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

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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 chemicals 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_{50} 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_{50} 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_{50} found in the study, deltamethrin plus (S)-methoprene and malathion for *T. confusum* aligned with the LC_{50} found in the study while the recommended label rate for pirimiphos-methyl exceeded the LC_{50} 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. (Erdoğuş, 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 Annapoorani, 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 (PH3), 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 granaries* (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. Daglish (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.

References

Abamecha, Nezif. 2021. "A Survey of Farmers' Perceptions on Maize and Sorghum Storage Duration and Level of Pest Infestations in the Case of Two Selected Districts of Jimma Zone, Ethiopia." *International Journal of Food Science* 2021. https://doi.org/10.1155/2021/5583387.

Abd El-Aziz, Shadia E. 2011. "Control Strategies of Stored Product Pests." *Journal of Entomology* 8 (2): 101–22. https://doi.org/10.3923/je.2011.101.122.

Abdullahi, Gambo, Rita Muhamad, and Hassan Sule. 2019. "Biology, Host Range and Management of Red Flour Beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae): A Review Insect Toxicology View Project Ecological Control of Tomato Leaf Miner (*Tuta absoluta*) in Nigeria View Project." https://www.researchgate.net/publication/337824598.

Adetunji, J F. 1984. "Studies on the Rice Weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) with Special Reference to Sorghum Cultivar Resistance." Berkshire: Imperial College of Science and Technology.

Ahmad, Rayees, Shafiya Hassan, Showkat Ahmad, Syed Nighat, Yendrambamb K. Devi, Kounser Javeed, Salma Usmani, et al. 2021. "Stored Grain Pests and Current Advances for Their Management." *Postharvest Technology - Recent Advances, New Perspectives and Applications*, December. https://doi.org/10.5772/INTECHOPEN.101503.

Arthur, Frank H. 1996. "Grain Protectants: Current Status and Prospects for the Future." *J. Stored Prod. Res*. Vol. 32.

Ashamo, M O, O O Odeyemi, and A A Olatunji. 2010. "Resistance of Strains of Rice Weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) to Pirimiphos Methyl." *10th International Working Conference on Stored Product Protection* 425. https://doi.org/10.5073/jka.2010.425.433.

Atanda, S A, S Agoda, G C Ihionu, and O E Usanga. 2018. "Protection of Grains and Cereals - a Review." https://papers.ssrn.com/abstract=3201531.

Athanassiou, C G, A S Papagregoriou, and C.Th Buchelos. 2004. "Insecticidal and Residual Effect of Three Pyrethroids against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) on Stored Wheat." *Journal of Stored Products Research* 40 (3): 289–97. https://doi.org/10.1016/S0022-474X(03)00025-0.

Attia, Manal A, Trandil F Wahba, Nehal Shaarawy, Fathia I Moustafa, Raul Narciso C Guedes, and Youssef Dewer. 2020. "Stored Grain Pest Prevalence and Insecticide Resistance in Egyptian Populations of the Red Flour Beetle *Tribolium castaneum* (Herbst) and the Rice Weevil *Sitophilus oryzae* (L.)." *Journal of Stored Products Research* 87 (May). https://doi.org/10.1016/j.jspr.2020.101611.

Awika, Joseph M. 2011. "Major Cereal Grains Production and Use around the World." *ACS Symposium Series* 1089: 1–13. https://doi.org/10.1021/BK-2011-1089.CH001/ASSET/IMAGES/LARGE/BK-2011-00480Z_G003.JPEG.

Baker, J E. 1994. “Sensitivities of Laboratory and Field Strains of the Parasitoid *Anisopteromalus Calartdrae* (Hymenoptera: Pteromalidae) and Its Host, *Sitophilus oryzae* (Coleoptera: Curculionidae), to Deltamethrin and Cyfluthrin.” *J. Entomol. Sci*. Vol. 29. http://meridian.allenpress.com/jes/article-pdf/29/1/100/1560651/0749-8004-29_1_100.pdf.

Baldwin, Rebecca, and Thomas R Fasulo. 2003. “Confused Flour Beetle, *Tribolium confusum* Jacquelin Du Val (Insecta: Coleoptera: Tenebrionidae) and Red Flour Beetle, *Tribolium castaneum*(Herbst) (Insecta: Coleoptera: Tenebrionidae) 1.” http://entomology.ifas.ufl.edu/creatures.PleasevisittheEDISwebsiteathttp://edis.ifas.ufl.edu.

———. 2005. “Confused Flour Beetle, *Tribolium confusum* Jacquelin Du Val and Red Flour Beetle, *Tribolium castaneum* (Herbst) (Insecta: Coleoptera: Tenebrionidae) 1.” http://creatures.ifas.ufl.edu.

———. 2014. “Confused Flour Beetle, *Tribolium confusum* Jacquelin Du Val (Insecta: Coleoptera: Tenebrionidae) and Red Flour Beetle, *Tribolium castaneum* (Herbst) (Insecta: Coleoptera: Tenebrionidae) 1.” http://entomology.ifas.ufl.edu/creatures.PleasevisittheEDISwebsiteathttp://edis.ifas.ufl.edu.

Befikadu, Dubale. 2014. “Factors Affecting Quality of Grain Stored in Ethiopian Traditional Storage Structures and Opportunities for Improvement.” *International Journal of Sciences: Basic and Applied Research* 18 (1): 235–57. http://gssrr.org/index.php?journal=JournalOfBasicAndApplied.

Bharathi, Vimala S K, J A Moses, and Alice Sujeetha. 2017. “Insect Infestation and Losses in Stored Food Grains Entomopathogenic Fungus View Project.” https://www.researchgate.net/publication/317804375.

Boeke, S. J., I. R. Baumgart, J. J.A. Van Loon, A. Van Huis, M. Dicke, and D. K. Kossou. 2004. “Toxicity and Repellence of African Plants Traditionally Used for the Protection of Stored Cowpea against *Callosobruchus maculatus*.” *Journal of Stored Products Research* 40 (4): 423–38. https://doi.org/10.1016/S0022-474X(03)00046-8.

Bonjour, E L. 2019. “Stored Grain Insect Pests That Feed inside Kernels and Seeds | Oklahoma State University.” 2019. https://extension.okstate.edu/fact-sheets/stored-grain-insect-pests-that-feed-inside-kernels-and-seeds.html.

Boudreaux, H Bruce. 1969. “The Identity of *Sitophilus oryzae*.” *Annals of the Entomological Society of America* 62 (1): 169–72. https://doi.org/10.1093/AESA/62.1.169.

Bramavath, Deepak, Sushma Rani Thiyyagura, Ram Prakash, and Rajesh Babu Nandam. 2017. “Fumigation of Stored-Products: Current Needs and Practices, and Future Outlook.” *~ 1499 ~ International Journal of Chemical Studies* 5 (5).

Brewbaker, James L, and T Hawaii. 2003. “Corn Production in the Tropics.” www.ctahr.hawaii.edu.

Brown, Susan J., Teresa D. Shippy, Sherry Miller, Renata Bolognesi, Richard W. Beeman, Marcé D. Lorenzen, Gregor Bucher, Ernst A. Wimmer, and Martin Klingler. 2009. “The Red Flour Beetle, *Tribolium castaneum* (Coleoptera): A Model for Studies of Development and Pest

Biology.” *Cold Spring Harbor Protocols* 2009 (8): pdb.emo126. https://doi.org/10.1101/PDB.EMO126.

