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## Toxicity of Different Insecticides to *Sitophilus oryzae*, *Tribolium castaneum* and *Tribolium confusum* Infesting Corn

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Toxicity of Different Insecticides to *Sitophilus oryzae*  
*Tribolium castaneum* and *Tribolium confusum* Infesting Corn

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science in Entomology

by

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University of Malawi  
Bachelor of Science in Agriculture, 2016

August 2023  
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This thesis is approved for recommendation to the Graduate Council.

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## Abstract

Corn is an important cereal crop cultivated across various countries, including the United States. Corn serves multiple purposes such as human and livestock food and raw material for industrial processes. Despite being an important crop, corn production faces challenges due to factors such as weather fluctuations and the presence of insect pests and diseases, both in the field and during storage. Among these challenges, insect infestation poses a major threat, as the insect damage not only reduces the quantity and quality of the grain but also renders it unfit for human and animal consumption, leading to a decrease in market value. To address the challenges of insect infestation in stored grain, several control methods have been employed which include cleaning and drying grain to eliminate debris, the use of airtight containers or hermetic storage facilities, the application of botanicals with insecticidal properties, heat treatment, and the use of chemical insecticides. Insecticides have been extensively used since 1950 to deal with various insect pests due to their effectiveness, ease of application, and long-lasting effects.

This research examined the toxicity of four different insecticides namely, pirimiphos-methyl, deltamethrin, deltamethrin plus (S)-methoprene and malathion against *Sitophilus oryzae*, *Tribolium castaneum*, and *Tribolium confusum* infesting corn. The toxicity of each insecticide was determined in terms of LC<sub>50</sub> by exposing target pests at different doses and the mortality was observed at 24 hours until 10 days to determine delayed mortality. In the first study, the toxicity of the mentioned insecticides to *S. oryzae* was evaluated and the results revealed that pirimiphos-methyl insecticide was more toxic to *S. oryzae*, followed by malathion, deltamethrin, and deltamethrin plus (S)-methoprene. When the LC<sub>50</sub> value of each insecticide formulation was compared with the label-recommended application rate, we found that only malathion was aligned with the label rate for this species.

In the second study, the toxicity of these insecticides was determined for *T. castaneum* and *T. confusum*. The results indicated that deltamethrin plus (S)-methoprene and deltamethrin displayed the highest toxicity against *T. castaneum*, followed by pirimiphos-methyl. Malathion displayed the lowest toxicity among all the insecticides tested. On the other hand, pirimiphos-methyl was highly toxic among all the insecticides, followed by deltamethrin plus (S)-methoprene, deltamethrin, and malathion against *T. confusum*. Although these two pests are closely related species, their susceptibility to the selected insecticides was different in such a way that *T. confusum* displayed higher susceptibility to pirimiphos-methyl compared to *T. castaneum* while *T. castaneum* exhibited greater susceptibility to deltamethrin, deltamethrin plus (S)-methoprene, and malathion compared to *T. confusum*. When comparing the label recommended application rate with the LC<sub>50</sub> found in the study, deltamethrin plus (S)-methoprene and malathion for *T. confusum* aligned with the LC<sub>50</sub> found in the study while the recommended label rate for pirimiphos-methyl exceeded the LC<sub>50</sub> value for both species of flour beetles. These findings contribute to our understanding of insecticide toxicity to these stored-grain pests and provide valuable insights for their management.

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## **Dedication**

Special dedication should go to my late mother, Mrs. Margret Twaibu. Though she is no longer with us, her spirit lives on in my heart, and I am forever grateful for the values she instilled in me. This achievement is a testimony to her love, guidance, and unwavering belief in my potential.

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## Chapter 1

### Introduction and Literature Review

#### History and Importance of Corn

Cereals are the most important crops in the world, with total annual grain yields exceeding 2 billion tonnes (mt) globally, hence playing a significant role in food insecurity problems (Shewry and Halford, 2002). Around the world, rice, wheat, and maize, and to a lesser extent, sorghum, and millets, are important staples critical to the daily diets and survival of billions of people (McKevith, 2004). More than 50% of the world's daily caloric intake is derived directly from cereal grain consumption (Awika, 2011).

Corn or maize (*Zea mays* L.) is a grain crop that has been grown by human societies for a very long time (García-Lara and Serna-Saldivar, 2019). The domestication of maize by indigenous people roughly 10,000 years ago can be attributed to the Americas, more specifically to Mexico and Central America (McCann, 2001). It is grown all over the world, in more than 170 different geographic regions and the top corn-producing nations in the world are the United States, China, Brazil, Argentina, Ukraine, India, Mexico, Indonesia, Russia, and South Africa (Kandil, 2016; Nafziger, 2019). The USA alone produces 46% of the total world's corn production on 23% of the total world land area devoted to corn production (Glover and Mertz, 2015).

In Mexico and the United States, corn is the largest crop in terms of production and consumption volume, for example, in Mexico, corn accounts for a large share of the population's caloric intake and is used to make tortillas (Narayan et al., 2013). In the United States, 38 percent of corn produced is used for ethanol production and 33 percent of corn is used as feedstock for livestock while 15 percent is exported to countries such as Japan, South Korea, and

Columbia (Ranum et al., 2014). Additionally, corn is used as raw materials in various industrial processes and many products such as corn syrup (Rausch and Belyea, 2006; Zhang et al., 2021) This has made corn an important crop for global food security and economic development (Jiao et al., 2022).

### **Grain Losses**

Corn is a warm-season crop, and its growth and productivity can be significantly impacted by several factors such as variations in temperature, rainfall (Hatfield et al., 2018), and extreme weather events like droughts, floods, or heatwaves that reduce corn yields or even destroy entire crops (Sivakumar, 2018). In addition, corn crops can be vulnerable to a range of pests and diseases such as corn borers, armyworms, and fungal diseases like gray leaf spots or rust which if left uncontrolled, can significantly reduce corn yields (Maureen et al., 1998; Brewbaker and Hawaii, 2003). There are several species of insects associated with stored grain products and many of them cause economic losses (Mandali, 2020). According to Srivastava and Subramanian (2016), stored grain pests infest grains to meet their food and shelter needs.

The infestation of insects alone may lead to a significant loss of 5-30% in stored food grains (Kale et al., 2021) globally, depending on geographical location, climate conditions (Das et al., 2013), and insect pest species (Singh et al., 2021). Worldwide economic losses due to insects are about 13 million tons of grains worth about \$1 trillion (Kumar and Kalita, 2017). The United States of America produces approximately 50 million tons of wheat and 250 million tons of corn each year worth over \$25 billion, and damage caused by insects and other pests can exceed \$1 billion per year (Throne et al., 2003a). On the other hand, tropical countries such as India, Nigeria, Indonesia, Thailand, Brazil, and many other African and Asian countries have the highest grain insect infestation because of favorable weather conditions such as high

temperatures and humidity which create ideal conditions for the development of stored-grain insect pests. In these geographic regions, more than 70% of the products are stored in traditional structures such as earthen pots and silos because most farmers cannot afford proper storage facilities as many are small-scale and do not have access to sufficient capital to invest in proper storage facilities (Mobolade et al., 2019). Also, the farmers have limited access to credit facilities making it difficult to obtain financing for such investments. This often results in economic losses caused by stored-grain damage due to the feeding of various insect pests (Nwaigwe, 2019).

To address these problems, farmers use different low-cost and traditional methods to protect their grains from damage (Upadhyay and Ahmad, 2011). For example, in many parts of the world, farmers dry their grains in the sun before storing them. This not only helps to reduce the moisture content in grains but also exposes any pests already present in the grain to the heat of the sun, which can kill them (Shankar and Abrol, 2012). Farmers also use natural substances such as neem tree leaves or tobacco plant extracts to fumigate their stored grains (Dougoud et al., 2019; Hikal et al., 2017). The fumes from these substances are known to repel or kill various insect pests (Chaudhary et al., 2017). The other method involves storing the grains in airtight containers or bags which prevents the entry of oxygen and moisture and as a result, pests like weevils cannot survive (Kalpna et al., 2022).

### **Mechanism of Insect Infestation**

In storage, insect pests cause huge damage to grains and pulses by directly consuming the kernels where they often chew a hole and feed inside the grain (Bonjour, 2019), and multiply through reproduction (Ahmad et al., 2021). In addition, insect infestation causes contamination of the grains through excretions, molting, body fragments, and webbing (Rosentrater, 2022). Furthermore, when insects infest stored grain, they often leave behind broken pieces of grain and

frass. This creates a damp, nutrient-rich micro-environment that can promote the growth of fungi and causes further losses to stored grains (Abamecha, 2021). Consequently, the infestation of stored grains by these pests results in unpleasant odors, rendering the grains unsuitable for consumption, and reducing their nutritional content and overall quality (Bharathi et al., 2017).

The overall loss of quality due to insect damage in stored grains has a significant impact on the market value because of the physical and chemical changes that affect the grain's nutritional quality, flavor, texture, and appearance (Taddese et al., 2020). Additionally, the growth of fungi further reduces the grain's value and safety (Mohapatra et al., 2017). As a result, buyers may be willing to pay less for insect-damaged grains or may require discounts to compensate for the lower quality and increased risk (Stathers et al., 2020; Sauer, 1988).

### **Types of stored grain insect pests**

Based on feeding habits, stored insect pests can be divided into two groups. The first group are primary pests or internal feeders (Petroff, 2004). Primary insect pests are those that are capable of penetrating and infesting intact grain kernels (Weaver et al., 2005) as they have adapted to feed on stored grains by developing specialized mouthparts and feeding habits that allow them to obtain nutrition from the grains (Harush et al., 2021). For example, weevils have a long snout (Atanda et al., 2018) that they use to pierce the outer layer of grain or seed and feed on their inner contents (Maciej Serda et al., 2022). In addition, primary insect pests lay eggs inside the grains, spending a part or entire larval and pupal life stages within the grain, emerge as adults (Edde, 2012; Tripathi, 2018) hence, contributing significantly to the loss of quantity, quality, and loss of germination (Tyagi et al., 2019).

The other group of stored grain pests is known as secondary insect pests or external feeders (Deshwal et al., 2020). As the name indicates they feed on cut or broken seeds and grain

debris damaged by primary insect pests (Hiruy and Degaga, 2018). Mouthparts of secondary insect pests are adapted for chewing and grinding food particles (Yaseen et al., 2019). For example, Indian meal moth larvae and flour beetles have strong mandibles that they use to chew and consume stored grains, and flour beetles have maxillae used for handling and digestion of food (Busvine, 1980; Nikolaou et al., 2021). Secondary stored insect pests contribute heavily to contamination through ball formation and webbing besides the deterioration of grains (Golob et al., 2002). Additionally, they lead to fungal activity and moisture migration from their excretions across the stored grains. (Rees, 2007; Srivastava and Subramanian, 2016). Some other examples of secondary insect pests are sawtoothed grain beetle, common mites, and cheese mites (Abd El-Aziz, 2011).

Both primary and secondary insect pests are found globally, especially in regions where grains are produced and stored. In the United States, the major primary pest of corn is the maize weevil (*Sitophilus zeamais*, Motschulsky), and the major primary pests of wheat are the lesser grain borer (*Rhyzopertha dominica*, Fabricius) and rice weevil (*Sitophilus oryzae* (L)) (Washington, 2016). Furthermore, the major secondary pests of stored corn and wheat are the red flour beetle (*Tribolium castaneum*, Herbs) and confused flour beetle (*Tribolium confusum*, Jacquelin du Val), saw-toothed grain beetle (*Oryzaephilus surinamensis* (L)), flat grain beetle (*Cryptolestes pusillus*, (Schonherr)) and Indian meal moth (*Plodia interpunctella*, Hubner) (Mohandass et al., 2007).

In Arkansas, the common insect pests in stored grains are the warehouse beetles (*Trogoderma variabile*, Dejean), lesser grain borers (*R. dominica*), rice weevils (*S. oryzae*) red flour beetle (*T. castaneum*), confused four beetles (*T. confusum*), and Angoumois grain moth (*Sitotroga cerealella* (Olivier)) (McKay et al., 2014; Joshi et al., 2020).

