resulted in complete suppression of adult progeny production of *S. oryzae*. The mean $\pm$ SE adult progeny produced when adults were exposed to silica powder 1 at rates of 0.05 to 0.20 g/kg for 14 d ranged from 5.7 $\pm$ 1.8 to 21.3 $\pm$ 5.8 (Table 11.5). Similarly, when silica powder 2 was combined with Gravista-D at 0.09 g/kg, the mean $\pm$ SE adult progeny produced ranged from 5.3 $\pm$ 1.5 to 16.0 $\pm$ 3.5. One-way ANOVA indicated significant differences in adult progeny production data among treatments for silica powder 1 ($F = 50.96$; $df = 11, 24$; $P < 0.0001$; REGWQ test). Notably, silica powder 1 combined with Gravista-D at 0.18 g/kg showed significant differences in adult progeny production compared to silica powder 1 alone and silica powder 1 combined with Gravista-D 0.09 g/kg. Complete suppression of adult progeny production was observed at 0.18 g/kg of Gravista-D alone and in combination with silica powder 2 at all application rates. The mean $\pm$ SE adult progeny produced when adults were exposed to silica powder 2 at rates of 0.05 to 0.20 g/kg for 14 d ranged from 3.7 $\pm$ 0.9 to 19.3 $\pm$ 2.2 (Table 11.6). When silica powder 2 was combined with 0.09 g/kg of Gravista-D, the mean $\pm$ SE number

of adult progeny produced ranged from 0.15 to 7.7 ± 3.5 . One-way ANOVA indicated significant differences in corrected mortality response among treatments for silica powder 2 ($F = 50.96$; $df = 11, 24$; $P < 0.0001$; REGWQ test). Notably, silica powder 2 combined with 0.09 g/kg of Gravista-D at showed significant differences in adult progeny production compared to silica powder 2 alone.

C. *Insect-damaged kernels*

The mean $\pm$ SE percentage insect-damaged kernels caused by *S. oryzae* adults in the control group was $17.5 \pm 2.0\%$. Treatment with 0.18 g/kg of Gravista-D, either alone or in combination with silica powders, resulted in close to 100% reduction in the percentage insect-damaged kernels. The mean $\pm$ SE percentage insect-damaged kernels caused by *S. oryzae* adults exposed to silica powder 1 at rates of 0.05 to 0.20 g/kg for 14 d ranged from 2.3 ± 0.5 to $7.8 \pm 1.4\%$ (Table 11.5). When silica powder 1 was combined with Gravista-D at 0.09 g/kg, the percentage insect-damaged kernels ranged from 0.7 ± 0.2 to $4.7 \pm 2.0\%$. One-way ANOVA indicated significant differences in percentage insect damaged kernels among treatments for tests with silica powder 1 ($F = 29.14$; $df = 11, 24$; $P < 0.0001$; REGWQ test). Notably, at 0.10 g/kg of silica powder 1 alone, the percentage insect-damaged kernels differed significantly from control and other treatment combination with 0.18 and 0.9 g/kg of Gravista-D. For silica powder 2, the mean $\pm$ SE percentage insect-damaged kernels caused by *S. oryzae* adults exposed to rates of 0.05 to 0.20 g/kg for 14 d ranged from 0.8 ± 0.1 to $9.1 \pm 1.5\%$ (Table 11.6). Complete inhibition of insect-damaged kernels was achieved at all application rates of silica powder 2 and when combined with 0.18 g/kg of Gravista-D. One-way ANOVA indicated significant differences in percentage insect damaged kernels among treatments for tests with silica powder 2 ($F = 68.14$; $df = 11, 24$; $P < 0.0001$; REGWQ test). Notably, at 0.05 g/kg of silica powder 2 alone, the

percentage insect-damaged kernels differed significantly from control and other treatment combinations with 0.18 and 0.9 g/kg of Gravista-D (Table 2).

D. Grain weight loss

The mean $\pm$ SE percentage grain weight loss caused by *S. oryzae* adults in the control group was $12.2 \pm 2.5\%$. Treatment with 0.18 g/kg of Gravista-D, either alone or in combination with silica powders, resulted in close to 100% reduction in the percentage grain weight loss kernels. The mean $\pm$ SE percentage grain weight loss caused by *S. oryzae* adults exposed to silica powder 1 at rates of 0.05 to 0.20 g/kg for 14 d ranged from 1.2 ± 0.3 to $5.0 \pm 0.5\%$ (Table 11.5). When silica powder 1 was combined with 0.09 g/kg of Gravista-D, the percentage grain weight loss ranged from 0.2 ± 0.1 to $3.0 \pm 1.5\%$. One-way ANOVA indicated significant differences in percentage grain weight loss among treatments for tests with silica powder 1 ($F = 22.51$; $df = 11, 24$; $P < 0.0001$; REGWQ test). At 0.05 g/kg of silica powder 1 alone, the percentage grain weight loss differed significantly from control and other treatment combinations with 0.18 and 0.9 g/kg of Gravista-D. For silica powder 2, the mean $\pm$ SE grain weight loss caused by *S. oryzae* adults exposed to rates of 0.05 to 0.20 g/kg for 14 d ranged from 0.3 ± 0.2 to $4.0 \pm 0.3\%$ (Table 11.6). Complete inhibition of grain weight loss was achieved at all application rates of silica powder 2 and when combined with 0.18 g/kg of Gravista-D. One-way ANOVA indicated significant differences in percentage insect damaged kernels among treatments for tests with silica powder 2 ($F = 35.07$; $df = 11, 24$; $P < 0.0001$; REGWQ test). The percentage grain weight loss in treatments differed significantly from control (Table 11.6). No significant differences were observed between silica powder 2 alone and silica powder 2 with 0.09 g/kg of Gravista-D.