Burks, Charles S, Muhammad Yasin, Hamadttu A F El-Shafie, and Waqas Wakil. 2015. “Pests of Stored Dates.” *Sustainable Pest Management in Date Palm: Current Status and Emerging Challenges*, 237–86. https://doi.org/10.1007/978-3-319-24397-9_9.

Busvine, James R. 1980. “Pests of Foodstuffs.” *Insects and Hygiene*, 306–77. https://doi.org/10.1007/978-1-4899-3198-6_8.

Chambers, Craig Brian. 2005. “Development of Fungal Biological Control of Four Agriculturally Important Pests, *Sitophilus oryzae*, *Trialeurodes vaporariorum*, *Planococcus ficus* and *Eldana saccharina*, in South Africa.” Pietermaritzburg: University of Kwazulu Natal. https://researchspace.ukzn.ac.za/handle/10413/4062.

Chaudhary, S, R K Kanwar, A Sehgal, D M Cahill, C J Barrow, R Sehgal, and J R Kanwar. 2017. “Progress on *Azadirachta indica* Based Biopesticides in Replacing Synthetic Toxic Pesticides.” *Progress on Azadirachta indica Based Biopesticides in Replacing Synthetic Toxic Pesticides. Front. Plant Sci* 8: 610. https://doi.org/10.3389/fpls.2017.00610.

Deshwal, Rajat, Visvash Vaibhav, Nitin Kumar, Ankit Kumar, and Rahul Singh. 2020. “Stored Grain Insect Pests and Their Management: An Overview.” *~ 969 ~ Journal of Entomology and Zoology Studies* 8 (5): 969–74. http://www.entomoljournal.com.

Donahaye, E J, S Navarro, and J Leesch. 2001. “Rice Milling as an Alternative to Methyl Bromide for the Control of Rice Weevil *Sitophilus oryzae* (L.).” *Executive Printing Services*.

Doud, Carl W. 1999. “Monitoring the Red Flour Beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and Other Stored-Product Insects with Traps in Flour Mills,” December. https://shareok.org/handle/11244/11718.

Dougoud, Julien, Stefan Toepfer, Melanie Bateman, and Wade H Jenner. 2019. “Efficacy of Homemade Botanical Insecticides Based on Traditional Knowledge. A Review.” *Agronomy for Sustainable Development 2019 39:4* 39 (4): 1–22. https://doi.org/10.1007/S13593-019-0583-1.

Edde, Peter A. 2012. “A Review of the Biology and Control of *Rhyzopertha dominica* (F.) the Lesser Grain Borer.” *Journal of Stored Products Research* 48 (January): 1–18. https://doi.org/10.1016/J.JSPR.2011.08.007.

Erdoğuş, Fatma Dolunay. 2021. “On the Efficiency of Entomopathogenic Nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) on Rust Red Flour Beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae).” *Egyptian Journal of Biological Pest Control* 31 (1). https://doi.org/10.1186/s41938-021-00461-y.

Fields, Paul G, and Noel D G White. 2002. “Alternatives to Methyl Bromide Treatment for Stored Products and Quarantine Insects.” *Annual Review of Entomology* 47: 331–59. www.annualreviews.org.

García-Lara, Silverio, and Sergio O Serna-Saldivar. 2019. "Corn History and Culture." *Corn: Chemistry and Technology, Third Edition*, January, 1–18. https://doi.org/10.1016/B978-0-12-811971-6.00001-2.

Glover, D V, and E T Mertz. 2015. "Corn," October, 183–336. https://doi.org/10.2134/AGRONMONOGR28.C7.

Golob, P, G Farrell, and John E. 2002. "Crop Post-Harvest: Science and Technology, Volume 1." *Crop Post-Harvest: Science and Technology, Volume 1*, November. https://doi.org/10.1002/9780470751015.

Hagstrum, D W, T W Phiilips, and G W Cuperus. 2012. *Stored Product Protection*. Kansas State University.

Halle, Snir, Anastasia Nowizki, and Inon Scharf. 2015. "The Consequences of Parental Age for Development, Body Mass and Resistance to Stress in the Red Flour Beetle" *Biological Journal of the Linnean Society*. Vol. 115. https://academic.oup.com/biolinnean/article/115/2/305/2235999.

Hamel, Darka, Vlatka Rozman, and Anita Liška. 2020. "Storage of Cereals in Warehouses with or without Pesticides." *Insects* 11 (12): 1–21. https://doi.org/10.3390/INSECTS11120846.

Hansen, J. D., J. A. Johnson, and D. A. Winter. 2011. "History and Use of Heat in Pest Control: A Review." *Http://Dx.Doi.Org/10.1080/09670874.2011.590241* 57 (4): 267–89. https://doi.org/10.1080/09670874.2011.590241.

Harush, Avichai, Elazar Quinn, Anatoly Trostanetsky, Aviv Rapaport, Moshe Kostyukovsky, and Daphna Gottlieb. 2021. "Integrated Pest Management for Stored Grain: Potential Natural Biological Control by a Parasitoid Wasp Community." *Insects* 12 (11). https://doi.org/10.3390/insects12111038.

Hatfield, Jerry L, Christian Dold, Jerry L Hatfield, and Christian Dold. 2018. "Climate Change Impacts on Corn Phenology and Productivity." *Corn - Production and Human Health in Changing Climate*, October. https://doi.org/10.5772/INTECHOPEN.76933.

Hikal, Wafaa M, Rowida S Baeshen, Hussein A H Said-, and Al Ahl. 2017. "Cogent Biology Botanical Insecticide as Simple Extractives for Pest Control under a Creative Commons Attribution (CC-BY) 4.0 License Botanical Insecticide as Simple Extractives for Pest Control." *Cogent Biology* 3: 1404274. https://doi.org/10.1080/23312025.2017.1404274.

Hiruy, Berhanu, and Emana Getu Degaga. 2018. "Insect Pests Associated to Stored Maize and Their Bio Rational Management Options in Sub-Sahara Africa." https://www.researchgate.net/publication/323143871.

Huang, Fangneng, and Bhadriraju Subramanyam. 2005. "Management of Five Stored-Product Insects in Wheat with Pirimiphos-Methyl and Pirimiphos-Methyl plus Synergized Pyrethrins." *Pest Management Science* 61 (4): 356–62. https://doi.org/10.1002/ps.968.

Hussain, Aftab. 1993. "Chemical Ecology of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae): Factors Affecting Biology and Application of Pheromone."

Jayakumar, Manickkam, Subramanian Arivoli, Rajasingh Raveen, and Samuel Tennyson. 2017. "Repellent Activity and Fumigant Toxicity of a Few Plant Oils against the Adult Rice Weevil *Sitophilus oryzae* Linnaeus 1763 (Coleoptera: Curculionidae)." *Journal of Entomology and Zoology Studies* 5 (2).

Jiao, Yan, Hao Dong Chen, He Han, and Ying Chang. 2022. "Development and Utilization of Corn Processing By-Products: A Review." *Foods*. MDPI. https://doi.org/10.3390/foods11223709.

Johnson, J. 2013. "Pest Control in Postharvest Nuts." *Improving the Safety and Quality of Nuts*, January, 56–87. https://doi.org/10.1533/9780857097484.1.56.