### **Biology, Behavior, and Ecology of *Sitophilus oryzae* (Rice weevil)**

*Sitophilus oryzae* is one of the primarily stored insect pests belonging to the Curculionidae family in the order Coleoptera (Jayakumar et al., 2017). According to Boudreaux (1969), it originated in India and has spread out globally primarily through trade (Ponce *et al.*, 2022). It is a serious pest in the southern United States and other developing countries, and it attacks crops such as wheat, rice, maize, sorghum, and other grains. Both adults and grubs damage the grain on which they feed voraciously (Zhang et al., 2021).

*Sitophilus oryzae* is small (2–3mm), stout-looking, and reddish-brown to black in color (Patole, 2017). It has four light yellow or reddish dots on the corners of its stiff protective forewings (elytra) (Masiko, 2018). Additionally, the elytra have rows of pits within longitudinal grooves, and the prothorax is heavily pitted (Chambers, 2005; Rahman, 2017). During its life cycle, the female *S. oryzae* produces 250–400 eggs. The development from egg to adult takes approximately 26–32 days at an average temperature of around 28 °C and a relative humidity of 70%. Female adult *S. oryzae* makes a hole inside the grain using its snout and lays a single egg inside (Adetunji, 1984) sealing the hole with secretions from the ovipositor (Stephensons, 1983). The eggs hatch into larvae in about three days, and it takes the larvae around 18 days going through four instars to mature into pupae (Swamy et al., 2014). Pupation takes place inside the grain and takes 6 -7 days to develop into an adult (Mackled, 2017). Adults leave the grain after 2 -4 days and are ready to mate and continue the life cycle again (Roy, 2021).

### **Biology, Behavior, and Ecology of Flour Beetles**

*Tribolium castaneum* (red flour beetle) and *Tribolium confusum* (confused flour beetle) belong to the family of darkling beetles (Tenebrionidae, Coleoptera) (Vinokurov et al., 2009).

Red and confused flour beetles can be found all over the world, and they typically infest warehouses, retail locations, feed mills, and flour mills (Via, 1999). Flour beetles are tiny insects with body lengths of 3-5 mm (Doud, 1999; Burks et al., 2015).

*Tribolium castaneum* (red flour beetle) is a cosmopolitan pest of stored products with a polyphagous feeding habit attacking a wide variety of stored products and their by-products. (Erdoğan, 2021). With Indo-Australian origins, it is also found in temperate areas (Thakur, Eradasappa and Chandla, 2012). Additionally, *T. castaneum* survives winter in protected places, especially where there is central heat (Makai Panezai et al., 2019), and in the United States, it is found primarily in the southern states. *T. castaneum* can develop at temperatures between 22 and 40 °C, and this has allowed the beetles to spread to many geographic regions (Muslim and Al-Zurfi, 2019).

Larvae and adults of *T. castaneum* feed on grain dust and broken grain and spend their entire life cycle outside of grains or among several stored grain products including grain flour (Romero et al., 2010). In addition, *T. castaneum* infestations leave their excretions in the products such as frass, carcasses, exuviae, and a defensive chemical called benzoquinones by abdominal glands which generate unpleasant odor and colors in the product (Phankaen *et al.*, 2017) making the product unsuitable for human consumption (Linz et al., 2016). *Tribolium castaneum* has been identified as a potential carrier of phytopathogenic microbial agents, including *Aspergillus*, *Pseudomonas*, and *Staphylococcus*, which can contaminate infested stored-grains and storage products (Ebadollahi et al., 2021). The presence of these microbial agents can result in a decline in both the quality and quantity of the stored grains and products (Pires et al., 2019).

*Tribolium castaneum* adults is measure 2.3–4mm long and are red brown on color with eleven segmented antennae with the last three antennal segments being slightly enlarged (Abdullahi et al., 2019). Additionally, *T. castaneum* has a polygynandrous mating system characterized by extreme promiscuity by both sexes. Females can mate multiple times an hour with no precopulatory courtship or competition. In contrast, ejaculates of numerous males can coincide within the female reproductive tract leading to very intense postcopulatory sexual selection (Halle et al., 2015).

The life cycle of *T. castaneum* is that of a typical holometabolous insect (Suzuki et al. 2008). Female red flour beetles will lay 200–450 eggs in food. Eggs hatch in 5–12 days while the larvae can mature within 30 days in warmer months and 120 days in cooler months (Murugesan and Annappoorani, 2021). Additionally, the oviposition rate and emergence of new adults depend on the quality and type of substrate available since different types of flour contain different nutrients. More adult beetles emerge in diets with high protein content than with high carbohydrate content. Additionally, the developmental period of *T. castaneum* is longer when they are reared on diets with high carbohydrate content (Wong and Lee, 2011). *T. castaneum* is commonly used as a model for different studies such as insect development and pest biology studies (Brown et al., 2009; Rösner et al., 2020).

*Tribolium confusum* is also a secondary insect pest that attacks stored grains and foods in the pantry. This insect has a worldwide distribution including the United States (Throne et al., 2003b). Adults and larvae feed on broken kernels and finely-ground materials in granaries, mills, warehouses, and other places where grain or grain products are stored. Additionally, *T. confusum* is an omnivore and can eat eggs and other stages of its own and other species (Park, 1934). Similar to *T. castaneum*, when disturbed or crowded, may secrete



chemicals called quinones that can cause the infested feed to turn pink and produce a pungent odor (Hussain, 1993).

*Tribolium confusum* is a shiny, flattened, oval, reddish-brown beetle about 3-4 mm long (Tang et al. 2007; Johnson 2013). It was named because of the confusion over its identity since it closely resembles *T. castaneum* (Baldwin and Fasulo, 2003; Ming et al., 2015) except for the antennae which are four-segmented and gradually thicken towards the tip. Another slight difference is in the shape of the thorax, *T. castaneum* has a curved thorax on the sides, whereas the thorax of the *T. confusum* is straight (Stack, 2015; Baldwin and Fasulo, 2005). Furthermore, female *T. confusum* lay an average of about 450–500 eggs in its life cycle. Eggs are laid directly in flour and other stored food and are white or colorless covered with a sticky material to which flour can adhere. Eggs generally hatch into larvae in 3–5 days and the larvae are a light honey color and about 6 mm long (Baldwin and Fasulo 2014).

### **Management of stored grain pests**

Farmers commonly employ various management practices to control insect damage to stored grains (Kiaya, 2014). Additionally, proper ventilation is encouraged in storage facilities to regulate temperature and moisture levels, thereby reducing the risk of mold growth and insect infestations (Befikadu, 2014). Farmers also utilize airtight containers or bags to protect grains from moisture and insect damage (Martin et al., 2015; Odjo et al., 2022), and use botanicals that help to repel insect attacks (Boeke et al. 2004; Rajashekar et al. 2012). Moreover, heat treatment, fumigation (Hansen et al., 2011; Tang, Mitcham, et al., 2007), and insecticide application are employed as additional measures to deal with insect pests in storage (Hagstrum et al., 2012).

For decades, various synthetic pesticides have been used for stored grain protection from different pests (Hamel et al., 2020) because of their effectiveness, ease of shipment, storage, and application, and long-term persistence (Stejskal et al., 2021). Insecticides may be classified according to the type of formulation for example fumigants, liquid, and powder (Talukder, 2009). Insecticidal products belong to different classes: organochlorines, organophosphates, carbamates, and pyrethroids, however, organophosphates and pyrethroids insecticides are the major insecticides used in storage facilities (Lima do Rêgo et al., 2021; Liu et al., 2023), including developing countries (Athanassiou et al. 2004).

Fumigants are gases that are used to kill insect pests within stored grains. Some examples of fumigants are phosphine and methyl bromide. Methyl bromide is a chemical that was widely used as a fumigant to kill pests until recently. It is highly effective at controlling a wide range of pests (Fields and White, 2002). However, methyl bromide is highly toxic to humans and can cause a range of health effects, including headaches, dizziness, and difficulty breathing. Additionally, methyl bromide contributes to the destruction of the ozone layer, the part of the earth's atmosphere that protects living organisms from harmful ultraviolet (UV) radiation (Bramavath et al., 2017). Hence, its use is highly restricted and regulated by the Environmental Protection Agency (EPA) in the United States (Petroff, 2004). On the other hand, for many years, hydrogen phosphide, also known as phosphine (PH<sub>3</sub>), a colorless, toxic, and flammable gas has been used to fumigate insect pests in stored grain (Dieterich et al., 1967). Phosphine is a distinctive fumigant known for its numerous positive attributes, including affordability, adaptability, ease of application, and most notably, its reputation as a residue-free treatment (Chigoverah et al., 2018). However, insect resistance to phosphine has been reported due to excessive application (Emery and Holloway, 2011). Additionally, acute inhalation exposure to

phosphine can lead to various adverse effects in humans, such as headaches, dizziness, fatigue, drowsiness, nausea, vomiting, and cough. The use of new fumigants such as carbonyl sulfide ethanedinitrile (EDN) and ethyl formate have been investigated as alternatives in protecting food and non-food commodities in storage environments (Donahaye et al., 2001). Pirimiphos-methyl and malathion are examples of organophosphate insecticides used to control pest insects. The toxicity related to acute exposure to organophosphate insecticides is a cholinergic crisis resulting from acetylcholinesterase inhibition (Ohbe et al., 2018). Studies have shown pirimiphos-methyl and malathion to be toxic to insect pests when ingested (Lagisz et al., 2010; Rusyniak and Nañagas, 2004). On the other hand, deltamethrin is a broad-spectrum synthetic pyrethroid insecticide widely used to control insect pests, including *S. oryzae* (Paudyal et al., 2016). Pyrethroids are synthetic chemicals that are structurally like naturally occurring pyrethrins which are derived from the flowers of *Chrysanthemum* species (Shrivastava et al., 2011). Deltamethrin, a common pyrethroid, works by disrupting the normal functioning of the nervous system in insects leading to paralysis and death (Palmquist et al., 2012). Methoprene acts as an insect growth regulator that disrupts the natural growth and development of insects. By preventing processes like molting, egg-laying, egg hatching, and maturation from immature to adult stages, it effectively inhibits insect reproduction (Shinoda, 2015).

**Toxicity and Resistance of Pirimiphos-methyl, Deltamethrin, Deltamethrin plus (S-) Methoprene and Malathion to *Sitophilus oryzae*, *Tibolium castaneum*, and *Tribolium confusum***

In the case of pirimiphos-methyl and *S. oryzae*, several studies have been conducted to assess the toxicity of different doses in laboratory bioassays (Lagisz et al., 2010). For example, pirimiphos-methyl applied to corn at the rate of 4, 6, and 8ppm was extremely effective against

*S. oryzae* and other beetles (Huang and Subramanyam, 2005). Another study also found that pirimiphos-methyl was highly toxic to *S. oryzae* when applied to corn, with lethal doses ranging from 10 – 100ppm, and could be a useful tool in the management of *S. oryzae* infestations in stored grains (Ashamo et al., 2010). A recent study showed a 5ppm dose of pirimiphos-methyl can cause 100% mortality of *S. oryzae* after seven days of exposure (Sakka and Athanassiou, 2021). However, Rumbos et al. (2013) found that pirimiphos-methyl at lower dose range (1-4 ppm) can cause 100% mortality of adult *S. oryzae* at seven days of exposure.

Despite pirimiphos-methyl being effective against *S. oryzae*, a risk of resistance has been observed over time (Arthur, 1996). For example, Attia et al. (2020) conducted bioassays to determine the susceptibility of *S. oryzae* to pirimiphos-methyl and found that the insects were highly resistant to the insecticide.

Similarly, for deltamethrin, several studies have investigated its toxicity to *S. oryzae*. For instance, in one study, deltamethrin was found to have high toxicity after 24–48 hours of exposure to *S. oryzae* when applied at a concentration of 0.48 ppm (Soltan, 2020). In another study conducted by (Vélez et al., 2017), the researchers assessed the effects of deltamethrin and spinosad on the survival, activity, and avoidance behavior of the grain weevils *Sitophilus granarius* and *S. zeamais*. The results revealed that a concentration of 0.25mg of deltamethrin and 0.5mg of spinosad led to mortality in both insect species. Notably, deltamethrin exhibited a quicker action compared to spinosad. Additionally, Baker (1994) assessed the response of *S. oryzae* to different concentrations of deltamethrin and cyfluthrin, *S. oryzae* exhibited significantly lower sensitivity to cyfluthrin compared to deltamethrin.

Overall, these studies suggest that deltamethrin is a highly effective chemical for controlling *S. oryzae* infestations in corn crops. However, repeated exposure to deltamethrin can

result in the development of resistance in *S. oryzae*. For instance, a study conducted by Singh et al. (2021) to evaluate the resistance levels to deltamethrin revealed that *S. oryzae* treated with 180ppm exhibited resistance to the insecticide.