11.5 Discussion

Diatomaceous earth formulations affect insect pests by adsorbing the waxy epicuticular layer causing death by desiccation (Ebeling, 1971; Subramanyam and Roesli, 2000). Moreover, this high adsorption properties of the powders especially at elevated humidities, can also lead to the absorption of water by the powders which can in turn influence their insecticidal efficacy (Ebeling 1971; Fields and Korunic 2000). We observed that the mortality of *S. oryzae* adults exposed to both the silica powders 1 and 2 at application rates of 0.15 to 0.35 g/kg were affected by relative humidity. Complete mortality of *S. oryzae* was achieved when adults were exposed to 0.35 g/kg of silica powder 1 and 2 at 28 °C and 30 ± 2% r.h. compared to experiments performed at 75 ± 4% r.h. Similarly, Arthur (2000) observed that exposure of *T. castaneum* adults for 8 -72 h to 0.5 g/m² of Protect-It at different humidity conditions provided variable results based on humidity. It was observed that the adult mortalities were 100, 98 and 40% when treated at 32 °C and at 40, 57, and 75% r.h. for 48 h, respectively. We observed that the mortality was < 50% when adults were exposed to 0.15 g/kg of silica powder 1 at 28 °C and 75 ± 4% r.h. compared to 70% mortality observed for silica powder 2 at similar application rate and humidity conditions. These varied results based on relative humidities augments the fact that increased relative humidity would reduce water loss induced by exposure to diatomaceous earth, consequently leading to a decrease in mortality (Arthur, 2000). Aldryhim (1990) also observed a decrease in toxicity of the confused flour beetle, *Tribolium confusum* Jacquelin du Val when silica dust was treated to wheat and held at increasing relative humidities.

More numbers of adult progenies were produced in untreated control at 75 ± 4% r.h. compared to other humidity conditions. Complete suppression of adult progeny was not observed at any application rates of silica powder 1 when the experiments were performed at 75 ± 4% r.h.

While no progeny production was observed when adults of *S. oryzae* were exposed to 0.35 g/kg of silica powder 2 at $75 \pm 4\%$ r.h., indicating silica powder to be more efficacious than silica powder 1. A 0.35 g/kg of silica powder 1 and 0.25 g/kg of silica powder 2 gave complete progeny inhibition at both the relative humidities of $30 \pm 2\%$ and $50 \pm 5\%$ r.h., respectively. This correlated with the adult mortalities observed at these application rates of the powders and the relative humidities they were exposed to. While 0.35 g/kg of silica powder 2 completely reduced the adult progeny production of *S. oryzae* exposed for 14 d at 28°C and 65% r.h (Manivannan and Subramanyam, unpublished data). The percentage insect-damaged kernels caused by *S. oryzae* were significantly higher at lower application rates compared to higher application rates when tested at all relative humidity conditions. We observed that the insect- damaged kernels were observed completely inhibited when *S. oryzae* adults were exposed to 0.25 g/kg of silica powder 1 and 0.35 g/kg of silica powder 2 tested at $30 \pm 2\%$ r.h and $50 \pm 5\%$ r.h. Similarly, the percentage grain weight loss was also observed to be completely inhibited at 0.25 g/kg of silica powder 1 and 0.35 g/kg of silica powder 2 tested at $30 \pm 2\%$ r.h and $50 \pm 5\%$ r.h. Complete suppression of grain weight loss at $75 \pm 4\%$ r.h. was only noticed at 0.35 g/kg of silica powder 2 and not to any application rate of silica powder 1 at this tested humidity conditions. Tadesse et al. (2019) observed complete inhibition of insect-damaged kernels and grain weight loss when *S. oryzae* adults were exposed to 1 g/kg of filter cake, a non-diatomaceous inert dust from Ethiopia after 7 and 14 d treated at 28°C and 65% r.h.