Joshi, N K, G Lorenz, N Bateman, G Studebaker, A Cato, A Plummer, G Felts, J Belsy, O Kline, and B Gibson. 2020. "Insect Management in On-Farm Grain Storage: Survey of Insect Pests Corn in on-Farm Storage in Arkansas." *Arkansas Corn and Grain Sorghum Research Studies 2020*, 22-26.

Kalpna, Younis Ahmad Hajam, and Rajesh Kumar. 2022. "Management of Stored Grain Pest with Special Reference to *Callosobruchus maculatus*, a Major Pest of Cowpea: A Review." *Heliyon* 8 (1). https://doi.org/10.1016/J.HELIYON.2021.E08703.

kandil, Amin. 2016. "Understanding the Effect of Non-Starch Grain Components on the Amylolysis of Starch in Whole Grains." University of Alberta.

Kiaya, K. 2014. "Post-Harvest Losses and Strategies to Reduce Them."

Kumar, Deepak, and Prasanta Kalita. 2017. "Reducing Postharvest Losses during Storage of Grain Crops to Strengthen Food Security in Developing Countries." *Foods* 6 (1): 1–22. https://doi.org/10.3390/foods6010008.

Lagisz, Malgorzata, Kirsten Wolff, and Gordon Port. 2010. "Time Matters: Delayed Toxicity of Pirimiphos-Methyl on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and Its Effects on Efficacy Estimation of Residual Treatments." *Journal of Stored Products Research* 46 (3): 161–65. https://doi.org/10.1016/j.jspr.2010.02.003.

Lima do Rêgo, Enoc, José Domingos Santos da Silva, Thamilin Costa Nakamura, Paulo Henrique Gonçalves Dias Diniz, Uldérico Rios Oliveira, Jurandir Rodrigues de Souza, Enoc R Lima do, et al. 2021. "Distribution of Organochlorine, Organophosphates, Carbamate, Thiocarbamate, Pyrethroids, and Strobilurins in Surface Sediments of the Rio de Ondas Watershed by GC-MS." *Https://Doi.Org/10.1080/03601234.2021.1885263* 56 (4): 357–69. https://doi.org/10.1080/03601234.2021.1885263.

Linz, David M., Alan W. Hu, Michael I. Sitvarin, and Yoshinori Tomoyasu. 2016. "Functional Value of Elytra under Various Stresses in the Red Flour Beetle, *Tribolium castaneum*." *Scientific Reports* 6 (October). https://doi.org/10.1038/srep34813.

Liu, Nannan, Qisheng Song, Jinsong Zhu, Maria F Araújo, Elisabete M S Castanheira, and Sérgio F Sousa. 2023. "The Buzz on Insecticides: A Review of Uses, Molecular Structures, Targets, Adverse Effects, and Alternatives." *Molecules 2023, Vol. 28, Page 3641* 28 (8): 3641. https://doi.org/10.3390/MOLECULES28083641.

Maciej Serda, Fernando Gertum Becker, Michelle Cleary, R M Team, Helge Holtermann, Disclaimer The, National Agenda, et al. 2022. “Review on Major Storage Insect Pests of Cereals and Pulses.” Edited by G. Balint, B. Antala, C. Carty, J-M. A. Mabieme, I. B. Amar, and A. Kaplanova. *Asian Journal of Advances in Research* 7 (1): 41–56. https://doi.org/10.2/JQUERY.MIN.JS.

Mackled, Marwa I. 2017. “Determining the Fitness Components of (*Sitophilus oryzae* L.) on Imported Wheat in Egypt.” *Egyptian Academic Journal of Biological Sciences. A, Entomology* 10 (4): 119–30. https://doi.org/10.21608/EAJBSA.2017.12516.

Makai Panezai, Gul, Mariam Javaid, Sadaf Shahid, Wasia Noor, Zohra Bibi, and Ambreen Ejaz. 2019. “Effects of Extracts against *Trigoderma granerium* and *Tribolium castaeneum*.” *Pak. J. Bot* 51 (3): 1149–53. https://doi.org/10.30848/PJB2019-3(40).

Mandali, Rajasri. 2020. “Stored Grain Pests and Their Identification National Institute of Plant Health Management Eri Culture View Project Castor Pest Management View Project.” https://www.researchgate.net/publication/341134532.

Martin, D T, D Baributsa, J E Huesing, S B Williams, and L L Murdock. 2015. “PICS Bags Protect Wheat Grain, *Triticum aestivum* (L.), against Rice Weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae).” *Journal of Stored Products Research* 63 (July): 22–30. https://doi.org/10.1016/J.JSPR.2015.05.001.

Masiko, Mahafuzi. 2018. “Storage Insect Pests Diversity at Different Levels of Maize Value Chain: Mukono District, Uganda.” http://dissertations.mak.ac.ug/handle/20.500.12281/6013.

Maureen, Authors O, Anastasia Becker, Armon Keaster, Laura Kabrick, Michael Chippendale, Darren Hoffman, Mary Jackson, et al. 1998. “Corn Insect Pests: A Diagnostic Guide (1998).” *Harvested from University of Missouri--Columbia, University Extension, Extension Website.* https://mospace.umsystem.edu/xmlui/handle/10355/16081.

McCann, James. 2001. “Maize and Grace: History, Corn, and Africa’s New Landscapes, 1500-1999.” *Comparative Studies in Society and History* 43 (2): 246–72. https://doi.org/10.1017/S0010417501003486.

McKevith, Brigid. 2004. “Nutritional Aspects of Cereals.” *Nutrition Bulletin* 29 (2): 111–42. https://doi.org/10.1111/J.1467-3010.2004.00418.X.

Ming, Qinglei, Jiafei Shen, Chao Cheng, and Zhaojun Feng. 2015. “Genetic Relationships between *Tribolium castaneum* and *T. confusum* Based on Mitochondrial DNA Sequences.” *Pakistan J. Zool* 47 (5): 1405–12.

Mobolade, Adesina Jacobs, Nameirakpam Bunindro, Dinabandhu Sahoo, and Yallappa Rajashekar. 2019. “Traditional Methods of Food Grains Preservation and Storage in Nigeria and India.” *Annals of Agricultural Sciences* 64 (2): 196–205. https://doi.org/10.1016/J.AOAS.2019.12.003.

Mohandass, S, F H Arthur, K Y Zhu, and J E Throne. 2007. “Biology and Management of *Plodia interpunctella* (Lepidoptera: Pyralidae) in Stored Products.” *Journal of Stored Products Research* 43 (3): 302–11. https://doi.org/10.1016/j.jspr.2006.08.002.

Mohapatra, Debabandya, Sunil Kumar, Nachiket Kotwaliwale, and Krishna Kumar Singh. 2017. “Critical Factors Responsible for Fungi Growth in Stored Food Grains and Non-Chemical Approaches for Their Control.” *Industrial Crops and Products* 108 (December): 162–82. https://doi.org/10.1016/J.INDCROP.2017.06.039.

Murugesan, Sindhu, and Annapoorani. C A. 2021. “Life Cycle, Morphometrics, Fungal Identification and Effect of Preventive Treatments in Sesame Seeds of *Tribolium castaneum* Image Analysis of Groundnut Pests Using Artificial Neural Network View Project Distribution and Population Dynamics of Vector and Non Vector Species of Mosquitoes Prevalent in Selected Areas of Coimbatore Using GIS View Project.” https://www.researchgate.net/publication/355702262.