The insecticidal activity of several insect growth regulators (IGRs) was assessed against susceptible and pirimiphos- methyl-resistant strains of *T. castaneum*, susceptible strains of *Rhyzopertha dominica*, and *S. oryzae* at the concentration range of 0.1–20 ppm (Kostyukovsky et al., 2000). The results showed that among the IGRs evaluated, pyriproxyfen demonstrated the highest effectiveness against the three major stored product insects in the study. Conversely, methoprene demonstrated high efficacy against *R. dominica* but was less effective against *S. oryzae*. Tebufenozide on the other hand, exhibited low toxicity against all three species (Kostyukovsky et al., 2000). In another study, three compounds with insect juvenile hormone activity were investigated for their effectiveness in protecting wheat grain against one insecticide-susceptible strain and two insecticide-resistant strains of *Sitophilus granarius* (Edwards and Short, 1984). The findings revealed that neither JH I nor methoprene provided complete control of *S. granarius* at a concentration of 100ppm, whereas ethyl[2-(p-phenoxy) ethyl] carbamate exhibited effectiveness at a significantly lower concentration of only 5ppm. Additionally, there was no indication that the two insecticide-resistant strains of *S. granarius* displayed cross-resistance to any of the tested compounds.

Loschiavo (1976) investigated the impact of methoprene and hydroprene on six species of insects commonly found in stored food products. The study involved rearing these insects on diets treated with varying concentrations (1, 5, 10, and 20 ppm) of these insecticides. The findings revealed that both compounds, particularly at a concentration of 20 ppm, effectively prevented the emergence of pupae in *T. castaneum* and reduced pupae emergence in *T.*

*confusum*. Additionally, concentrations of 5 ppm or higher inhibited oviposition in both species. Padhee et al. (2002) investigated the resistance of *T. castaneum* to deltamethrin in their study and successfully generated six deltamethrin-resistant strains of *T. castaneum* through multiple generations. They started with an initial deltamethrin concentration of 5 ppm in the wheat flour medium for the first generation and gradually increased the concentration for each subsequent generation. By the sixth generation, the deltamethrin concentration reached 200 ppm. The study findings revealed that the deltamethrin resistance level in *T. castaneum* increased by 78.6-fold compared to the initial strain after six generations of selection. DGLISH (2008) studied the effectiveness of binary combinations of different insecticides against resistant strains of stored-grain beetles: *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum*, and *Cryptolestes ferrugineus* in wheat. The results of this study demonstrated that a combination of chlorpyrifos-methyl and (S)-methoprene at 10 ppm and 0.6 ppm, respectively, effectively controlled all strains, except for methoprene-resistant *R. dominica*. Similarly, spinosad and chlorpyrifos-methyl at 1 ppm and 10 ppm, respectively, controlled all strains, except for organophosphate-resistant *O. surinamensis*. This study emphasizes the significance of considering resistance when evaluating the efficacy of insecticide combinations.

### **Study Objective**

The primary objective of this study was to assess the toxicity of four different insecticides against *S. oryzae*, *T. castaneum*, and *T. confusum*, which are known to infest corn grain. The application of insecticides in managing pests in grain storage facilities is a justified approach to protect stored food products, minimize economic losses, and reduce health hazards. Stored pests like *S. oryzae*, *T. castaneum*, and *T. confusum* can cause damage and contamination in stored food products, resulting in a decrease in quality, quantity, and an increase in health risks. By

employing insecticides in a controlled and regulated manner, it becomes possible to effectively eliminate or minimize pest populations, ensuring the safety and preservation of stored food products. The study holds significant importance as it provides insights into the toxicity and resistance of pirimiphos-methyl, deltamethrin, deltamethrin plus (S)-methoprene, and malathion against *S. oryzae*, *T. castaneum*, and *T. confusum*. The findings of these studies will contribute to refinement of chemical management recommendations for these insect pests.

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## Chapter 2

### Toxicity Assessment of Four Commonly Used Insecticides to the Corn-Infesting Rice

#### Weevil *Sitophilus oryzae* (Coleoptera: Curculionidae)

##### Abstract

Rice weevil (*Sitophilus oryzae*) is one of the most significant pests of corn and other stored grains. For a long time, synthetic insecticides have been widely used to control pest populations due to their effectiveness, convenience of storage and application, and long-lasting effects. This study aimed to assess the toxicity profiles of four commonly used insecticides, including pirimiphos-methyl, deltamethrin, deltamethrin plus (S)-methoprene, and malathion to mature rice weevils. During our experiment, rice weevils were exposed to a range of doses of each insecticide to generate response curves, and LC<sub>50</sub> and LC<sub>90</sub> values were determined. The results showed that pirimiphos-methyl had the highest toxicity, with an LC<sub>50</sub> of 0.74 ppm (95% CL 0.57–0.92), followed by malathion, deltamethrin, and deltamethrin plus (S)-methoprene at 7.08 ppm (95% CL 5.44–9.01), 9.11 ppm (95% CL 6.89–12.6), and 13.94 ppm (95% CL 9.71–18.60), respectively. Subsequently, we compared the LC<sub>50</sub> value of each insecticide formulation with the label-recommended application rate and found that only malathion aligned with the label rate. Pirimiphos-methyl was over-applied, while deltamethrin and deltamethrin plus (S)-methoprene were under-applied.

## Introduction

Food grains and pulses are the primary sources of nutrition for the global population, particularly in tropical regions, making them vital in the fight against food insecurity comprising a major portion of daily diets in many countries across the globe (Singh and Singh, 1992). *Triticum aestivum* (wheat), *Zea mays* (corn), and *Oryza sativa* (rice) are the world's topmost staple cereals (Erenstein et al., 2022). Corn, scientifically known as *Zea mays* (L.), is a cereal crop that has been cultivated by human societies for thousands of years (Danforth, 2009). Corn has become a vital food source for billions of people and serves as a primary ingredient in animal feed (Serna-Saldivar and Carrillo, 2019). Moreover, it acts as a critical raw material in various industrial processes, such as the production of ethanol and corn syrup (Zahniser et al., 2019). Despite its essential role in food and industrial production, corn cultivation faces several challenges, including pest and disease damage, which have resulted in significant losses over the year (Balderacchi et al., 2018).

The rice weevil, *Sitophilus oryzae* (L) 1763 (Coleoptera: Curculionidae) is a highly destructive pest that infests grain products, including corn (Davis, 2011). Due to its preference for newly harvested grain, it is classified as a primary pest (Srivastava and Subramanian, 2016). Bhargude et.al. (2021) suggested that *S. oryzae* originated in India and subsequently spread globally through trade (Ashamo et al., 2010). This pest poses a significant threat to agricultural production in the southern United States and developing countries as it attacks a wide range of crops such as wheat, rice, maize, sorghum (*Sorghum bicolor*), and other grains (Irabagon, 1959). Both the adult weevils and larvae are responsible for causing severe and extensive damage to grains( Zhang et al., 2021b). *Sitophilus oryzae* is characterized by its small size (2 to 3 mm) and stout, reddish-brown to black appearance, with four light yellow or reddish spots located at the

corners of the elytra (wing cases) (Hong et al., 2018). The prothorax exhibits significant pitting, while rows of pits can be found within longitudinal grooves on the elytra. The larvae are plump, fleshy, and legless measuring 5mm in length, with a cream-colored body and a dark head capsule (Koehler, 2022). The feeding behavior of *S. oryzae* involves chewing a hole into the grain where they deposit a single egg inside the hole, and subsequently sealing it with secretions from its ovipositor (Funsho, et al., 1984). After approximately three days, the eggs hatch into larvae, which undergo four instars over a period of approximately 18 days before pupating inside the grain (Bhuiyah et al., 1990). The pupation stage lasts for six days before the larvae emerge as adult weevils. These newly emerged adults leave the grain within 2 to 4 days and are ready to mate again (Fouad, et al., 2021). Female *S. oryzae* lay approximately 250–400 eggs during their lifetime and complete their lifecycle around 26–32 days at a temperature of 28<sup>0</sup>C and relative humidity of 70% (Howe, 1952; Maciej Serda et al., 2022). Studies on the biology of *S. oryzae* on rice have shown that the developmental stages and duration of the life cycle can vary depending on the season (Devi et al., 2017). The life cycle duration is longer during winter and shorter during summer (Okram and Hath, 2019).

To control *S. oryzae* infestations, farmers commonly employ a range of management practices from cultural methods to chemical interventions (Shankar and Abrol, 2012). Some of the common practices include thorough cleaning and drying of grain to eliminate debris and prevent mold growth (Bhattacharyya et al., 2022). Additionally, ensuring adequate ventilation in storage facilities to regulate temperature and moisture levels plays a crucial role in preventing mold growth and insect infestations. The use of airtight containers or bags is also recommended to mitigate moisture-related damage and deter insect activity. Utilizing heat treatment, fumigation, and insecticide application has also proven effective in *S. oryzae* management

(Hagstrum et al., 2012; Bhattacharyya et al., 2022). The use of insecticides in managing stored pests is a reasonable approach for preserving food products in storage, minimizing economic losses, and reducing hazards (Chulze, 2010). Pests such as *S. oryzae* can infest food products during storage, resulting in damage and contamination that can adversely affect the quality and quantity, and pose health risks (Kumar and Kalita, 2017). However, the regulated application of insecticides can effectively control pest populations, thereby ensuring the safety and preservation of stored food products (Damalas and Eleftherohorinos, 2011).

Since the 1950s, synthetic pesticides and fumigants have been used to protect stored grain due to their effectiveness, ease of storage and application, and long-term persistence (Attia et al., 2020). The commonly used fumigants are phosphine and methyl bromide. However, methyl bromide poses significant risks to human health, causing a range of health issues and contributing to ozone layer depletion, thereby necessitating stringent restrictions on its use (Rajendran, 2001; Bramavath et al., 2017). Presently insecticides commonly used include organophosphates, pyrethroids, and carbamates (Andrić, 2006). However, resistance to these insecticides is common in stored grain pests, including *S. oryzae*, underscoring the importance of regular monitoring. The main objective of this study was to assess the toxicity of four commonly used insecticide formulations, including pirimiphos-methyl, deltamethrin, deltamethrin plus (S)-methoprene (insect growth regulator), and malathion to *S. oryzae*. From the findings of this study, we can evaluate the efficacy of the current application rates and provide recommendations for enhancing the management strategies for controlling *S. oryzae* infestations in corn and other stored grains.

## **Materials and Methods**

### ***Insect Collection and Handling for Laboratory Bioassays***

The field population of *S. oryzae* was collected from different corn-growing regions in Arkansas from 2018 – 2020- and brought to the toxicology laboratory at the Department of Entomology and Plant Pathology, University of Arkansas (Fayetteville, AR). The test insects were reared in 4.4L plastic containers (Rubbermaid, Atlanta, GA), wherein whole kernels of corn were provided as the food source. The containers were modified with mesh lids to ensure proper airflow. During the study, the laboratory maintained a constant temperature of 75<sup>0</sup>F and a relative humidity of 60–65%. This approach closely mimicked their natural diet and conditions to ensure the growth and reproduction of the insect population throughout the experimental period.

### ***Bioassay Protocol***

The candidate insecticides were those commonly used for stored grain pest control (Table 1). These pesticide formulations came in two physical forms: liquid and powder. Liquid insecticides were mixed with distilled water to get the desired concentrations, then applied to the rice weevils by using a specially designed Lab Spray Tower (Figure 1) with a spray nozzle (Burkard Scientific, Uxbridge, United Kingdom), and distilled water was used as the control for these liquid treatments. Meanwhile, powder insecticide was mixed with white corn flour to get the desired concentrations (Gazab Products, United Trading Inc., Des Plaines, IL), and the control was corn flour alone. Treatment concentrations were chosen first based on the recommended application rate, then adjusted gradually based on the results of a series of pilot studies. The primary goal of these pilot bioassays was to determine the range of concentration that caused 5–95% mortality. To generate a response curve representing each insecticide's

toxicity profile, at least five different concentrations (each with three replications of 15 individuals/replication) (Robertson et al. 2007) were used.