We evaluated the efficacy of two amorphous silica powders 1 and 2 combined with Gravista-D which is registered for use as a grain protectant and for structural treatment. In the present study, we tested the efficacy of these two silica powders at their sub-lethal doses for *S. oryzae* based on our previous research with these two powders. Furthermore, the combination of

these two powders with Gravista-D was evaluated at two different application rates of 0.18 g/kg which is the industry recommended rate and at 0.09 g/kg which is a sublethal dose. These treatments combination was chosen in order to reduce the amount of chemical insecticide used in the grain storages and also to reduce the amount of powder required for treating bulk grains. This is the first report to study the effect of the two amorphous silica powders in combination with Gravista-D.

We observed that none of the tested sublethal doses of silica powder 1 or silica powder 2 gave mortality of *S. oryzae* when tested alone. The mortality was close to 100% at the industrial recommended rate of Gravista-D either alone or in combination with silica powder 1 or silica powder 2. Further we noticed that exposure of adults to the sub-lethal doses of Gravista-D gave more than 98% mortality when combined with 0.20 g/kg of silica powder 1. While for the same combination with silica powder 2 gave complete mortality at the similar application rate. Even a lower application rate of 0.05 g/kg gave close to 90% mortality when combined with 0.09 g/kg of silica powder 2. None of the application rates of silica powder 1 was capable of completely suppressing the adult progeny production either when tested alone or in combination with 0.09 g/kg of Gravista-D. In contrary, we observed that no adult progeny production when 0.20 g/kg of silica powder 2 was combined with 0.09 g/kg of Gravista-D. Arthur (2004) evaluated the efficacy of S-methoprene EC (0 to 0.001 g/kg) in combination with commercial diatomaceous earth (DE) Protect-It (0 to 0.3 g/kg). His results indicated that within each methoprene concentration, survival tends to decrease with increasing DE concentration, with higher survival rates generally observed at 75% relative humidity compared to 57% relative humidity. No adult progeny production was observed after exposure to DE-methoprene combination even at the lower dose. Insect-damaged kernels and grain weight loss were completely inhibited when the *S.*

oryzae were tested at 0.18 g/kg of Gravista-D either alone or in combination with silica powder 1 or silica powder 2. Conversely, 0.09 g/kg of Gravista-D either alone or in combination with 0.05 to 0.20 g/kg of silica powder 1 did not completely reduce the insect-damaged kernels or the grain weight loss. But for the tests with silica powder at 0.20 g/kg in combination with 0.09 g/kg of silica powder 2, complete inhibition of both insect-damaged kernels and grain weight loss was observed.

Our study investigated the efficacy of two different silica powders, silica powder 1 and silica powder 2, against *S. oryzae* under varying humidity conditions. The efficacy of both powders was influenced by application rates and relative humidity, with higher application rates and lower humidity levels leading to better outcomes. Furthermore, our study evaluated the combined effects of the silica powders with Gravista-D, a registered grain protectant. The combination of silica powders with Gravista-D resulted in enhanced efficacy, leading to better mortality, suppression of adult progeny, inhibition of insect-damaged kernels and grain weight loss, compared to individual application of the powders. Overall, our findings highlight the potential of silica powders in combination with chemical protectants like Gravista-D, as effective tools for controlling *S. oryzae* infestations in grain storage facilities, reducing the required application rate of silica powders.

11.6 References

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Table 11.1. Effect of relative humidity on the mortality of *S. oryzae* exposed to silica powders 1 and 2 for 14 d.

Relative humidity (%)	Mean $\pm$ SE corrected mortality (%) ^a		
	Application rate (g/kg)	Silica powder 1	Silica powder 2
30	0	0 $\pm$ 0b	0 $\pm$ 0b
	0.15	93.3 $\pm$ 4.2a	99.0 $\pm$ 1.0a
	0.25	99.0 $\pm$ 1.0a	100 $\pm$ 0a
	0.35	100 $\pm$ 0a	100 $\pm$ 0a
50	0	0 $\pm$ 0c	1.9 $\pm$ 1.9b
	0.15	89.5 $\pm$ 3.4b	97.1 $\pm$ 1.6a
	0.25	100 $\pm$ 0a	99.0 $\pm$ 1.0a
	0.35	100 $\pm$ 0a	100 $\pm$ 0a
75	0	3.8 $\pm$ 3.8d	0 $\pm$ 0c
	0.15	42.9 $\pm$ 12.9c	70.5 $\pm$ 2.5b
	0.25	79.0 $\pm$ 6.9b	93.3 $\pm$ 3.4a
	0.35	100 $\pm$ 0a	100 $\pm$ 0a

^aMeans in a column followed by different letters are significantly different between silica powders 1 and 2 ($P < 0.05$; by REGWQ test).

Mortality of adults among application rates for different relative humidities treated to silica 1 were significantly different ($F_{\text{range}} = 38.68 - 139.45$; $df = 3, 8$; $P < 0.0001$).