Muslim, Sienaa, and Abid Al-Zurfi. 2019. “Biological Control of the Red Flour Beetle, *Tribolium castaneum* Using Entomopathogenic Fungi.”

Nafziger, Emerson. 2019. “Cultivation and Environmental Impact of Corn.” *Oxford Research Encyclopedia of Environmental Science*, December. https://doi.org/10.1093/ACREFORE/9780199389414.013.220.

Narayan, Aditya, Dilip Kumar, and Aditya Narayan Jhariya. 2013. “Mini Review Paper Nutritional, Medicinal and Economical Importance of Corn.” *Research Journal of Pharmaceutical Sciences* 2 (7): 1–6. https://doi.org/10.13140/RG.2.2.30058.62408.

Nikolaou, Polyxeni, Paweł Marciniak, Zbigniew Adamski, and Nikoletta Ntalli. 2021. “Controlling Stored Products’ Pests with Plant Secondary Metabolites: A Review.” *Agriculture 2021, Vol. 11, Page 879* 11 (9): 879. https://doi.org/10.3390/AGRICULTURE11090879.

Nwaigwe, Kevin N. 2019. “An Overview of Cereal Grain Storage Techniques and Prospects in Africa.” *International Journal of Bioengineering & Biotechnology*. Vol. 4. http://www.opensicenceonline.com/journal/ijbb.

Odjo, Sylvanus, Natalia Palacios-Rojas, Juan Burgueño, Marina Corrado, Tim Ortner, and Nele Verhulst. 2022. “Hermetic Storage Technologies Preserve Maize Seed Quality and Minimize Grain Quality Loss in Smallholder Farming Systems in Mexico.” *Journal of Stored Products Research* 96 (March): 101954. https://doi.org/10.1016/J.JSPR.2022.101954.

Ohbe, Hiroyuki, Taisuke Jo, Hiroki Matsui, Kiyohide Fushimi, and Hideo Yasunaga. 2018. “Cholinergic Crisis Caused by Cholinesterase Inhibitors: A Retrospective Nationwide Database Study.” *Journal of Medical Toxicology* 14 (3): 237–41. https://doi.org/10.1007/S13181-018-0669-1/TABLES/4.

Palmquist, Katherine, Johanna Salatas, and Anne Fairbrother. 2012. “Pyrethroid Insecticides: Use, Environmental Fate, and Ecotoxicology.” In *Insecticides - Advances in Integrated Pest Management*. InTech. https://doi.org/10.5772/29495.

Park, T. 1934. “Observations on the General Biology of the Flour Beetle, *Tribolium confusum*.” *Https://Doi.Org/10.1086/394454* 9 (1): 36–54. https://doi.org/10.1086/394454.

Patole, S S. 2017. “Review on Beetles (Coleopteran): An Agricultural Major Crop Pests of the World.” *Int. J. Life. Sci. Scienti. Res* 3 (6): 1424–32. https://doi.org/10.21276/ijlssr.2017.3.6.1.

Paudyal, S, G P Opit, F H Arthur, G V Bingham, and S G Gautam. 2016. “Contact Toxicity of Deltamethrin against *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Sitophilus oryzae* (Coleoptera: Curculionidae), and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) Adults.” *Journal of Economic Entomology* 109 (4): 1936–42. https://doi.org/10.1093/jee/tow107.

Petroff, Reeves. 2004. “Pest Management for Grain Storage and Fumigation Seed Treatment-Pest Control-Grain Storage & Seed Treatment Facilities Montana Department of Agriculture.”

Phankaen, Yaowanan, Auraya Manaprasertsak, Wanchai Pluempanupat, Opender Koul, Yooichi Kainoh, and Vasakorn Bullangpoti. 2017. “Toxicity and Repellent Action of Coffea Arabica against *Tribolium castaneum* (Herbst) Adults under Laboratory Conditions.” *Journal of Stored Products Research* 71 (March): 112–18. https://doi.org/10.1016/J.JSPR.2017.01.006.

Ponce, Marco A, Sandra Lizarraga, Alexander Bruce, Tania N Kim, and William R Morrison. 2022. “Grain Inoculated with Different Growth Stages of the fungus, *Aspergillus flavus*, Affect the Close-Range Foraging Behavior by a Primary Stored Product Pest, *Sitophilus oryzae* (Coleoptera: Curculionidae).” *Environmental Entomology* 51 (5): 927–39. https://doi.org/10.1093/EE/NVAC061.

Rahman Sabuj, Zaidur. 2017. “Control Potentials of Two Medicinal Plants *Morus Alba* L. and *Saraca indica* L. against Three Stored Product Pests.” https://www.researchgate.net/publication/321035418.

Rajashekar, Yallappa, Nandagopal Bakthavatsalam, and Thimmappa Shivanandappa. 2012. “Botanicals as Grain Protectants.” *Psyche (London)*. https://doi.org/10.1155/2012/646740.

Ranum, Peter, Juan Pablo Peña, Pe˜ Peña-Rosas, Maria Nieves Garcia-Casal, and Maria Nieves Garcia-Casal. 2014. “Global Maize Production, Utilization, and Consumption.” *Annals of the New York Academy of Sciences* 1312 (1): 105–12. https://doi.org/10.1111/NYAS.12396.

Rausch, Kent D, and Ronald L Belyea. 2006. “Coproducts from Corn Processing 47 The Future of Coproducts From Corn Processing.” *Applied Biochemistry and Biotechnology*. Vol. 128.

Rees, D. 2007. *Insects of Stored Grain: A Pocket Reference*. CSIRO Pub.

Romero, Susan A, James F Campbell, James R Nechols, and Kimberly A With. 2010. “Movement Behavior of Red Flour Beetle: Response to Habitat Cues and Patch Boundaries.” *Environmental Entomology* 39 (3): 919–29. https://doi.org/10.1603/EN09324.

Rosentrater, Kurt A. 2022. “Insects in Grains: Identification, Damage, and Detection.” *Storage of Cereal Grains and Their Products*, January, 607–46. https://doi.org/10.1016/B978-0-12-812758-2.00014-3.

Rösner, Janin, Benedikt Wellmeyer, and Hans Merzendorfer. 2020. “*Tribolium castaneum*: A Model for Investigating the Mode of Action of Insecticides and Mechanisms of Resistance.” *Current Pharmaceutical Design* 26 (29): 3554–68. https://doi.org/10.2174/1381612826666200513113140.

Roy, M. 2021. “Efficacy of Essential Oil of *Eucalyptus maculata* on the Protein Profile of Rice Weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae).” 2021. http://www.annalsofrscb.ro/index.php/journal/article/view/328/278.

Rusyniak, Daniel E, and Kristine A Nañagas. 2004. “Organophosphate Poisoning.” *Seminars in Neurology* 24 (2): 197–204. https://doi.org/10.1055/S-2004-830907/ID/27/BIB.

Sakka, Maria K, and Christos G Athanassiou. n.d. “Insecticidal Effect of Diatomaceous Earth and Pirimiphos-Methyl against Phosphine-Susceptible and Phosphine-Resistant Populations of Two Stored Product Beetle Species.” https://doi.org/10.1007/s11356-021-12971-1/Published.