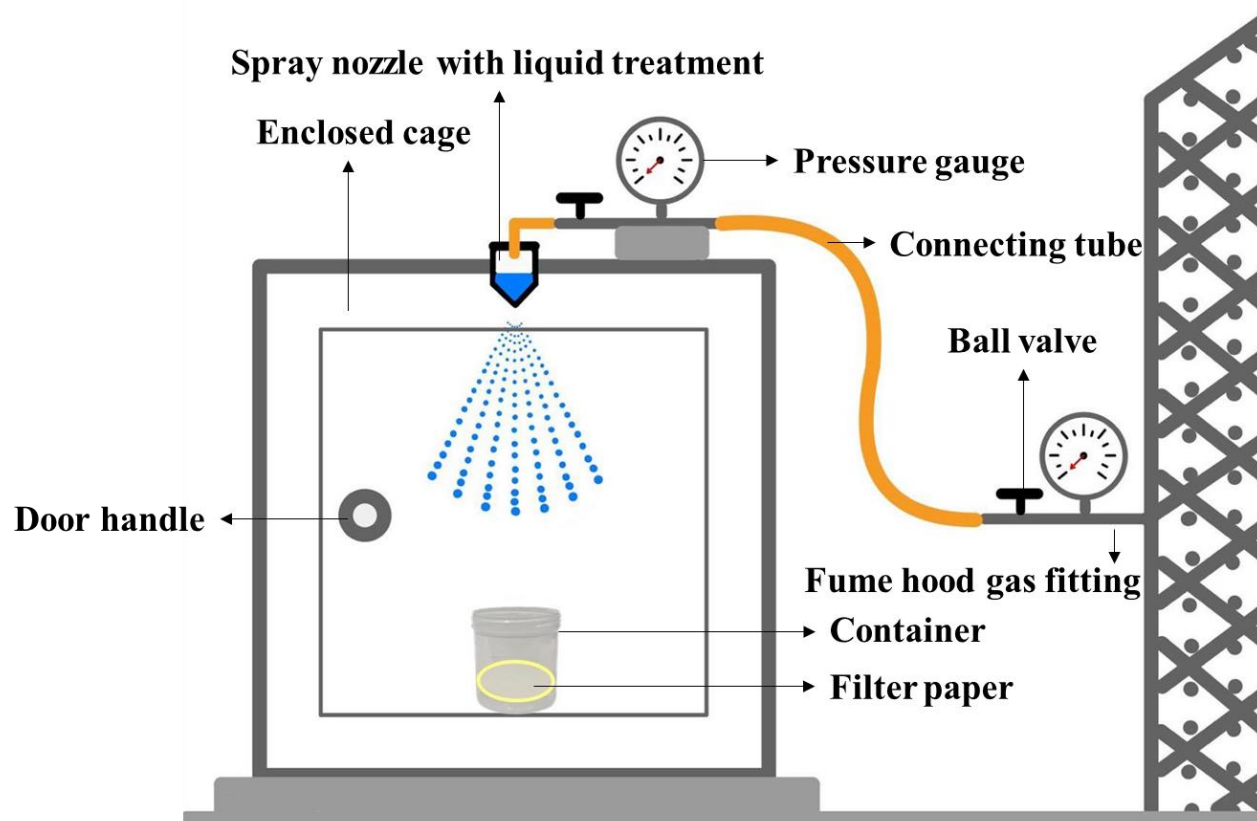
For the bioassays, the specific insecticide treatment or control was sprayed onto Whatman filter paper Grade 1 WHA1001-045 (Sigma-Aldrich Inc., St. Louis, MO) and placed in a 120mL polypropylene jar PLA-03346 (Qorpak, Clinton, PA) to ensure uniform distribution (Figure 1). In the following step, a group of 15 rice weevils was introduced into the jar with a twisted cap as a barrier to prevent the escapees (Figure 2). This methodology aligns with the guidelines provided by Haliscak and Beeman (1983), which is consistent with the guidelines provided by (Busvine and the Food and Agriculture Organization of the United Nations, 1980). On the other hand, for the powder formulation, desired treatments were mixed with corn flour and assigned to the prepared jars. Thereafter, insects were introduced in the jars and then shaken vigorously.



**Table 1. List of pesticides used in the bioassays.**

The commercial name, common name, mode of action group, and physical form for each pesticide used for insect bioassay efficacy studies in Arkansas

<b>Product</b>	<b>Formulation</b>	<b>Chemical Group (IRAC Code)</b>	<b>Mode of Action</b>	<b>Physical Form</b>
Actellic 5E (WinField United, St. Paul, MN, USA)	pirimiphos-methyl 57%	1B - organophosphate	Acetylcholinesterase Inhibitors	Liquid
Centynal EC (Central Life Sciences, Schaumburg, IL, USA)	deltamethrin 4.75%	3A - pyrethroids, pyrethrins	Sodium Channel Modulators	Liquid
Diacon IGR Plus (Central Life Sciences, Schaumburg, IL, USA)	deltamethrin 4.75% + (S)-methoprene 11.4%	3A - pyrethroids, pyrethrins + juvenile hormone	Sodium Channel Modulators + Insect Growth Regulator	Liquid
Malathion Big-6 Dust (Balcom Chemicals, Inc., Greeley, CO, USA)	malathion 6%	1B - organophosphate	Acetylcholinesterase Inhibitors	Powder



**Figure 1. Design and illustration of a customized lab spray tower for application of liquid pesticide formulations used in the experiment.**



**Figure 2. Design of Experimental container used in the study.**

### ***Data Collection and Statistical Analysis***

In each treatment, the mortality data of *S. oryzae* was recorded every 24 hours after exposure and continued for 10 days to determine if there was delayed mortality (Biddinger, Hull, and Rajotte, 1998; Phan et al., 2020). The data sets were analyzed by POLO Plus 2.0 (LeOra Software LLC, 2005) as described by Robertson et al. (2007). This statistical approach is used to determine the 50% lethal concentration value ( $LC_{50}$ ) and 95% confidence interval. These values are crucial for understanding insecticide toxicity and enhancing the current pest control strategies.

## Results and Discussion

We found the toxicity profiles of the selected insecticides as follows: pirimiphos-methyl > malathion  $\geq$  deltamethrin  $\geq$  deltamethrin plus (S)-methoprene, by comparing the LC<sub>50</sub> values and their 95% confidence limits. Pirimiphos-methyl had the strongest effect, being ~15 times more toxic than the other three insecticides, followed by malathion and deltamethrin.

Deltamethrin plus (S)-methoprene was the least toxic insecticides among all (Table 2). Mortality reached the maximum at 48 hours after exposure. Although the test insects were maintained and mortality reading was conducted for 10 days after exposure, there was no delayed mortality.

The variations in the toxicity profiles among the selected insecticides can be attributed to their differences in their modes of action and target specificity. For example, pirimiphos-methyl and malathion, classified as organophosphate insecticides, exert their insecticidal activity by inhibiting the action of acetylcholinesterase, an essential enzyme for proper nervous system function in insects (Brown, 2000). Acetylcholinesterase is responsible for breaking down the neurotransmitter acetylcholine, which is crucial for the transmission of nerve signals (Pope, 2005). By inhibiting this enzyme, pirimiphos-methyl causes an accumulation of acetylcholine, leading to overstimulation of the nervous system and subsequent paralysis or death of the targeted insects (Lagisz, Wolff, and Port, 2010). Deltamethrin disrupts the normal functioning of the nervous system (Tapia et al., 2020) by affecting the voltage-gated sodium channels, leading to an influx of sodium ions and subsequent nerve excitation (Pitzer et al., 2021). This disrupts the transmission of nerve signals, resulting in paralysis and ultimately causing the death of the insects (Gupta and Milatovic, 2014; Magby and Richardson, 2017). On the other hand, (S)-methoprene, functioning as an insect growth regulator, operates by impeding chitin synthesis (Ghosat, 2018). Chitin is a crucial component of the exoskeleton in insects. By inhibiting its

synthesis, (S)-methoprene interferes with the proper growth and development of insects, ultimately leading to their control (Merzendorfer and Zimoch, 2003; Fulton et al., 2013).

The effectiveness of pirimiphos-methyl in controlling *S. oryzae* has been demonstrated in several studies. Huang and Subramanyam (2005) found that pirimiphos-methyl at the rate of 4–6ppm was toxic to *S. oryzae*, while Rumbos et al. (2013) reported 100% mortality of adult *S. oryzae* after 7 days of exposure to doses of 1–4 ppm of pirimiphos-methyl. In a recent study conducted by Soltan et al. (2020), the acute toxicity of different insecticides, including pirimiphos-methyl and deltamethrin, was compared where researchers found that concentrations ranging from 0.35 to 0.85ppm of both pirimiphos-methyl and deltamethrin were effective against *S. oryzae*.

In the study by Derbalah et al. (2021), the researchers evaluated different methods for controlling *S. oryzae* by considering parameters such as adult mortality, offspring production, mode of action, and grain quality. They found that a treatment rate of malathion at 0.06ppm was effective to control *S. oryzae*. On the other hand, previous studies by Hasan Iqbal et al. (2012) evaluated the toxicity of cypermethrin and malathion to *S. oryzae*. The results of this study indicated that malathion at the rate of 5ml with an  $LC_{50}$  of 7.54ppm after 48 hours of exposure was effective. Regarding (S)-methoprene, Wijayaratne et al. (2018) found limited toxicity of (S)-methoprene towards mature stored pests. Its effectiveness in managing adult insects is comparatively lower than its efficacy against juvenile insects. Another possible reason for the difference in toxicity could be the development of resistance within the *S. oryzae* population towards deltamethrin plus (S)-methoprene, deltamethrin, and malathion due to the repeated use of these pesticides thereby reducing the effectiveness of these chemicals, in contrast, there is

currently no evidence of widespread resistance to pirimiphos-methyl in *S. oryzae* (Khan et al., 2022).

In a separate step, we compared the label-recommended application of these insecticides with their respective LC<sub>50</sub> values and found that the recommended rate for pirimiphos-methyl (6–10 ppm) not only exceeded its LC<sub>50</sub> at 0.74 ppm (95% CL 0.57–0.92) but also surpassed its LC<sub>90</sub> at 1.68 ppm (95% CL 1.31–2.56) by ~4 times. On the other hand, deltamethrin and deltamethrin plus (S)-methoprene were under-applied, as their recommended rates (0.5–1.0 ppm) were lower than their respective LC<sub>50</sub> values at 9.11 (95% CL 6.89–12.6) and 13.94 (95% CL 9.71–18.60), respectively. Only the recommended application rate of malathion aligned with the toxicity profile.

The difference between the recommended label rates and the LC<sub>50</sub> values may arise from several factors. Firstly, the recommended label rates are typically established as technical rates, designed to address a range of insect pests beyond those specifically mentioned in the study. As a result, these rates might be either overapplied or under-applied when applied to the insect species under investigation. However, it's possible that these rates align well with other groups of insect pests listed in the approved labels of these pesticides. Moreover, these recommended rates are applied as preventative methods, and therefore may have higher rates. Furthermore, in this study, we used formulated concentrations of the insecticides which could lead to variations in the effective to the target insects and influence the observed outcomes.