Mortality of adults among application rates for different relative humidities treated to silica 2 were significantly different ($F_{\text{range}} = 138.18 - 752.29$; $df = 3, 8$; $P < 0.0001$).

Table 11.2. Effect of relative humidity on the adult progeny production of *S. oryzae* exposed to silica powders 1 and 2 for 14 d.

Relative humidity (%)	Mean $\pm$ SE adult progeny production ^a		
	Application rate (g/kg)	Silica powder 1	Silica powder 2
30	0	360.3 $\pm$ 41.6a	274.7 $\pm$ 40.5a
	0.15	50.7 $\pm$ 14.1b	9.0 $\pm$ 1.7b
	0.25	9.7 $\pm$ 1.2c	0 $\pm$ 0c
	0.35	0 $\pm$ 0d	0 $\pm$ 0c
50	0	272.3 $\pm$ 49.1a	452.3 $\pm$ 55.5a
	0.15	25.7 $\pm$ 5.7b	4.3 $\pm$ 0.7b
	0.25	20.7 $\pm$ 14.7b	0 $\pm$ 0c
	0.35	0 $\pm$ 0c	0 $\pm$ 0c
75	0	569.0 $\pm$ 82.9a	295.0 $\pm$ 68.0a
	0.15	17.3 $\pm$ 2.3b	23.0 $\pm$ 4.7b
	0.25	11.7 $\pm$ 2.7b	4.3 $\pm$ 2.2c
	0.35	3 $\pm$ 3c	0 $\pm$ 0c

^aMeans in a column followed by different letters are significantly different between silica powders 1 and 2 ($P < 0.05$; by REGWQ test).

Adult progeny production among application rates at different relative humidities treated to silica 1 were significantly different ($F_{\text{range}} = 32.41 - 257.12$; $df = 3, 8$; $P < 0.0001$).

Adult progeny production among application rates at different relative humidities treated to silica 2 were significantly different ($F_{\text{range}} = 26.67 - 468.98$; $df = 3, 8$; $P < 0.0003$).

Table 11.3. Effect of relative humidity on the insect-damaged kernels caused by *S. oryzae* exposed to silica powders 1 and 2 for 14 d.

Relative humidity (%)	Mean $\pm$ SE insect-damaged kernels (%) ^a		
	Application rate (g/kg)	Silica powder 1	Silica powder 2
30	0	12.5 $\pm$ 0.9a	11.2 $\pm$ 1.7a
	0.15	9.0 $\pm$ 2.1ab	6.4 $\pm$ 2.0a
	0.25	5.6 $\pm$ 1.1b	0 $\pm$ 0b
	0.35	0 $\pm$ 0c	0 $\pm$ 0b
50	0	14.1 $\pm$ 1.4a	15.7 $\pm$ 4.0a
	0.15	6.1 $\pm$ 1.5b	2.3 $\pm$ 0.4b
	0.25	3.9 $\pm$ 1.5b	0 $\pm$ 0c
	0.35	0 $\pm$ 0b	0 $\pm$ 0c
75	0	16.0 $\pm$ 3.8a	9.3 $\pm$ 0.9a
	0.15	5.5 $\pm$ 0.7ab	1.8 $\pm$ 0.8b
	0.25	1.7 $\pm$ 0.5b	0.8 $\pm$ 0.4b
	0.35	1.5 $\pm$ 1.5b	0 $\pm$ 0b

^aMeans in a column followed by different letters are significantly different between silica powders 1 and 2 ($P < 0.05$; by REGWQ test).

Percentage insect-damaged kernels among application rates at different relative humidities treated to silica 1 were significantly different ($F_{\text{range}} = 10.11 - 46.85$; $df = 3, 8$; $P < 0.0058$).

Percentage insect-damaged kernels among application rates at different relative humidities treated to silica 2 were significantly different ($F_{\text{range}} = 3.40 - 40.62$; $df = 3, 8$; $P < 0.0003$).

Table 11.4. Effect of relative humidity on the grain weight loss caused by *S. oryzae* exposed to silica powders 1 and 2 for 14 d.

Relative humidity (%)	Mean $\pm$ SE grain weight loss (%) ^a		
	Application rate (g/kg)	Silica powder 1	Silica powder 2
30	0	8.5 $\pm$ 0.8a	6.4 $\pm$ 1.3a
	0.15	4.7 $\pm$ 1.6a	5.0 $\pm$ 2.5a
	0.25	4.2 $\pm$ 1.0a	0 $\pm$ 0b
	0.35	0 $\pm$ 0b	0 $\pm$ 0b
50	0	11.0 $\pm$ 1.7a	13.1 $\pm$ 4.3a
	0.15	2.8 $\pm$ 1.5b	1.1 $\pm$ 0.2b
	0.25	0.2 $\pm$ 0.2c	0 $\pm$ 0b
	0.35	0 $\pm$ 0c	0 $\pm$ 0b
75	0	11.4 $\pm$ 2.4a	6.5 $\pm$ 0.9a
	0.15	2.7 $\pm$ 0.8b	1.2 $\pm$ 0.6b
	0.25	0.6 $\pm$ 0.3bc	0.5 $\pm$ 0.3bc
	0.35	0.1 $\pm$ 0.1c	0 $\pm$ 0c

^aMeans in a column followed by different letters are significantly different between silica powders 1 and 2 ($P < 0.05$; by REGWQ test).