Sauer, David B. 1988. “Effects of Fungal Deterioration on Grain: Nutritional Value, Toxicity, Germination.” *International Journal of Food Microbiology* 7 (3): 267–75. https://doi.org/10.1016/0168-1605(88)90045-1.

Shankar, U, and D P Abrol. 2012. “Integrated Pest Management in Stored Grains. | Integrated Pest Management: Principles and Practice.” 2012. https://www.cabidigitallibrary.org/doi/abs/10.1079/9781845938086.0386.

Shewry, Peter R, and Nigel G Halford. 2002. “Cereal Seed Storage Proteins: Structures, Properties and Role in Grain Utilization.” *Journal of Experimental Botany* 53 (370): 947–58. https://academic.oup.com/jxb/article/53/370/947/537249.

Shinoda, Tetsuro. 2015. “Juvenile Hormone.” *Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research*, January, 564,e99-1-565,e99-3. https://doi.org/10.1016/B978-0-12-801028-0.00099-4.

Shrivastava, Bhanu, Shrivastava Bhanu, Shrivastava Archana, Kumar Ajay, Parihar Surendra Singh, and Bhatnagar Vandana. 2011. “Impact of Deltamethrin on the Environment, Use as an Insecticide and Its Bacterial Degradation-A Preliminary Study Typhoid Fever View Project Effect of Insecticide.” *International Journal of Environmental Science* 1 (5). https://doi.org/10.6088/ijessi.0010520027.

Sivakumar, Mannava V K. 2018. “Climate Extremes and Impacts on Agriculture.” *Agroclimatology*, January, 621–47. https://doi.org/10.2134/AGRONMONOGR60.2016.0003.

Soltan, H R, A S Elbakary, M S Shawir, and T AlHoumudi. 2020. “Comparative Acute Toxicity of Five Insecticide against Rice Weevil.” *Modern Concepts & Developments in Agronomy* 7 (3). https://doi.org/10.31031/mcda.2020.07.000665.

Srivastava, Chitra, and S Subramanian. 2016. “Storage Insect Pests and Their Damage Symptoms: An Overview.” *Indian Journal of Entomology* 78 (special): 53. https://doi.org/10.5958/0974-8172.2016.00025.0.

Stack, Camilla. n.d. *Beetles: Biodiversity, Ecology and Role in the Environment*.

Stathers, Tanya E, Sarah E J Arnold, Corinne J Rumney, and Clare Hopson. 2020. “Measuring the Nutritional Cost of Insect Infestation of Stored Maize and Cowpea.” *Food Security* 12 (2): 285–308. https://doi.org/10.1007/s12571-019-00997-w.

Stejskal, Vaclav, Tomas Vendl, Radek Aulicky, and Christos Athanassiou. 2021. "Synthetic and Natural Insecticides: Gas, Liquid, Gel and Solid Formulations for Stored-Product and Food-Industry Pest Control." *Insects 2021, Vol. 12, Page 590* 12 (7): 590. https://doi.org/10.3390/INSECTS12070590.

Stephensons, Maria Christina. 1983. "A Study of Population Growth of *Sitophilus oryzae* L. and *Sitophilus granarius* L. in Single and Mixed Culture in Wheat and Rice: A Thesis Presented in Partial Fulfilment of the Requirements for the Degree of Master of Science in Plant Science at Massey University." https://mro.massey.ac.nz/handle/10179/13751.

Suzuki, Yuichiro, James W Truman, and Lynn M Riddiford. 2008. "The Role of Broad in the Development of *Tribolium castaneum*: Implications for the Evolution of the Holometabolous Insect Pupa." *Development* 135 (3): 569–77. https://doi.org/10.1242/DEV.015263.

Swamy, K C Narayana, G P Mutthuraju, E Jagadeesh, and G T Thirumalaraju. 2014. "Biology of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) on Stored Maize Grains." *Current Biotica* 8 (1): 76–81. www.currentbiotica.com.

Taddese, Marid, Kumela Dibaba, Wakuma Bayissa, Debela Hunde, Esayas Mendesil, Menale Kassie, Christopher Mutungi, and Tadele Tefera. 2020. "Assessment of Quantitative and Qualitative Losses of Stored Grains Due to Insect Infestation in Ethiopia." *Journal of Stored Products Research* 89 (December): 101689. https://doi.org/10.1016/J.JSPR.2020.101689.

Talukder, Farid. 2009. "Pesticide Resistance in Stored-Product Insects and Alternative Biorational Management: A Brief Review." *Agricultural and Marine Sciences*. Vol. 14.

Tang, Juming, Elizabeth Mitcham, Shaojin Wang, and Susan Lurie. 2007. "Heat Treatments for Postharvest Pest Control: Theory and Practice," July, 1–349. https://doi.org/10.1079/9781845932527.0000.

Tang, Juming, Shaojin Wang, and J A Johnson. 2007. "Biology and Thermal Death Kinetics of Selected Insects," July, 133–61. https://doi.org/10.1079/9781845932527.0133.

Thakur, Meena, Eradasappa Eradasappa, and V K Chandla. 2012. "A New Incidence of Red Flour Beetle (*Tribolium castaneum* Herbst) in Stored True Potato Seeds." http://biosciencesjournal.info/.

Throne, James E., Guy J. Hallman, Judy A. Johnson, and Peter A. Follett. 2003a. "Post-Harvest Entomology Research in the United States Department of Agriculture-Agricultural Research Service." *Pest Management Science*. https://doi.org/10.1002/ps.690.

———. 2003b. "Post-Harvest Entomology Research in the United States Department of Agriculture-Agricultural Research Service." *Pest Management Science*. https://doi.org/10.1002/ps.690.

Tripathi, Arun Kumar. 2018. "Pests of Stored Grains." *Pests and Their Management*, August, 311–59. https://doi.org/10.1007/978-981-10-8687-8_10/COVER.

Tyagi, S K, P N Guru, Aarti Nimesh, Akhoon Asrar Bashir, Pulin Patgiri, Vandana Mohod, and Anju B Khatkar. 2019. "Post-Harvest Stored Product Insects and Their Management," December. http://krishi.icar.gov.in/jspui/handle/123456789/31390.

Upadhyay, R K, and S Ahmad. 2011. "Management Strategies for Control of Stored Grain Insect Pests in Farmer Stores and Public Ware Houses." *World Journal of Agricultural Sciences* 7 (5): 527–49.

Vélez, M, W F Barbosa, J Quintero, M Chediak, and R N C Guedes. 2017. "Deltamethrin- and Spinosad-Mediated Survival, Activity and Avoidance of the Grain Weevils *Sitophilus granarius* and *S. zeamais*." *Journal of Stored Products Research* 74 (December): 56–65. https://doi.org/10.1016/J.JSPR.2017.10.002.

Via, Sara. 1999. "Cannibalism Facilitates the Use of a Novel Environment in the Flour Beetle, *Tribolium castaneum*." *Heredity* 82 (3): 267–75. https://doi.org/10.1038/sj.hdy.6884820.