**Table 2. Toxicity response of *Sitophilus oryzae* to selected pesticides at 48h after treatment.**

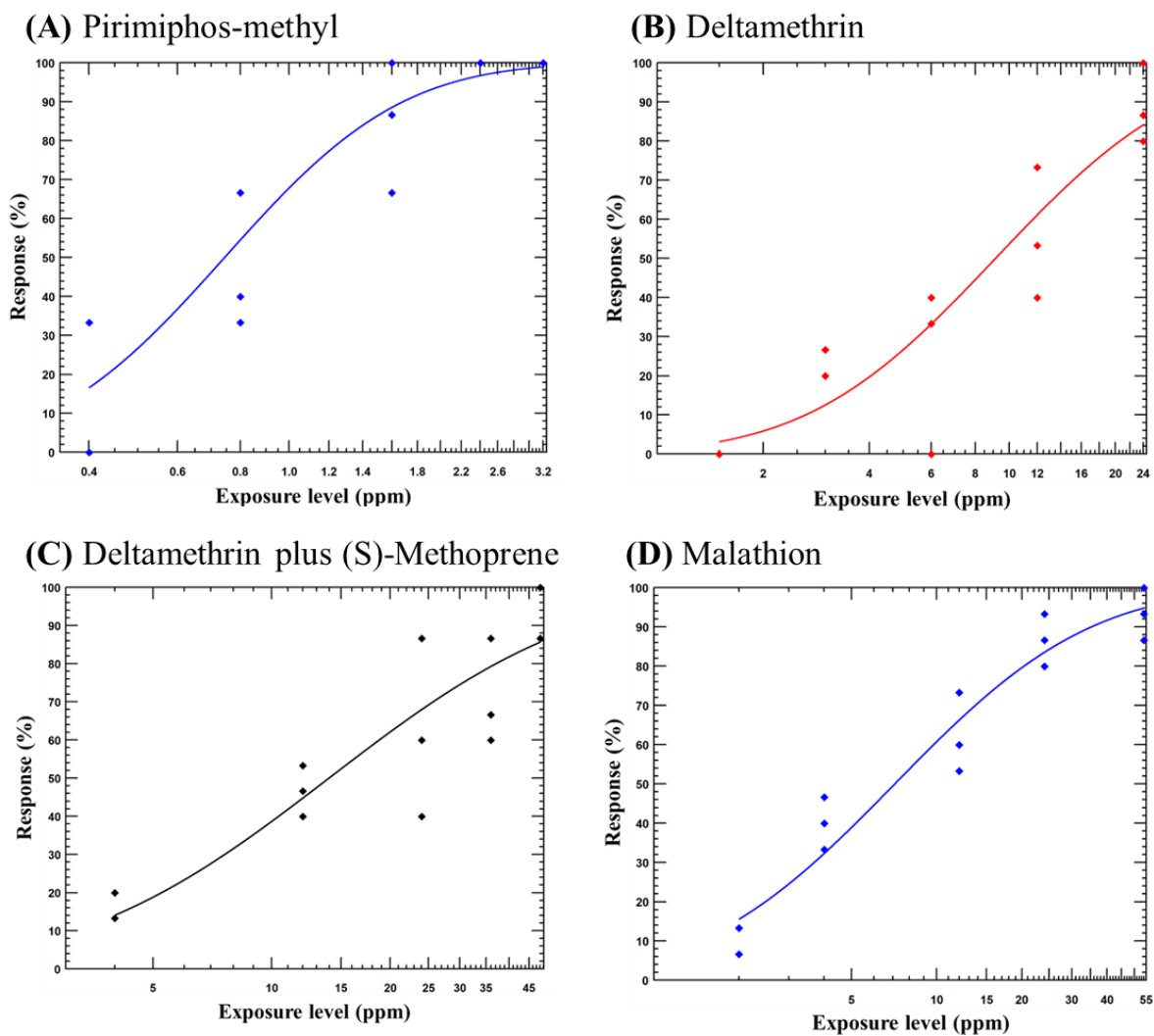
Active ingredient <sup>1</sup>	N <sup>2</sup>	Slope ± SE*	LC <sub>50</sub> (ppm) (95% CL)	LC <sub>90</sub> (ppm) (95% CL)	Recommended application rate (ppm) <sup>#</sup>
Pirimiphos-methyl	225	3.608 ± 0.411	0.74 (0.57 – 0.92)	1.68 (1.31 – 2.56)	6 – 8
Malathion	225	1.842 ± 0.214	7.08 (5.44 – 9.01)	35.13 (25.00 – 57.58)	10
Deltamethrin	225	2.381 ± 0.283	9.11 (6.89 – 12.6)	31.45 (20.34 – 70.50)	0.5 – 1.0
Deltamethrin + (S)-methoprene	225	1.984 ± 0.262	13.94 (9.71 – 18.60)	61.69 (41.05 – 131.0)	0.5 – 1.0

<sup>1</sup>The products are listed based on toxicity profile, from high to low.

<sup>2</sup>N is the number of individuals tested for each product. Response regression lines are presented by Slope ± SE, LC<sub>50</sub> (in ppm), and LC<sub>90</sub> (in ppm).

\*Control mortality was 0% during the study period.

<sup>#</sup> Recommended application rates were obtained from the pesticide product labels (Balcom Chemicals Inc., 1975; Winfield Solutions LLC, 2015; Central Garden & Pet Company, 2016a, 2016b).



**Figure 3. Toxicity response of *Sitophilus oryzae* to selected pesticides: (A) pirimiphos-methyl, (B) deltamethrin, (C) deltamethrin plus (S)-methoprene, and (D) malathion at 48h after treatment.**



## Conclusion and Recommendation

In conclusion, this study highlights the importance of selecting appropriate insecticides for controlling stored grain pests. All pesticides evaluated in this study varied in toxicity. Pirimiphos-methyl was more toxic to *S. oryzae* than malathion, deltamethrin, and deltamethrin plus (S)-methoprene. However, comparing the label-recommended application of these insecticides with their respective LC<sub>50</sub> values, the recommended rate for pirimiphos-methyl exceeded its LC<sub>50</sub> by ~4 times. This finding indicated that pirimiphos-methyl was over-applied. On the other hand, deltamethrin and deltamethrin plus (S)-methoprene were under-applied, as their recommended rates were lower than the lower ends of their 95% CL of LC<sub>50</sub>. Only the recommended application rate for malathion aligned with the toxicity profile. The difference in toxicity between the four insecticides could be attributed to the difference in the mode of action and the development of resistance of *S. oryzae* to some of these insecticides. Additionally, the recommended rates are applied as preventative methods and designed technically for a number of insects apart from *S. oryzae*. Therefore, this study contributes to our understanding of the relative toxicity and effectiveness of pirimiphos-methyl, deltamethrin, deltamethrin plus (S)-methoprene, and malathion in controlling *S. oryzae* populations. However, further research is needed to determine long-term efficacy and potential resistance of these insecticides for different populations of *S. oryzae*.

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### Chapter 3

#### **Toxicity of Different Insecticides to Red Flour Beetle *Tribolium castaneum* and Confused Flour Beetle *Tribolium confusum* (Coleoptera: Tenebrionidae)**

##### **Abstract**

Flour beetles such as *Tribolium castaneum*, the red flour beetle, and *Tribolium confusum*, the confused flour beetle are major pests of cereal grains and their products. Different insecticides that provide rapid and effective control for various insect pests can help reduce flour beetle infestations. This study evaluated the toxicity of commonly used insecticides (pirimiphos-methyl, deltamethrin, deltamethrin plus (S)-methoprene, and malathion) on flour beetles. The flour beetles were exposed to these insecticides at different ranges of concentration to determine toxicity. In terms of the LC<sub>50</sub> values, the toxicity of the four insecticides to *T. castaneum* was as follows: deltamethrin plus (S)-methoprene  $\geq$  deltamethrin > pirimiphos-methyl > malathion. This means deltamethrin plus (S)-methoprene and deltamethrin displayed the highest toxicity against *T. castaneum* with LC<sub>50</sub> values of 0.025ppm and 0.026ppm, respectively, followed by pirimiphos-methyl at 0.785ppm. Malathion displayed the lowest toxicity among all the insecticides tested, with an LC<sub>50</sub> value of 4.090ppm. On the other hand, the toxicity of these insecticides against *T. confusum* were as follows: pirimiphos-methyl > deltamethrin plus (S)-methoprene > deltamethrin  $\geq$  malathion. However, considering the LC<sub>50</sub> values of these insecticides, pirimiphos-methyl was highly toxic among all the insecticides, 0.148ppm, followed by deltamethrin plus (S)-methoprene 0.512ppm, deltamethrin 4.247ppm and malathion 7.842ppm. Although these two are closely related species, they responded differently to the selected insecticides. For instance, *T. confusum* displayed higher susceptibility to pirimiphos-methyl compared to *T. castaneum* while *T. castaneum* exhibited greater susceptibility to

deltamethrin, deltamethrin plus (S)-methoprene, and malathion compared to *T. confusum*. These findings suggest the need for different chemicals to be included in the management programs aimed at controlling these two *Tribolium* species.



## Introduction

Flour beetles, including the red flour beetle *Tribolium castaneum* and its closely related species, the confused flour beetle *Tribolium confusum* (Coleoptera: Tenebrionidae), are of significant global concern due to their status as pests in stored food products such as flour, grains, and other commodities (Duehl et al., 2011; Athanassiou et al., 2016; Via, 1999). Since both species are widely distributed across various regions worldwide, they are widely used as model organisms in different scientific studies including those focusing on ecology and evolution (Holditch and Smith, 2020; Milutinović et al., 2013; Grünwald et al., 2013; Mason 2018; Kumar et al., 2018). Flour beetles are small insects that are about 3–5 mm long and have a reddish-brown color (Brown et al., 2009; Muhamad and Sule, 2019).

Despite being closely related species, *T. castaneum* and *T. confusum* exhibit distinct characteristics. For example, adult *T. castaneum* is 2.3–4 mm long and red brown in color (Klingler and Bucher, 2022). The beetle's antennae consist of eleven segments, with the final three segments slightly enlarged (Umar, Shirama and Turak, 2015). Male individuals possess a slender projection, known as a setaceous lesion, on the ventral surface of the front femur (Chaubey, 2023). On the other hand, *T. confusum* is an oval-shaped, flattened, shiny, reddish-brown beetle, approximately 3–4 mm in length (Baldwin and Fasulo, 2020). It was named due to the confusion regarding its identification with *T. castaneum*, except for the four-segmented antennae that gradually thicken towards the tip (Athanassiou et al., 2008). Another slight distinction lies in the shape of the thorax, with *T. castaneum* exhibiting curved sides, while the thorax of *T. confusum* is straight (Stack, 2015).

Infestation by *T. castaneum* could leave noticeable effects on the affected grains such as the presence of frass, carcasses, and exuviae (Gao et al., 2022), leading to a gray discoloration

and strong odor (Lü et al., 2022; Campbell and Runnion, 2003). This is attributed to the release of defensive chemicals called benzoquinones (Lis et al., 2011; Buckman et al., 2013) which are synthesized by the insect's prothoracic and abdominal glands (Agarwal and Agashe, 2020; Davyt-Colo et al., 2022). As a result, these effects render the infested product unsuitable for human and animal consumption (Linz et al., 2016). Furthermore, *T. castaneum* can act as a vector for transmitting phytopathogenic microbial agents such as *Aspergillus*, *Pseudomonas*, and *Staphylococcus* to stored products (Yun et al., 2018). Similarly, infestations by *T. confusum* cause great damage to stored products that may reach 9% in developed countries and more than 20% in developing countries (Tawfeeq Al-Ani et al., 2018). Furthermore, *T. confusum* infestations in stored flour and grains increase due to contamination with frass, body fragments, and metabolic by-products. As a result, the grain and flour become unfit for human and animal consumption as well as reduce the market value ((Ebadollahi et al., 2021; Pires et al., 2019) ). Additionally, when disturbed or in crowded conditions, *T. confusum* secretes quinones, leading to a pink coloration and pungent odor in the infested feed (Hussain 1993).

There are different control methods that are used to reduce the damage caused by flour beetle for example, proper sanitation to remove their source of food, the use of natural repellents such as neem leaves, and heat treatment that kill some stages of the beetles (Jilani et al., 1988; Dowdy and Fields, 2002). Insecticides such as organophosphates, pyrethroids, and carbamates on the other hand, are now commonly utilized to reduce infestation by different stored product pests including flour beetles (Andrić, 2006; Reina et al., 2017). Insecticides offer rapid, effective, and long-lasting control methods for insect pests (Attia et al., 2020; Athanassiou et al., 2021) However, the development of resistance among the stored grain insect pests, including *T.*

*castaneum* and *T. confusum*, is very common and requires regular monitoring (Singh and Prakash, 2013).

In this study, we assessed the toxicity profiles of four insecticide formulations, including pirimiphos-methyl, deltamethrin, deltamethrin plus (S)-methoprene, and malathion, on *T. castaneum* and *T. confusum* to find the toxicity profiles of the insecticides in terms of 50% lethal concentration and 95% confidence limits. In addition, the toxicity response of these closely related species to these insecticides was compared. The findings of this study would be helpful in refining current recommended pesticide application rates for cost-effective stored-grain pest management.

## **Materials and Methods**

### ***Insects Collection and Handling for Lab Bioassays***

The field populations of the two flour beetle species *T. castaneum* and *T. confusum* were obtained from several corn-growing regions in Arkansas from 2018 – 2020. These insects were reared in the Insect Toxicology and Behavior Lab at the Department of Entomology and Plant Pathology, University of Arkansas (Fayetteville, AR). To ensure optimal conditions for the insects' growth and development, modified large (4.4L) plastic containers (Rubbermaid, Atlanta, GA) with mesh lids were used. These containers were half-filled with white corn flour (Gazab Products, United Trading Inc., Des Plaines, IL), which served as the primary food source for both species. This choice of substrate aimed to mimic the conditions prevalent in storage facilities and warehouses where infestations commonly occur. During the rearing process, the ambient temperature was maintained within the range of 70–75°F, while the relative humidity was regulated and maintained between 60% and 65%.