Percentage grain weight loss among application rates at different relative humidities treated to silica 1 were significantly different ($F_{\text{range}} = 22.22 - 28.25$; $df = 3, 8$; $P < 0.0004$).

Percentage grain weight loss among application rates at different relative humidities treated to silica 2 were significantly different ($F_{\text{range}} = 19.18 - 24.62$; $df = 3, 8$; $P < 0.0006$).

Table 11.5. Efficacy of silica powder 1 plus Gravista-D (GV) combination against *S. oryzae*.

Treatment (g/kg)	Mean $\pm$ SE			
Silica 1 plus Gravista-D	Corrected mortality (%)	Adult progeny production	Insect-damaged kernels (%)	Grain weight loss (%)
0.05 + 0.18	98.9 $\pm$ 1.1a	0.7 $\pm$ 0.7c	0.2 $\pm$ 0.2fg	0.1 $\pm$ 0.1e
0.10 + 0.18	100 $\pm$ 0a	0 $\pm$ 0c	0 $\pm$ 0g	0 $\pm$ 0e
0.20 + 0.18	100 $\pm$ 0a	0 $\pm$ 0c	0 $\pm$ 0g	0 $\pm$ 0e
0.05 + 0.09	85.8 $\pm$ 2.8b	16.0 $\pm$ 3.5a	4.7 $\pm$ 2.0def	3.0 $\pm$ 1.5bcd
0.10 + 0.09	84.8 $\pm$ 5.8b	10.3 $\pm$ 1.5b	1.7 $\pm$ 0.3efg	0.9 $\pm$ 0.2de
0.20 + 0.09	97.8 $\pm$ 1.1b	5.3 $\pm$ 1.5bc	0.7 $\pm$ 0.2efg	0.2 $\pm$ 0.1e
0.05	29.3 $\pm$ 3.9c	21.3 $\pm$ 5.8b	7.8 $\pm$ 1.4b	5.0 $\pm$ 0.5b
0.10	39.1 $\pm$ 5.8c	9.7 $\pm$ 2.7b	6.0 $\pm$ 1.4bc	3.9 $\pm$ 1.1bcd
0.20	82.6 $\pm$ 2.2b	5.7 $\pm$ 1.8bc	2.3 $\pm$ 0.5cde	1.2 $\pm$ 0.3cde
0.18 (GV)	98.9 $\pm$ 1.1a	0 $\pm$ 0c	0 $\pm$ 0g	0 $\pm$ 0e
0.09 (GV)	67.4 $\pm$ 3.8b	13.3 $\pm$ 2.4b	6.2 $\pm$ 0.4ab	4.2 $\pm$ 0.2bc
Control	-	381.7 $\pm$ 11.6a	17.5 $\pm$ 2.0a	12.2 $\pm$ 2.5a

Values mentioned in columns mean $\pm$ SE.

^aMeans in a column followed by different letters are significantly different between silica powders 1 and 2 ($P < 0.05$; by REGWQ test).

Table 11.6. Efficacy of silica powder 2 plus Gravista-D (GV) combination against *S. oryzae*.

Treatment (g/kg)	Mean $\pm$ SE			
Silica 2 plus Gravista-D	Corrected mortality (%)	Adult progeny production	Insect-damaged kernels (%)	Grain weight loss (%)
0.05 + 0.18	100 $\pm$ 0a	0 $\pm$ 0f	0 $\pm$ 0e	0 $\pm$ 0c
0.10 + 0.18	100 $\pm$ 0a	0 $\pm$ 0f	0 $\pm$ 0e	0 $\pm$ 0c
0.20 + 0.18	100 $\pm$ 0a	0 $\pm$ 0f	0 $\pm$ 0e	0 $\pm$ 0c
0.05 + 0.09	88.0 $\pm$ 2.9ab	7.7 $\pm$ 1.5cd	4.3 $\pm$ 1.5c	2.6 $\pm$ 1.1b
0.10 + 0.09	84.8 $\pm$ 5.8ab	4.7 $\pm$ 0.9de	1.0 $\pm$ 0.6d	0.4 $\pm$ 0.3b
0.20 + 0.09	100 $\pm$ 0a	0 $\pm$ 0f	0 $\pm$ 0e	0 $\pm$ 0c
0.05	58.7 $\pm$ 4.7b	19.3 $\pm$ 2.2b	9.1 $\pm$ 1.5b	4.0 $\pm$ 0.3b
0.10	55.4 $\pm$ 6.6b	12.3 $\pm$ 3.2b	5.7 $\pm$ 0.4bc	2.5 $\pm$ 0.8b
0.20	96.7 $\pm$ 1.9a	3.7 $\pm$ 0.9e	0.8 $\pm$ 0.1d	0.3 $\pm$ 0.2b
0.18 (GV)	98.9 $\pm$ 1.1a	0 $\pm$ 0f	0 $\pm$ 0e	0 $\pm$ 0c
0.09 (GV)	67.4 $\pm$ 3.8b	13.3 $\pm$ 2.4bc	6.2 $\pm$ 0.4bc	4.2 $\pm$ 0.2
Control	-	381.7 $\pm$ 11.6a	17.5 $\pm$ 2.0a	12.2 $\pm$ 2.5a