Vinokurov, K S, E N Elpidina, D P Zhuzhikov, B Oppert, D Kodrik, and F Sehnal. 2009. "Digestive Proteolysis Organization in Two Closely Related Tenebrionid Beetles: Red Flour Beetle (*Tribolium castaneum*) and Confused Flour Beetle (*Tribolium confusum*)." *Archives of Insect Biochemistry and Physiology* 70 (4): 254–79. https://doi.org/10.1002/ARCH.20299.

Washington, D C. 2016. "Marketing and Regulatory Programs Agricultural Marketing Service Federal Grain Inspection Service Stored-Grain Insect Reference."

Weaver, David K, and A Reeves Petroff. 2005. "Introduction-Stored Grain Pest Management Pest Management for Grain Storage and Fumigation."

Wong, Nellie, and Chow Yang Lee. 2011. "Relationship between Population Growth of the Red Flour Beetle *Tribolium castaneum* and Protein and Carbohydrate Content in Flour and Starch." *Journal of Economic Entomology* 104 (6): 2087–94. https://doi.org/10.1603/EC11234.

Yaseen, Mifftha, Tahreem Kausar, Bushra Praween, Shafaq Javaid Shah, Yasmeena Jan, Surendra Singh Shekhawat, Muneeb Malik, and Z. R. Azaz Ahmad Azad. 2019. "Insect Pest Infestation during Storage of Cereal Grains, Pulses and Oilseeds." *Health and Safety Aspects of Food Processing Technologies*, December, 209–34. https://doi.org/10.1007/978-3-030-24903-8_8/COVER.

Zhang, Liu, Heng Sun, Hao Li, Zhenhong Rao, and Haiyan Ji. 2021. "Identification of Rice-Weevil (*Sitophilus oryzae* L.) Damaged Wheat Kernels Using Multi-Angle NIR Hyperspectral Data." *Journal of Cereal Science* 101 (September). https://doi.org/10.1016/j.jcs.2021.103313.

Zhang, Runyang, Sen Ma, Li Li, Minghui Zhang, Shuangqi Tian, Dongying Wang, Kunlun Liu, Huamin Liu, Wenxue Zhu, and Xuede Wang. 2021. "Comprehensive Utilization of Corn Starch Processing By-Products: A Review." *Grain & Oil Science and Technology* 4 (3): 89–107. https://doi.org/10.1016/J.GAOST.2021.08.003.

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_{50} and LC_{90} values were determined. The results showed that pirimiphos-methyl had the highest toxicity, with an LC_{50} 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_{50} 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^{0}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 75^0F 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

Product	Formulation	Chemical Group (IRAC Code)	Mode of Action	Physical Form
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

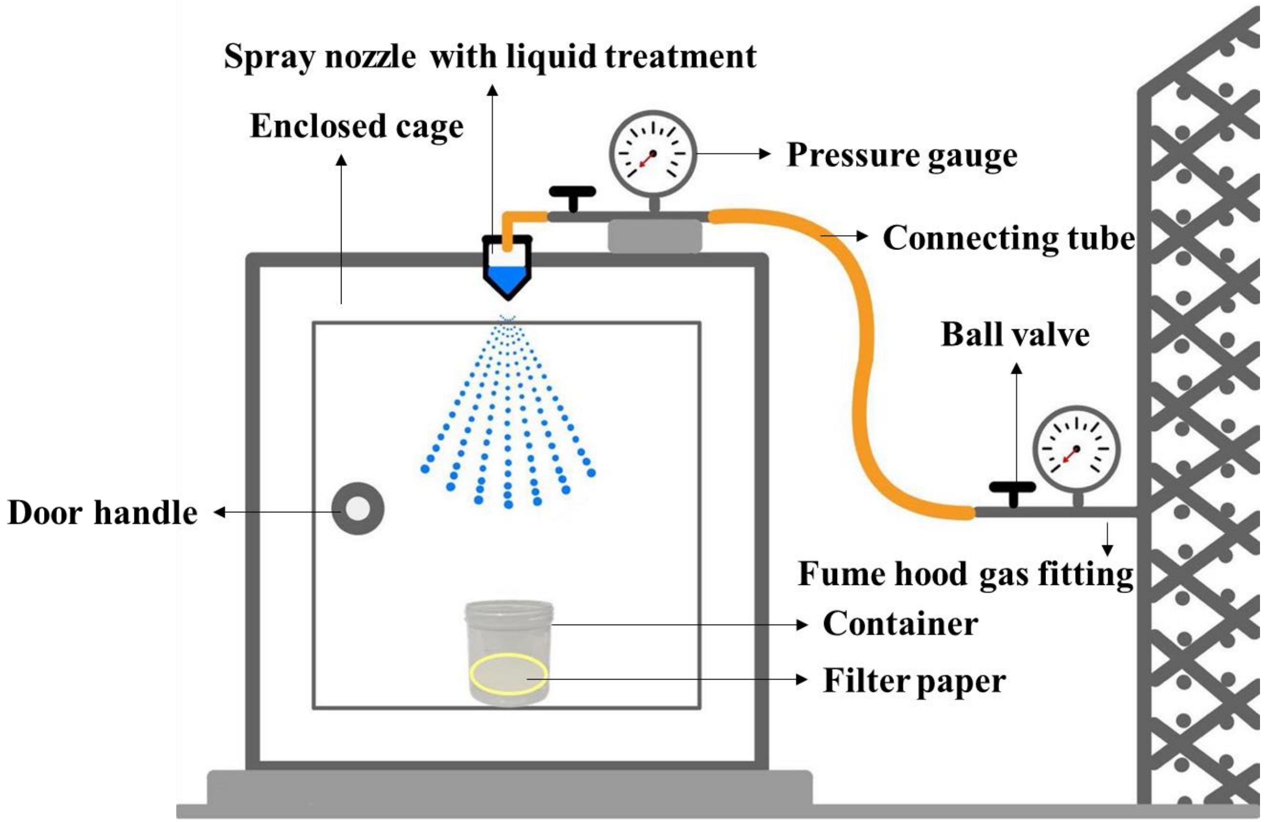

Figure 1. Design and illustration of a customized lab spray tower for application of liquid pesticide formulations used in the experiment.

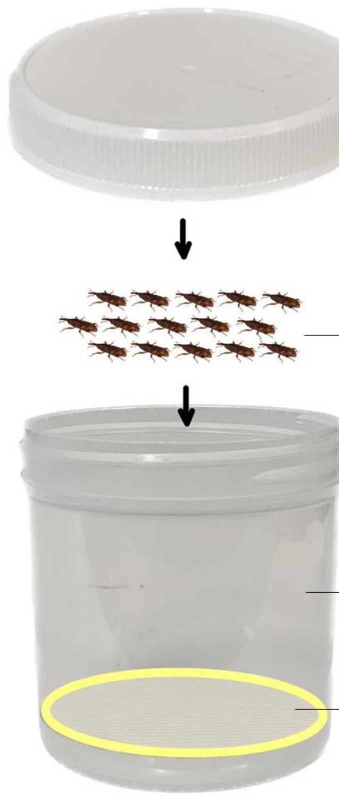

Figure 2. Design of Experimental container used in the study.

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 ≥ deltamethrin ≥ deltamethrin plus (S)-methoprene, by comparing the LC_{50} 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_{50} values and found that the recommended rate for pirimiphos-methyl (6–10 ppm) not only exceeded its LC_{50} at 0.74 ppm (95% CL 0.57–0.92) but also surpassed its LC_{90} 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_{50} 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_{50} 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[1]	N[2,]	Slope ± SE*	LC_{50} (ppm) (95% CL)	LC_{90} (ppm) (95% CL)	Recommended application rate (ppm)[#]
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

[1]The products are listed based on toxicity profile, from high to low.