### ***Bioassay Protocols***

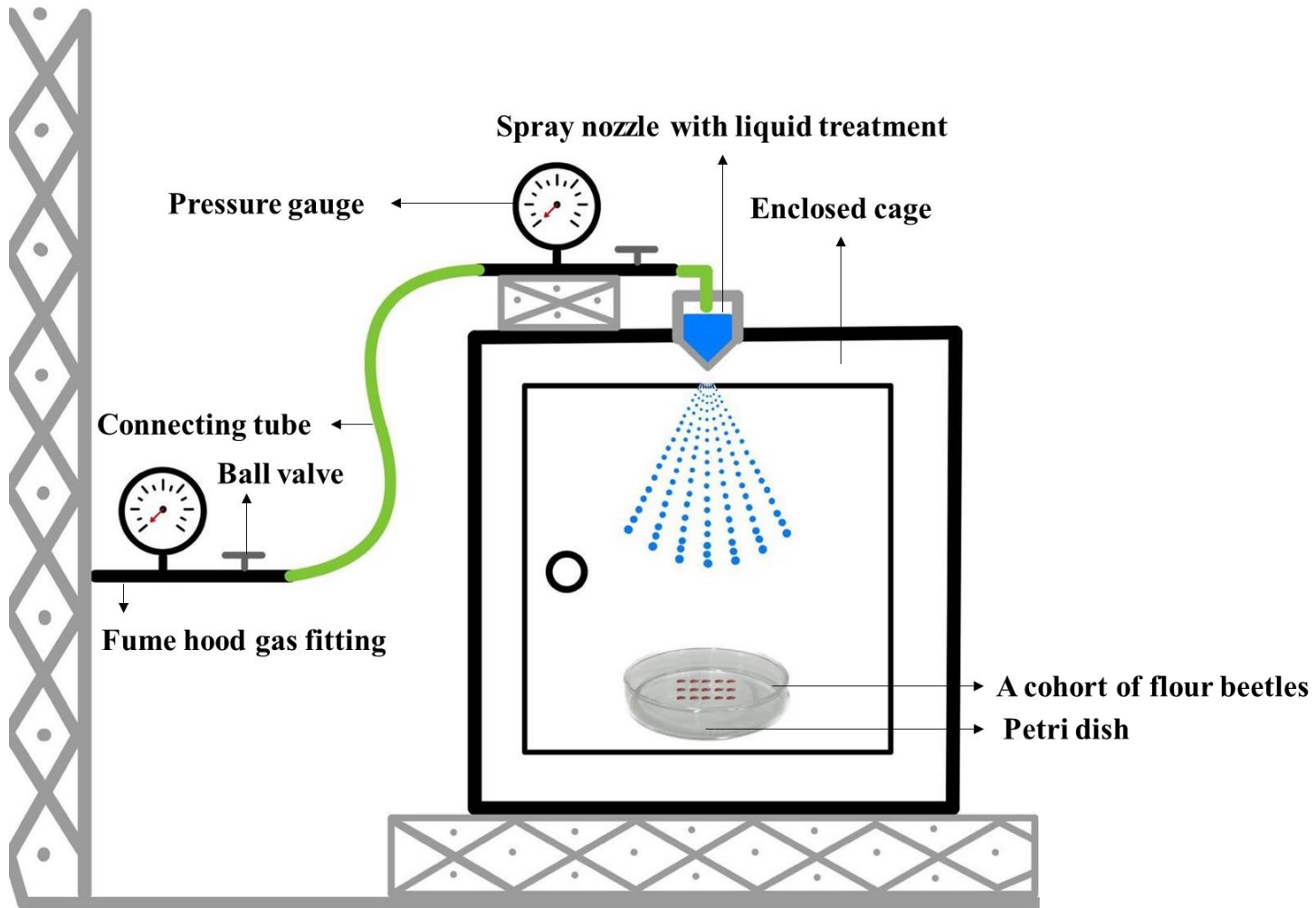
In laboratory experiments the toxicity of four insecticide formulations, including pirimiphos-methyl, deltamethrin, deltamethrin plus (S)-methoprene, and malathion against *T. castaneum* and *T. confusum* was evaluated. These insecticide products were selected based on their current use in stored pest management in Arkansas and elsewhere. Specific formulations and known modes of action as well as other related information are presented in Table 1. The initial selection of treatment concentrations was based on the suggested application rate, then we gradually adjusted these ranges based on the preliminary results of pilot studies. The primary goal of these pilot bioassays was to determine the range of concentration that caused mortality rates between 5–95%.

The selected insecticides were applied to the cohort of insects by different methods depending on their physical form (liquid or powder). For liquid pesticides, we dissolve them in water and diluted the solution to get the desired concentrations, then sprayed on the prepared cohort of insects in Mono Petri Dish VWR25384-302 (Avantor Inc., Radnor, PA) by using a spray nozzle in a Lab Spray Tower (Burkard Scientific, Uxbridge, United Kingdom) (Figure 1). For powder insecticides, we mixed the pesticide formulations with corn flour if needed and applied the desired concentrations directly inside Pyrex® Petri Dishes VWR25354-047 (Corning Inc., Glendale, AZ) (Figure 2). The use of glass containers for powder treatments was to prevent static electricity, as we observed that dust tends to cling to plastic containers and thus does not ensure equal exposure of the insect cohort to powder treatments.

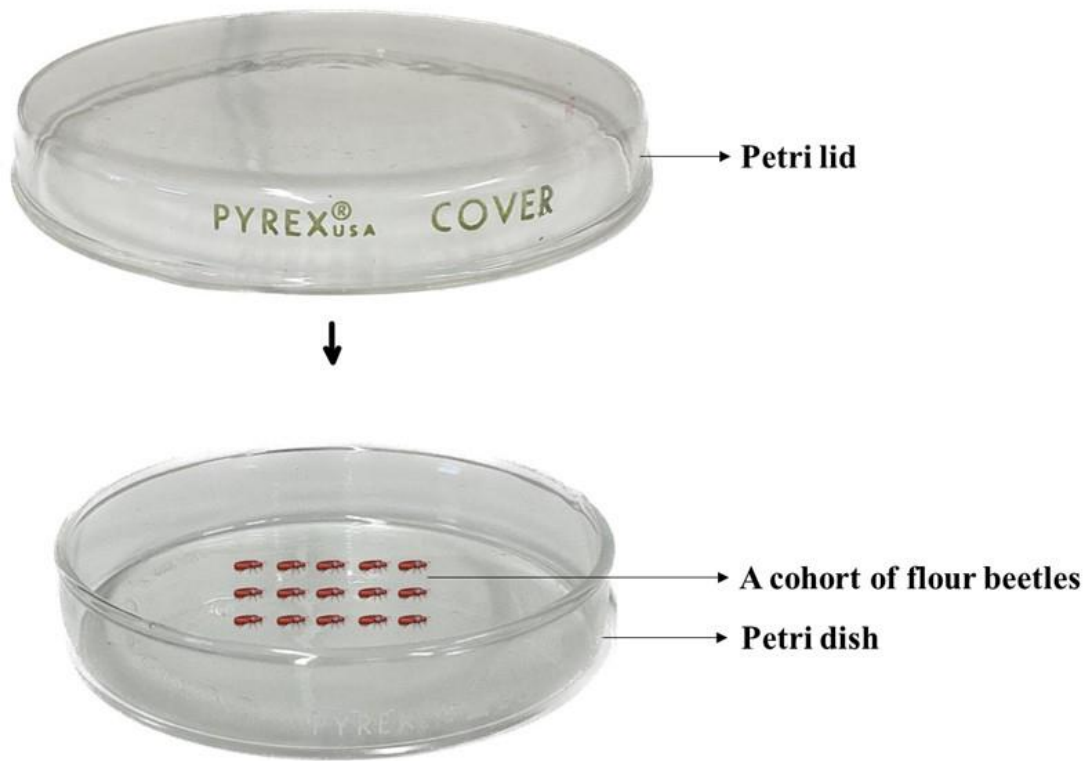
**Table 1. List of different insecticides used in the laboratory bioassays.**

<b>Product</b>	<b>Formulation</b>	<b>Chemical Group (IRAC Code)</b>	<b>Mode of Action</b>
Actellic 5E <i>(WinField United, St. Paul, MN, USA)</i>	pirimiphos-methyl 57%	1B - organophosphate	Acetylcholinesterase Inhibitors
Malathion Big-6 Dust <i>(Balcom Chemicals, Inc., Greeley, CO, USA)</i>	malathion 6%	1B - organophosphate	Acetylcholinesterase Inhibitors
Centynal EC <i>(Central Life Sciences, Schaumburg, IL, USA)</i>	deltamethrin 4.75%	3A - pyrethroids, pyrethrins	Sodium Channel Modulators
Diacon IGR Plus <i>(Central Life Sciences, Schaumburg, IL, USA)</i>	deltamethrin 4.75% + (S)-methoprene 11.4%	3A - pyrethroids, pyrethrins + juvenile hormone	Sodium Channel Modulators + Insect Growth Regulator

The products are listed based on chemical group order.



**Figure 1. Design of the customized lab spray tower used for the application of insecticides in liquid formulations.**



**Figure 2. Experimental container used in the study.**

### ***Data Collection and Analysis***

In all experiments, mortality was recorded post-treatment at 24h intervals until 10 days or all insects were dead (after Biddinger et al. 1998, and Phan et al. 2020). Response regressions were generated based on response variables (state of the insects: dead or alive) and explanatory variables (pesticide concentrations) with POLOPlus 2.0 using the probit model (LeOra Software LLC, 2005). Response regression lines, which are presented by Slope  $\pm$  SE, and LC<sub>50</sub> (in ppm), and allowed us to compare the differences in pesticide responses of the two insect species (Robertson et al. 2007).

### **Results and Discussion**

This study highlighted the toxicity difference of pirimiphos-methyl, malathion, deltamethrin, and deltamethrin plus (S)-methoprene on both *T. castaneum* and *T. confusum* after 48 hours of exposure (**Table 2, Figures 2, 3**). Based on the results, the toxicity profiles of the four insecticides to *T. castaneum* could be ranked as follows: deltamethrin plus (S)-methoprene  $\geq$  deltamethrin  $>$  pirimiphos-methyl  $>$  malathion. Among them, deltamethrin plus (S)-methoprene exhibited the highest toxicity to *T. castaneum*, with an LC<sub>50</sub> value of 0.025ppm (95% CL 0.021–0.029) followed by deltamethrin and pirimiphos-methyl with LC<sub>50</sub> values of 0.026ppm (95% CL 0.002–0.052) and 0.785ppm (95% CL 0.609–1.049), respectively (**Table 2, Figure 2**). Malathion on the other hand, displayed the lowest toxicity among the tested insecticides, with an LC<sub>50</sub> value of 4.090ppm (95% CL 2.908–5.785). There was no significant difference in the toxicity profiles of deltamethrin plus (S)-methoprene and deltamethrin, as their 95% confidence limits overlapped (**Table 2**).



Similarly, for *T. confusum*, the toxicity profiles for the four insecticides were as follows: pirimiphos-methyl > deltamethrin plus (S)-methoprene > deltamethrin  $\geq$  malathion (**Table 2, Figure 3**). However, based on the LC<sub>50</sub> values and the 95% confidence limits found in this study, pirimiphos-methyl exhibited the lowest LC<sub>50</sub> value (0.148ppm (95% CL 0.095–0.200) indicating higher toxicity among all the insecticides, followed by deltamethrin plus (S)-methoprene 0.512ppm (95% CL 0.430–0.605), deltamethrin 4.247ppm (95% CL 2.422–7.800) and malathion 7.842ppm (95% CL 5.838–10.462). There was no significant difference between insecticides deltamethrin and malathion due to the overlap in their 95% confidence limits (**Table 2**).