Values mentioned in columns mean $\pm$ SE.

^aMeans in a column followed by different letters are significantly different between silica powders 1 and 2 ($P < 0.05$; by REGWQ test).

Chapter 12 - General Conclusions and Future Work

12.1 General Conclusions

Our studies consistently demonstrated that both the silica powders exhibited better insecticidal activity against the tested stored product insects at lower application rates compared to the industry-recommended rates of diatomaceous earth formulations. On concrete, *R. dominica* adults were highly susceptible to silica powder 2 compared to silica powder 1. The lethal concentrations for 99% mortality of *R. dominica* exposed for 24, 36, and 48 h exposures to silica powder 1 were 4.32, 1.62, and 1.34 g/m², respectively. While the estimated lethal concentrations required for 99% mortality of *R. dominica* adults were comparatively lower for silica powder 2, with 0.63, 0.62, and 0.19 g/m² for 4, 8, and 10 -h exposures. The lethal times required for 99% mortality of *R. dominica* adults exposed to 1.5 g/m² of silica powder 1 and 0.25 g/m² of silica powder 2 were 60.25 and 12.13 h, respectively. The effective concentrations (ECs) of silica powder 1 to reduce 99% of adult progeny production of *R. dominica* were 5.88, 4.64, 3.83, 0.27, and 0.19 g/m² for 4, 8, 12, 24, and 36 h exposures, respectively. The ECs to reduce 99% of *R. dominica* adult progeny production were 0.26 and 0.14 g/m² of silica powder 2 for 4 and 8 h, respectively. The estimated LC₉₉ values for 99% mortality of *S. oryzae* adults exposed for 12 - 48 h exposures to silica powder 1 were 15.81, 13.65, 9.35, 5.35 g/m², respectively. The corresponding LC₉₉ values for 99% mortality of *S. oryzae* adults exposed for 12 - 48 h exposures to silica powder 2 were 13.31, 9.68, 4.67, and 4.65 g/m², respectively. The lethal times needed for 99% mortality of *S. oryzae* exposed to 7 g/m² of silica powder 1 and 5 g/m² of silica powder 2 were 28.74 and 41.30 h respectively. The effective concentrations (ECs) needed for 99% suppression of adult progeny production in *S. oryzae* exposed for 12 - 48 h to silica powder 1 were 36.92, 9.28, 5.44, and 2.34 g/m², respectively. The corresponding effective concentrations

(ECs) needed for 99% suppression of adult progeny production in *S. oryzae* exposed for 12 - 48 h to silica powder 2 were 10.52, 4.96, 1.52, and 1.69 g/m². The effective times (ETs) required for 99% reduction of adult progeny production of *S. oryzae* exposed to 7 g/m² of silica powder 1 and to 5 g/m² of silica powder 2 were 27.39 and 18.38 h respectively. Unlike other species, the adults of *T. castaneum* were more susceptible to silica powder 1 compared to silica powder 2, indicating smaller particle diameter can exhibit better pick up of powders for a resilient species like *T. castaneum*. The minimum concentrations required to achieve complete mortality of *T. castaneum* when adults were exposed to concrete arenas treated with silica powder 1 for 24, 36, and 48 h were 5.0, 1.5, and 1.5 g/m², respectively. While complete mortality was only achieved after 48 h exposure to 0.75 g/m² of silica powder 2. Complete mortality was observed when *T. castaneum* adults were exposed for 22 h at 5.0 g/m² of silica powder 1. However, complete mortality was achieved only when *T. castaneum* adults were exposed to 6.0 g/m² of silica powder 2 for 24 h. The minimum concentrations required to achieve complete inhibition of adult progeny production of *T. castaneum* when adults were exposed to concrete arenas treated with silica powder 1 for 24, 36, and 48 h were 2.0, 0.75, and 0.75 g/m². While complete suppression of adult progeny production was observed when adults were exposed for 36 and 48 h to 3.0 and 0.75 g/m² of silica powder 2. Complete suppression of adult progeny production was observed when adults were exposed for 18-24 h to 5.0 g/m² of silica powder 1 and 6.0 g/m² of silica powder 2. The minimum application rates required to achieve complete suppression of adult progeny production of *T. confusum* were 4.5, 1.5, and 1.0 g/m² when adults were exposed for 24, 36, and 48 h to silica powder 1. The minimum application rates required to achieve complete mortality of *T. confusum* were 4.0, 2.5, and 3.0 g/m² when adults were exposed for 24, 36, and 48 h to silica powder 2. Minimum application rates required for 100% mortality of *O.*