[2]N is the number of individuals tested for each product. Response regression lines are presented by Slope ± SE, LC_{50} (in ppm), and LC_{90} (in ppm).

*Control mortality was 0% during the study period.

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

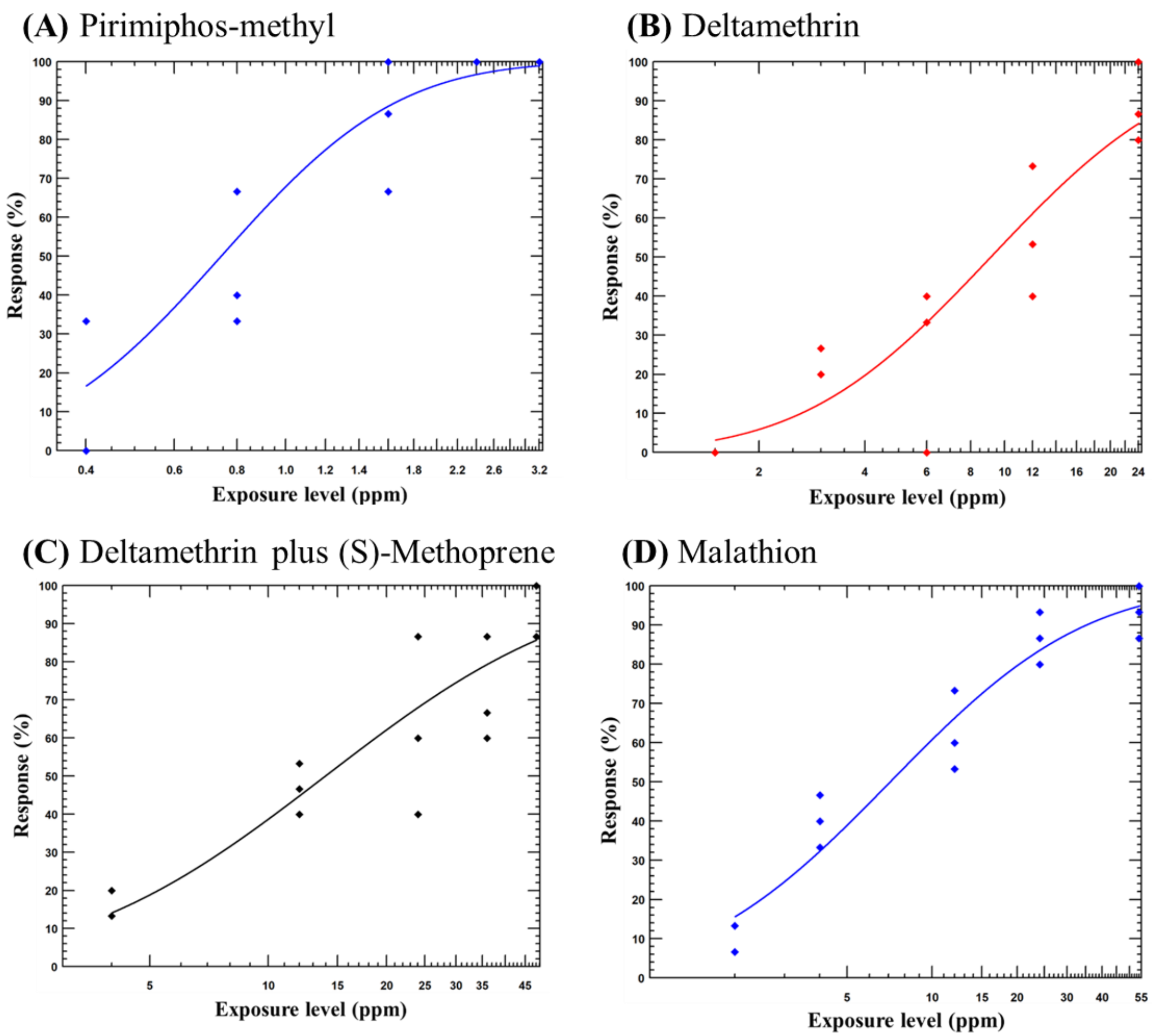

Figure 3. Toxicity response of *Sitophilus oryzae* to selected pesticides: (A) pirimiphos-methyl, (B) deltamethrin, (C) deltamethrin plus (S)-methoprene, and (D) malathion at 48h after treatment.

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_{50} values, the recommended rate for pirimiphos-methyl exceeded its LC_{50} 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_{50}. 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*.

References

Andrić, G. (2006) *Effects of several contact insecticides on adults of three Sitophilus species Development of Integrated Management of Harmful Organisms in Plant Production in Order to Overcome Resistance and Improve Food Quality and Safety" Petar Kljajić Pesticide and Environment Research Institute*. Available at: https://www.researchgate.net/publication/255607450.

Ashamo, M.O., Odeyemi, O.O. and Olatunji, A.A. (2010) 'Resistance of strains of rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) to pirimiphos methyl', 425. Available at: https://doi.org/10.5073/jka.2010.425.433.

Attia, M.A. *et al.* (2020) 'Stored grain pest prevalence and insecticide resistance in Egyptian populations of the red flour beetle *Tribolium castaneum* (Herbst) and the rice weevil *Sitophilus oryzae* (L.)', *Journal of Stored Products Research*, 87. Available at: https://doi.org/10.1016/j.jspr.2020.101611.

Balcom Chemicals Inc. (1975) *Malathion Big-6 Dust label, US EPA Pesticide Product Label*. Greeley, CO, USA. Available at: https://www3.epa.gov/pesticides/chem_search/ppls/000960-00202-19750610.pdf (Accessed: 8 March 2023).

Balderacchi, M. *et al.* (2018) 'Heat and drought stresses in crops and approaches for their mitigation', *Frontiers in Chemistry | www.frontiersin.org*, 6, p. 26. Available at: https://doi.org/10.3389/fchem.2018.00026.

Bhargude, A.R., Patil S K and Kadam D R (2021) 'Eco-friendly management of rice weevil (*Sitophilus oryzae* Linnaeus) on sorghum during storage', *~ 1647 ~ Journal of Entomology and Zoology Studies*, 9(1), pp. 1647–1652. Available at: http://www.entomoljournal.com.

Bhattacharyya, P. *et al.* (2022) 'Rice based production system climate resilient technologies for rice based production systems in eastern India', in, pp. 355–422. Available at: www.icar-nrri.in (Accessed: 26 March 2023).

Bhuiyah, M.I.M. *et al.* (1990) 'Biology of the rice weevil, *Sitophilus oryzae* (Linnaeus).', *Bangladesh Journal of Zoology*, 18(1), pp. 67–73.

Biddinger, D.J., Hull, L.A. and Rajotte, E.G. (1998) 'Stage specificity of various insecticides to tufted apple bud moth larvae (Lepidoptera: Tortricidae)', *Journal of Economic Entomology*, 91(1), pp. 200–208. Available at: https://doi.org/10.1093/JEE/91.1.200.