Despite belonging to the same family and being closely related species, the toxicity response of *T. castaneum* and *T. confusum* varied and these species exhibited different susceptibilities to insecticides. We compared the LC<sub>50</sub> ratio, or ratio of concentrations causing 50% mortality, through dividing the LC<sub>50</sub> of *Tribolium castaneum* by LC<sub>50</sub> of *Tribolium confusum*. Species was considered significantly more susceptible to a pesticide when LC<sub>50</sub> ratio was less than 1.0, and where the 95% confident limit of LC<sub>50</sub> did not include the value 1.0 (Robertson et al. 2007). The results indicated that *T. castaneum* was ~7 times more tolerant to pirimiphos-methyl than *T. confusum* (**Table 2**). In contrast, for the other three selected insecticides, deltamethrin, deltamethrin plus (S)-methoprene, and malathion, *T. castaneum* was more susceptible than *T. confusum*, with LC<sub>50</sub> ratios ranging from 1/10 to 1/2 (**Table 2**). These variations in response to different insecticides can be attributed to their physiological differences, genetic variations, and differing insecticide resistance mechanisms (Smith et al., 2022).

In this study, pirimiphos- methyl concentration at the rate of 0.1,0.25, 0.5, 1, 2.5, and 5ppm for *T. castaneum* and 0.1, 0.3, 0.6,0.9,1.2, and 1.8ppm for *T. confusum* was effective against *T. castaneum* population and caused 50% mortality, however, in a study regarding the

toxicity and repellency of dimethoate, pirimiphos-methyl, and deltamethrin against *T. castaneum*, Velki et al. (2014) found that 1 ppm of pirimiphos-methyl was highly toxic compared to dimethoate and deltamethrin. Another study that showed positive results of pirimiphos-methyl was done by Sakka and Athanassiou (2021), where pirimiphos-methyl at 5ppm exhibited higher toxicity against *T. castaneum*. Pirimiphos-methyl a short-term protectant of grain against stored-product insects, and in a study LaHue (1975) found that pirimiphos-methyl (at rates of 5, 10, and 20ppm) was effective against different stored insect pests such as *S. oryzae*, *T. castaneum*, *T. confusum*, and *Rhizopertha dominica* at 24 hours and one month after treatment. Similarly, Rumbos et al. (2015) evaluated efficacy of two pirimiphos-methyl formulations for the control of three stored-product beetle species and reported higher toxicity to both *T. castaneum* and *T. confusum*.

Toxicity of insecticide active ingredients vary greatly, and a wide range of concentration may cause similar mortality in population of test species. In our study, we found that deltamethrin at the concentration rate of 0.005, 0.01, 0.025, 0.05, and 0.25ppm caused at least 50% mortality of *T. castaneum*. However, in another study, Paudyal et al., (2016) evaluated the contact toxicity of deltamethrin against *T. castaneum*, *Sitophilus oryzae*, and *R. dominica* where they discovered that 1–3000ppm of deltamethrin was highly toxic to all the species, including *T. castaneum*. On the other hand, insect regulators also showed toxicity in a study conducted by McGregor and Kramer (1975) on the insecticidal activity of hydroprene and methoprene in wheat and corn against several stored-grain insects. These authors found that 2–10 ppm of both methoprene and hydroprene exhibited toxicity against *T. castaneum* and *T. confusum*. In contrast in our study, deltamethrin plus (S)-methoprene at the rate of 0.005, 0.015, 0.03, 0.06, and

0.09ppm for *T. castaneum* and 0.1, 0.3, 0.6, 0.9, and 1.5ppm for *T. confusum* caused 50% mortality of these insect species.

Despite the differences in the mode of action of the four insecticides evaluated in this study, one other important reason for the difference in toxicity is the development of resistance of insect species to insecticides due to repeated use (Hawkins et al., 2019). For example, in a study conducted by Attia et al. (2020), the prevalence of stored grain pests and their resistance to pirimiphos-methyl, malathion, and cypermethrin in Egyptian populations of *T. castaneum* and *S. oryzae* (L.). In this study, authors found that *S. oryzae* exhibited higher resistance to malathion compared to *T. castaneum*. On the other hand, *T. castaneum* populations displayed greater resistance to pirimiphos-methyl than the *S. oryzae* populations. However, both populations of *T. castaneum* and *S. oryzae* were found to be susceptible to cypermethrin (Attia et al., 2020).

Another laboratory study evaluating the resistance of malathion in *T. castaneum* from five different locations by Anusree et al. (2023), showed resistance to malathion in all field-collected populations which was uniform and homogenous. In the past, organophosphate insecticides had been used widely for agricultural pest control including stored grain pests, but their effectiveness varies from species to species. In a study, Mensah, and Watters (1979) compared four organophosphorus insecticides for the control of susceptible and malathion-resistant strains of the red flour beetle in stored wheat. It was observed that pirimiphos-methyl at a rate of 4–6ppm effectively controlled both strains of *T. castaneum*, while malathion at 8–12ppm was ineffective against the resistant strain.

Insecticide effectiveness also depends on the population strains of target insects collected from various field locations. Zettler (1991), evaluated the resistance of field strains of *T. castaneum* and *T. confusum* to malathion and found that out of the 17 *T. confusum* strains tested,

82% exhibited resistance to malathion, while among the 28 *T. castaneum* strains tested, 93% displayed resistance to malathion. Overall, *T. castaneum* demonstrated higher resistance compared to *T. confusum*. Furthermore, in another study conducted by Zettler and Arthur (1997), dose-response tests were carried out on these both flour beetles collected from various flour mills in the United States by assessing the resistance of 14 field strains of *T. castaneum* and 10 strains of *T. confusum* to malathion and dichlorvos insecticides through topical application. In this case all strains of *T. castaneum* exhibited resistance to both malathion and dichlorvos, while half of the *T. confusum* strains tested also displayed resistance to these insecticides. However, *T. castaneum* demonstrated a higher level of resistance compared to *T. confusum* for both malathion and dichlorvos.

In our study, we evaluated adult stages of both flour beetles for their susceptibility to insecticides and found differences in the susceptibility for certain insecticides. However, susceptibility may also be different for different life stages of the target pests. Yao et al. (2019) examined the susceptibilities of late-stage larvae of *T. castaneum*, and *T. confusum* to five insecticides: esfenvalerate, pyrethrins, dichlorvos, methoprene, and pyriproxyfen, and found differences in susceptibility between the two species as *T. castaneum* larvae showed lower susceptibility (resulting in low larval mortality) to dichlorvos and esfenvalerate compared to *T. confusum*, whereas the reverse was observed for treatment with pyrethrins. In addition, these researchers carried out another bioassay in which *T. castaneum* and *T. confusum* larvae were exposed to methoprene and pyriproxyfen, two insect growth regulators (IGRs). Compared to *T. castaneum*, *T. confusum* displayed increased tolerance to insect growth regulators. When exposed to either insect growth regulator, all *T. castaneum* larvae either died in the larval or pupal stages when exposed to either insect growth regulators. On the other hand, only

pyriproxyfen completely caused mortality in *T. confusum* larvae while almost 70% of *T. confusum* larvae successfully pupated despite receiving the maximum dose of methoprene, and 4.5% of adults emerged. These findings show that despite the close kinship between the two species, they exhibit distinct pesticide susceptibilities.

In this study, since the response of two species of flour beetles to pesticides was significantly different (**Table 2, Figures 2, 3**), so there should not be a “one fits all” recommended application rate for both species in general. For instance, the recommended rate for deltamethrin plus (S)-methoprene (0.5–1.0ppm) and malathion (10ppm) for *T. confusum* were aligned with the toxicity profiles, indicating appropriate application levels and hence no change required. In contrast, the recommended application rate for pirimiphos-methyl (6–10 ppm) was found to exceed the LC<sub>50</sub> value for both species at 0.785ppm (0.609–1.049) for *T. castaneum* and 0.148ppm (0.095–0.20) for *T. confusum*, while deltamethrin, deltamethrin plus (S)-methoprene (0.5–1.0ppm), and malathion (10ppm) were also higher than the LC<sub>50</sub> values for *T. castaneum* only 0.026ppm (0.002–0.052), 0.025ppm (0.021–0.029) and 4.090ppm (2.908–5.785) respectively. This indicates the overapplication which could potentially lead to adverse consequences such as the development of pest resistance (Aktar et al., 2009), health risks (Nicolopoulou-Stamati et al., 2016), financial burdens, and environmental contamination (Damalas and Koutroubas, 2016). On the other hand, the recommended rate for deltamethrin was found to be lower than the LC<sub>50</sub> value for *T. confusum*, which was 4.247ppm (2.422–7.800). This suggests an underapplication of deltamethrin for *T. confusum*, potentially leading to ineffective pest control, crop damage, yield losses, and economic losses. Such variations between the recommended label rates and the LC<sub>50</sub> obtained in this study could be attributed to the method of

application as the recommended rates are technically designed for a number of insect pests listed in the label and also used as preventative methods.

### **Conclusion and Recommendation**

The findings of this study revealed differences in the toxicity response of *T. castaneum* and *T. confusum*. Insecticide deltamethrin plus (S)-methoprene exhibited the highest toxicity to *T. castaneum*, followed by deltamethrin and pirimiphos-methyl. There was no significant difference in the toxicity level of deltamethrin and deltamethrin plus (S)-methoprene since their 95% confidence limits overlapped. Malathion was found to be the least toxic among all the insecticides. For *T. confusum*, pirimiphos-methyl exhibited higher toxicity, followed by deltamethrin plus (S)-methoprene. The toxicity response of these two closely related species to pirimiphos-methyl was different as *T. castaneum* was approximately 7 times more tolerant to pirimiphos-methyl exposure than *T. confusum*. However, *T. castaneum* was more susceptible to deltamethrin, deltamethrin plus (S)-methoprene, and malathion, with LC<sub>50</sub> ratios ranging from 1/10 to 1/2 compared to *T. confusum*. These variations in toxicity profiles were likely due to differences in the mode of action and the development of insecticide resistance, as previously highlighted in other studies (e.g., Liu, 2015).

Additionally, we assessed the current recommended pesticide application rates of these four insecticides based on their respective LC<sub>50</sub> values for both *T. castaneum* and *T. confusum*. The results indicated that the recommended rates for deltamethrin plus (S)-methoprene and malathion for *T. confusum* were appropriate and did not require any changes. However, the recommended rate for pirimiphos-methyl exceeded the LC<sub>50</sub> value for both species of flour beetles, and the rates for deltamethrin, deltamethrin plus (S)-methoprene, and malathion were also higher than the LC<sub>50</sub> value for *T. castaneum*. On the other hand, the recommended rate for

deltamethrin was lower than the  $LC_{50}$  value for *T. confusum*, indicating underapplication. Such difference could be due to the reason that the recommended label rates are applied as preventative methods and technically designed for numerous insects listed in the label. Hence, the recommended rates could also be aligned with the other groups of insect pests other than the ones in the study. This study enhances our understanding of chemical control of flour beetles and provides important insights for future stored-grain pest control programs. However, further research is required to investigate the underlying mechanisms and potential development of resistance to these insecticides in stored grain pests.