surinamensis exposed for 24, 36, and 48 hours to silica powder 1 were 3.0, 3.5, and 2.5 g/m², respectively. Conversely, the minimum application rates required for complete mortality of *O. surinamensis* when adults were exposed for 12, 24, 36, and 48 h to silica powder 2 were 4.0, 3.0, 1.5, and 1.0 g/m², respectively. Complete reduction of adult progeny production of *O. surinamensis* was observed when adults were exposed for 24, 36, and 48 h to silica powder 1 at 3.0, 3.5, and 2.0 g/m², respectively. Application rates of 3.5, 3.0, 1.5, and 1.0 g/m² were required for achieving complete suppression of adult progeny when adults were exposed for 12, 24, 36, and 48 h to silica powder 2. At 3.5 g/m², complete mortality of adults of *O. surinamensis* was achieved after 20-24 h exposure to both powders. Similarly, 3.5 g/m² of silica powder 1 and 2 gave complete progeny suppression of *O. surinamensis* after 20-24 h. On wheat, complete mortality of *R. dominica*, no adult progeny production, no insect-damaged kernels, and percentage of grain weight loss was achieved only for silica powder 2 at 0.20 g/kg. Silica powder 2 above 0.25 g/kg on wheat produced more than 99% mortality of *S. oryzae* adults after 14 d. Complete suppression of *S. oryzae* progeny production, no insect-damaged kernels, and grain weight loss was observed for both powders at 0.30 g/kg at 42 d. On wheat, 0.5 g/kg of silica powder 1 produced more than 99% mortality of *O. surinamensis* and *T. castaneum* adults. However, ≥ 0.35 g/kg of silica powder 2 on wheat gave more than 99% of *O. surinamensis* and *T. castaneum* adults. Complete suppression of *O. surinamensis* and *T. castaneum* adult progeny was achieved at ≥ 0.35 g/kg of silica powder 1 and 0.25 g/kg of silica powder 2. The bulk density of wheat in silica powder 1 and silica powder 2 treated wheat significantly reduced at all exposure periods compared to the untreated control. The exposure of wheat to silica powder 1 and silica powder 2 at 0.35 g/kg for 3 months reduced the bulk density of wheat by 12 and 11%, respectively. Wheat treated with 0.35 g/kg of silica powder 1 and silica powder 2 showed high

angle of repose values. Notably, wheat treated with 0.35 g/kg of silica powder 1 for 3 months had an angle of repose value of 41°, indicating transitioning of wheat from good to passable flowability. Carr index values were below 4 for controls, while silica powder 1 and 2 treated wheat exposed for 3 months had Carr index values between 21 to 22 making the wheat evolve from excellent to passable flowability. After 3 months, the Hausner Ratio for wheat treated with silica powder 1 and 2 transitioned from excellent (1.00 to 1.11) to passable (1.26 to 1.34). The stability index values for wheat treated with 0/35 g/kg of silica powder 1 or silica powder 2 after 3 months were > 1.1 indicating that the wheat samples were not stable. The aeration ratios for all the silica powder treated wheat were > 2.1 indicating that the materials were sensitive to air. Overall, the results showed that silica powder 2 was more efficacious on all the stored product insect species both on concrete surfaces and on wheat. Both the silica powders performed better against *S. oryzae* at 28° C and 30% r.h. compared to 28° C and 75% r.h. At 0.25 g/kg of silica powder 2, complete mortality, no adult progeny production, insect-damaged kernels, and grain weight loss were achieved when experiments were performed at 28 °C and 30% r.h. While 0.35 g/kg rate was required for a similar effect against *S. oryzae* exposed to wheat treated to silica powder 2 at 28 °C and 30% r.h. The combination of silica powders with Gravista-D (0.09 g/kg) resulted in enhanced efficacy, leading to better mortality, suppression of adult progeny, inhibition of insect-damaged kernels and grain weight loss, compared to individual application of the powders. Both the powders are effective at lower application rates than the current industry recommendation. The superior efficacy of silica powder 2 can be linked to its high oil adsorption capability, lower pH (< 8.5), and higher silicon dioxide content. However, silica powder 1 had a significantly smaller particle size diameter, this did not influence the insecticidal activity of the stored product insects, except for *T. castaneum* which exhibited better responses on concrete

surfaces. However, the treatment of wheat with 0.35 g/kg of silica powders 1 and 2 exhibited decrease in bulk density and flowability of wheat after 3 months, they had generally acceptable flowability.

12.2 Future work

Our investigation into two amorphous silica powders, 1 and 2, indicated their insecticidal activity against major stored product insects, both on concrete surfaces and when applied as grain protectants on wheat, at lower application rates compared to the recommended dosages for other diatomaceous earth formulations. Given the increasing concerns surrounding the use of residual insecticides in post-harvest management systems, the application of silica powders, particularly in amorphous forms, can offer benefits by reducing the adverse effects caused by diatomaceous earth formulations on grain quality, including a decrease in bulk density and grain flowability. Based on the current results, the two amorphous silica powders are effective at lower application rates and exhibit significant potential to be registered with the Environmental Protection Agency for their usage in grain storage facilities. Future endeavors should prioritize conducting field trials to validate their efficacy under diverse real-world storage conditions across various geographic regions and grain storage facilities.

Our findings regarding the influence of changing relative humidity conditions on the efficacy of these powders suggest that lower humidity levels favor better insecticidal activity, highlighting the importance of evaluating their performance at grain storage facilities in different agro-climatic conditions to understand their efficiency under varying temperature and humidity settings. Given that the effectiveness of the powders may rely on factors like particle size and shape characteristics, such as particle roughness, which can enhance the insecticidal activity of diatomaceous earth formulations, utilizing Atomic Force Microscopy can assist in

comprehending the intricate particles (Starostina et al., 2008) of these two powders that contribute to improved insecticidal activity. In addition, the efficacy of various diameter fractions of the two silica powders should be assessed separately against stored product insects to determine if smaller particles exhibit superior efficacy against stored product insects or not. Our studies consistently showed that silica powder 2, with higher oil adsorption capability, lower pH value, and higher silicon dioxide content, outperformed silica powder 1 in exhibiting better responses on treated stored product insects, both on concrete surfaces and on wheat. However, additional research is warranted to determine the key properties of these powders that contribute to enhanced insecticidal activity. This entails comparing them with different commercial diatomaceous earth formulations, each possessing unique elemental compositions and properties. Evaluating the effectiveness of the two powders in comparison to commercially available diatomaceous earth formulations will help confirm our study's findings. This validation process will shed light on whether the observed insecticidal activity can be attributed to specific factors, thereby offering valuable insights relevant to the commercial utilization of diatomaceous earth.

Moreover, while we assessed the efficacy of these silica powders on wheat grains, further research into their effectiveness on other commodities and their adherence ratio in relation to grain moisture content and commodity type is warranted to explore their broader applicability. Additionally, considering that diatomaceous earth formulations have been reported to reduce the mechanical efficiency of grain handling equipment, field studies examining the impact of these powders on the mechanical performance of such equipment could be instrumental in persuading milling operators to widely accept silica powder treated wheat for milling. Furthermore, comprehensive studies are necessary to assess the long-term efficacy of silica powders on stored product insect populations, including the evaluation of their persistence over extended periods to

determine optimal application intervals for sustained pest control. Diatomaceous earth particles are also likely to lose effectiveness by lipid absorption from the surface of the kernels. Hence, ensuring proper cleaning and sanitation before applying these powders is crucial in pest management practices involving structural treatment, as the presence of food can boost insect survival rates. While dry diatomaceous earth applications were favorable in laboratory studies, commercial practice might find slurry solutions preferable (Athanassiou and Korunić, 2007; Athanassiou et al., 2011). Slurries are easier for personnel to apply, as they help avoid exposure to the dusty atmospheres generated by dry-blown methods for surface treatments (Gowers and Le Patourel, 1984) but can have reduced efficacy compared to dry dust formulations. Hence, field-oriented studies are required to confirm the feasible method for application of these powders for structural treatments. In addition, the ways in which diatomaceous earths are applied to grains are also a significant concern. Hence, studies pertaining to the exploration of various application methods, such as treating specific layers of food grains or application of powders during conveyance, need investigation. Exploring alternative application methods including rotation with other insecticides, or combination treatments is essential to mitigate the risk of resistance emergence and for resistance management strategies against conventional insecticides.

Our studies potentially involved evaluating the insecticidal activity of these two powders at a small scale, ensuring uniform mixing with grains. Furthermore, the quality of grains post-exposure to these powders indicated that the bulk density and flowability of the grains were not greatly affected. Nevertheless, it is essential to conduct large-scale trials to showcase the efficacy of these two powders in controlling stored product insects. These trials will also elucidate whether integrating silica powder treatments into comprehensive pest management strategies in the food industry, agriculture, and urban environments can be achieved without compromising

grain quality. Field-oriented studies can reveal the efficacy of these two powders for surface treatment, grain protectants, or a combination of both methods to yield the best results. These studies will offer valuable insights into enhancing the efficacy of pest management strategies in grain storage facilities.

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