**Table 2. Toxicity response of *Tribolium castaneum* and *Tribolium confusum* to selected pesticides at 48h after pesticide treatment.**

Active ingredient <sup>1</sup>	Species	N <sup>‡</sup>	Slope ± SE	LC <sub>50</sub> (ppm) (95% CL)	LC <sub>50</sub> ratio <sup>2</sup> (95% CL)	Recommended application rate (ppm) <sup>#</sup>
Pirimiphos-methyl	<i>Tribolium castaneum</i>	270	4.55 ± 0.60	0.785 (0.609 – 1.049)	7.142 (5.203 – 9.805)	6 – 8
	<i>Tribolium confusum</i>	270	2.32 ± 0.28	0.148 (0.095 – 0.200)		
Deltamethrin	<i>Tribolium castaneum</i>	225	1.55 ± 0.25	0.026 (0.002 – 0.052)	0.010 (0.007 – 0.015)	0.5 – 1.0
	<i>Tribolium confusum</i>	225	1.91 ± 0.20	4.247 (2.422 – 7.800)		
Deltamethrin + (S)-methoprene	<i>Tribolium castaneum</i>	225	2.88 ± 0.28	0.025 (0.021 – 0.029)	0.049 (0.039 – 0.062)	0.5 – 1.0
	<i>Tribolium confusum</i>	225	2.74 ± 0.33	0.512 (0.430 – 0.605)		
Malathion	<i>Tribolium castaneum</i>	225	1.30 ± 0.16	4.090 (2.908 – 5.785)	0.546 (0.353 – 0.842)	10
	<i>Tribolium confusum</i>	225	1.54 ± 0.19	7.842 (5.838 – 10.462)		

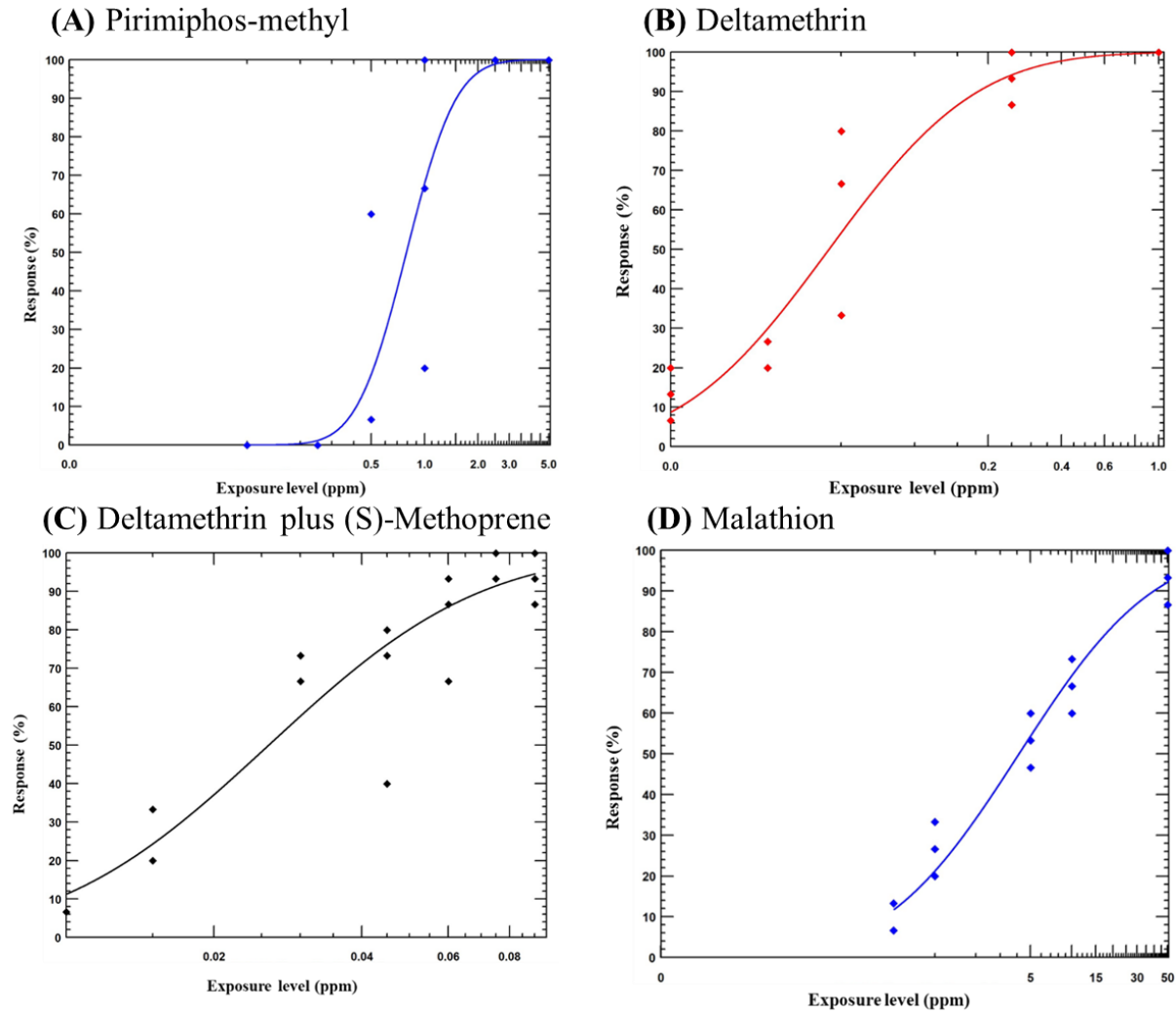
<sup>1</sup> The products are listed based on chemical group order.

<sup>‡</sup>N is the number of individuals tested for each product. Response regression lines are presented by Slope ± SE, and LC<sub>50</sub> (in ppm). Control mortality was 0% during the study period.

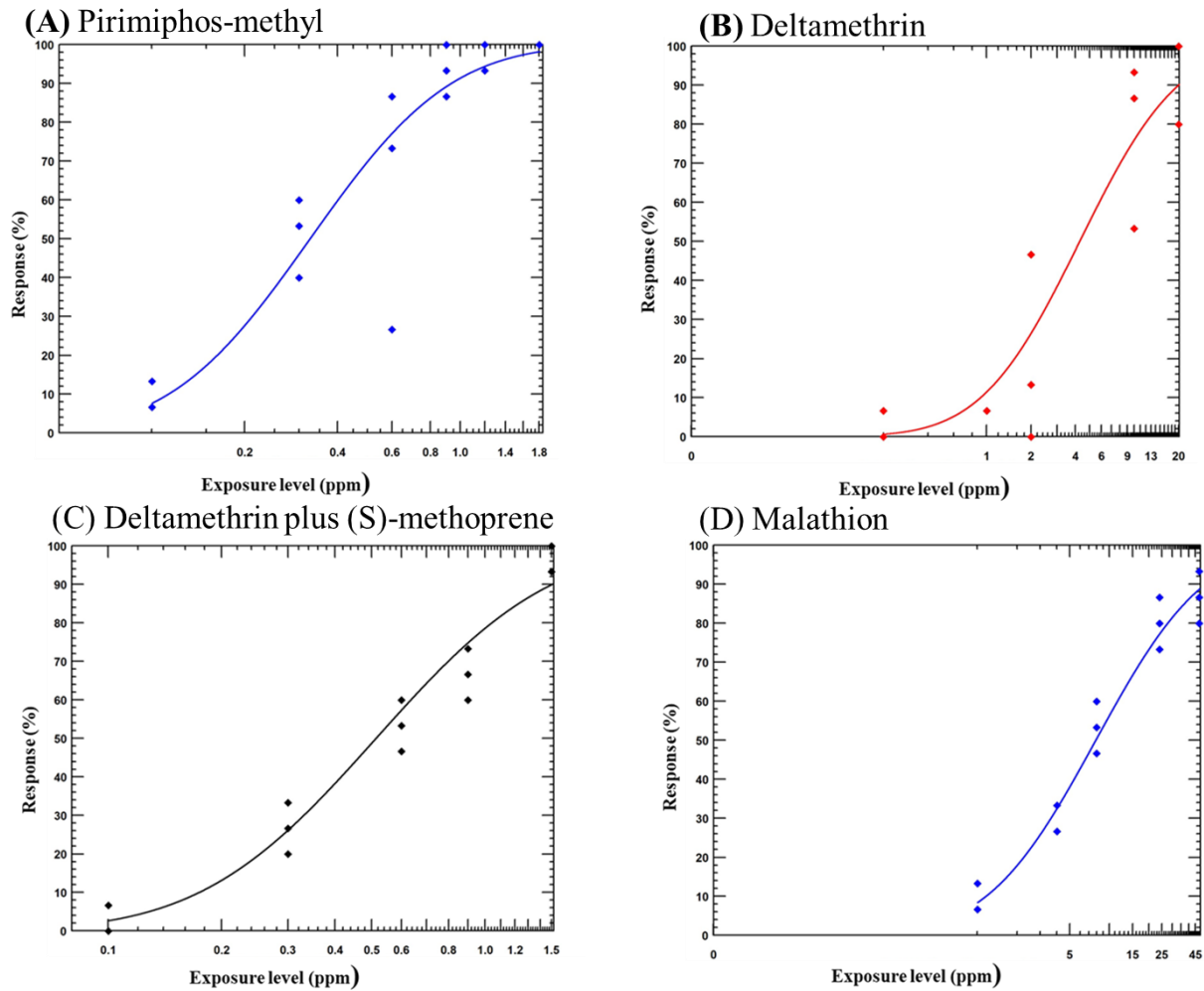
<sup>2</sup> LC<sub>50</sub> ratio, or ratio of concentrations causing 50% mortality, is LC<sub>50</sub> of *Tribolium castaneum* ÷ LC<sub>50</sub> of *Tribolium confusum*. One species is significantly more susceptible to a pesticide when: (1) LC<sub>50</sub> ratio < 1.0, and (2) 95% CL of LC<sub>50</sub> does not include the value 1.0 (Robertson *et al.*, 2007).

<sup>#</sup>Recommended application rates were obtained from the pesticide product labels (Balcom Chemicals Inc., 1975; Winfield Solutions LLC, 2015; Central Garden & Pet Company, 2016a, 2016b).





**Figure 3. Toxicity response of *Tribolium castaneum* to selected pesticides: (A) pirimiphos-methyl, (B) deltamethrin, (C) deltamethrin plus (S)-methoprene, and (D) malathion (at 48h after treatment).**



**Figure 4. Toxicity response of *Tribolium confusum* to selected pesticides: (A) pirimiphos-methyl (after 24h treatment), (B) deltamethrin, (C) deltamethrin plus (S)-methoprene, and (D) malathion (at 48h after treatment).**

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## Chapter 4

### Conclusion

In this study, we evaluated the toxicity of pirimiphos-methyl, deltamethrin, and deltamethrin plus (S)-methoprene against *S. oryzae*, *T. castaneum*, and *T. confusum* in two laboratory studies. The results of the first study showed that the insecticide pirimiphos-methyl was more toxic to *S. oryzae* than malathion, deltamethrin, and deltamethrin plus (S)-methoprene and there was no significant difference in toxicity between malathion, deltamethrin, and deltamethrin plus (S)-methoprene. However, comparing the label-recommended application of these insecticides with their respective LC<sub>50</sub> values, the recommended rate for pirimiphos-methyl exceeded its LC<sub>50</sub>. This finding indicated that pirimiphos-methyl was over-applied and might lead to various consequences. On the other hand, deltamethrin and deltamethrin plus (S)-methoprene were under-applied, as their recommended rates were lower than the lower ends of their 95% CL of LC<sub>50</sub>. Only the recommended application rate for malathion aligned with the toxicity profile.

The findings of the second study demonstrated that the insecticide premix deltamethrin plus (S)-methoprene was more effective against *T. castaneum*, followed by deltamethrin and pirimiphos-methyl insecticides. Deltamethrin and deltamethrin plus (S)-methoprene were not significantly different in terms of their toxicity. Malathion was found to be the least toxic among all the insecticides. On the other hand, pirimiphos-methyl showed higher toxicity, followed by deltamethrin plus (S)-methoprene for *T. confusum*. However, there was no significant difference in toxicity profiles between deltamethrin and malathion. The toxicity response of these two closely related species revealed that *T. castaneum* was approximately seven times more tolerant to pirimiphos-methyl than *T. confusum*. However, *T. castaneum* was more susceptible to deltamethrin, deltamethrin plus (S)-methoprene, and malathion, with LC<sub>50</sub> ratios ranging from



1/10 to 1/2 compared to *T. confusum*. We also assessed the current recommended pesticide application rates of these four insecticides based on their respective LC<sub>50</sub> values for both *T. castaneum* and *T. confusum*. The results indicated that the recommended rates for deltamethrin plus (S)-methoprene and malathion for *T. confusum* were appropriate. However, the recommended rate for pirimiphos-methyl exceeded the LC<sub>50</sub> value for both species of flour beetles, and the rates for deltamethrin, deltamethrin plus (S)-methoprene, and malathion were higher than the LC<sub>50</sub> value for *T. castaneum*. On the other hand, the recommended rate for deltamethrin was lower than the LC<sub>50</sub> value for *T. confusum*. It is very important to note that these insecticides are applied as a preventative measure and labeled for multiple insect pests. The labeled rate is to cover all potential insect pests and may be in line with other species than in these studies.

This research contributes to our understanding of the relative toxicity and effectiveness of pirimiphos-methyl, deltamethrin, deltamethrin plus (S)-methoprene, and malathion in controlling populations of *S. oryzae*, *T. castaneum*, and *T. confusum*. The difference in toxicity between these insecticides could be attributed to the difference in the mode of action and the development of insect resistance to some of these insecticides. However, further research is needed to determine the long-term efficacy of these insecticides and potential resistance development in local populations of stored-grain insect pests.