Bramavath, D. *et al.* (2017) 'Fumigation of stored-products: Current needs and practices, and future outlook', *~ 1499 ~ International Journal of Chemical Studies*, 5(5).

Brown, P. (2000) 'Insecticides | Gas Chromatography', *Encyclopedia of Separation Science*, pp. 3110–3118. Available at: https://doi.org/10.1016/B0-12-226770-2/06371-7.

Busvine, J.R. and Food and Agriculture Organization of the United Nations. (1980) 'Recommended methods for measurement of pest resistance to pesticides', p. 132.

Central Garden & Pet Company (2016a) *Centynal EC label*. Schaumburg, IL. Available at: https://www.bugfreegrains.com/products/centynal-ec-insecticide?gclid=Cj0KCQjw7aqkBhDPARIsAKGa0oIfSYlXJ4r4NkdmlUJvCxCAUHmOiC7QJEltx-gho0lRoRp8FFuIGwUaAvboEALw_wcB (Accessed: 14 June 2023).

Central Garden & Pet Company (2016b) *Diacon IGR Plus label*. Schaumburg, IL. Available at: https://www.bugfreegrains.com/products/diacon-igr-plus (Accessed: 8 March 2023).

Chulze, S.N. (2010) 'Strategies to reduce mycotoxin levels in maize during storage: a review', *http://dx.doi.org/10.1080/19440040903573032*, 27(5), pp. 651–657. Available at: https://doi.org/10.1080/19440040903573032.

Damalas, C.A. and Eleftherohorinos, I.G. (2011) 'Pesticide exposure, safety issues, and risk assessment indicators', *International Journal of Environmental Research and Public Health 2011, Vol. 8, Pages 1402-1419*, 8(5), pp. 1402–1419. Available at: https://doi.org/10.3390/IJERPH8051402.

Danforth, A.T. (2009) *Corn crop production: growth, fertilization and yield*. Nova Science Publishers.

Davis, S.R. (2011) 'Rostrum structure and development in the rice weevil *Sitophilus oryzae* (Coleoptera: Curculionoidea: Dryophthoridae)', *Arthropod Structure and Development*, 40(6), pp. 549–558. Available at: https://doi.org/10.1016/J.ASD.2011.06.002.

Derbalah, A. *et al.* (2021) 'New trends for controlling *Sitophilus oryzae* concerning adult mortality, offspring production, mode of action, and grain quality', *Journal of Consumer Protection and Food Safety*, 16, pp. 343–351. Available at: https://doi.org/10.1007/s00003-021-01339-9.

Devi, R.S. *et al.* (2017) 'Biology, morphology and molecular characterization of *Sitophilus oryzae* and *S. zeamais* (Coleoptera: Curculionidae)', *Journal of Stored Products Research*, 73, pp. 135–141. Available at: https://doi.org/10.1016/J.JSPR.2017.08.004.

Erenstein, O. *et al.* (2022) 'Global maize production, consumption and trade: trends and R&D implications', *Food Security*. Springer Science and Business Media B.V., pp. 1295–1319. Available at: https://doi.org/10.1007/s12571-022-01288-7.

Fouad, A.H., de Souza Tavares, W. and Zanuncio, J.C. (2021) 'Toxicity and repellent activity of monoterpene enantiomers to rice weevils (*Sitophilus oryzae*)', *Pest Management Science*, 77(7), pp. 3500–3507. Available at: https://doi.org/10.1002/ps.6403.

Fulton, M.H., Key, P.B. and DeLorenzo, M.E. (2013) 'Insecticide toxicity in fish', *Fish Physiology*, 33, pp. 309–368. Available at: https://doi.org/10.1016/B978-0-12-398254-4.00006-6.

Funsho, J., Adetunji B and Abu, P.G.D.E. (1984) 'Studies on the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) with special reference to sorghum cultivar resistance'.

Ghosat, A. (2018) *Mode of action of insecticides*. Available at: https://www.researchgate.net/publication/327580308.

Gupta, R.C. and Milatovic, D. (2014) 'Insecticides', *Biomarkers in Toxicology*, pp. 389–407. Available at: https://doi.org/10.1016/B978-0-12-404630-6.00023-3.

Hagstrum, D.W., Phiilips, T.W. and Cuperus, G.W. (2012) *Stored product protection*. Kansas State University.

Haliscak, J.P. and Beeman, R.W. (1983) 'Status of malathion resistance in five genera of beetles infesting farm-stored corn, wheat, and oats in the United States', *Journal of Economic Entomology*, 76(4), pp. 717–722. Available at: https://doi.org/10.1093/JEE/76.4.717.

Hasan Iqbal, M. *et al.* (2012) 'Toxicity of Cypermethrin and Malathion on rice weevil, *Sitophilus oryzae* (L.) and their effect on esterase isozymes nucleotide sequence of 16S rRNA gene of *Clarias gariepinus* and its molecular phylogeny with other catfishes view project MS project on " Toxic effects of Malathion on *Channa punctatus* (Bloch, 1793) and tissue esterase variability'. Available at: www.jcbsc.org (Accessed: 16 June 2023).

Hong, K.J. *et al.* (2018) 'The first confirmation of the distribution of rice weevil, *Sitophilus oryzae*, in South Korea', *Journal of Asia-Pacific Biodiversity*, 11(1), pp. 69–75. Available at: https://doi.org/10.1016/J.JAPB.2017.12.005.

Howe, R.W. (1952) 'The Biology of the rice weevil, *Calandra oryzae* (L.)', *Annals of Applied Biology*, 39(2), pp. 168–180. Available at: https://doi.org/10.1111/J.1744-7348.1952.TB00895.X.

Huang, F. and Subramanyam, B. (2005) 'Management of five stored-product insects in wheat with pirimiphos-methyl and pirimiphos-methyl plus synergized pyrethrins', *Pest Management Science*, 61(4), pp. 356–362. Available at: https://doi.org/10.1002/ps.968.

Irabagon, T.A. (1959) 'Rice weevil damage to stored corn', *Journal of Economic Entomology*, 52(6), pp. 1130–1136. Available at: https://doi.org/10.1093/JEE/52.6.1130.

Khan, T., Haider, M.S. and Khan, H.A.A. (2022) 'Resistance to grain protectants and synergism in Pakistani strains of *Sitophilus oryzae* (Coleoptera: Curculionidae)', *Scientific Reports 2022 12:1*, 12(1), pp. 1–8. Available at: https://doi.org/10.1038/s41598-022-16412-y.

Koehler P G (2022) 'Rice weevil, *Sitophilus oryzae (Coleoptera: Curculionidae)*. Available at: https://edis.ifas.ufl.edu/publication/IG120 (Accessed: 25 March 2023).

Kumar, D. and Kalita, P. (2017) 'Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries', *Foods 2017, Vol. 6, Page 8*, 6(1), p. 8. Available at: https://doi.org/10.3390/FOODS6010008.

Lagisz, M., Wolff, K. and Port, G. (2010) 'Time matters: delayed toxicity of pirimiphos-methyl on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and its effects on efficacy estimation of residual treatments', *Journal of Stored Products Research*, 46(3), pp. 161–165. Available at: https://doi.org/10.1016/j.jspr.2010.02.003.

LeOra Software LLC (2005) *PoloPlus 2.0*. Available at: https://leora-software-company-poloplus.software.informer.com/2.0/.

Maciej Serda *et al.* (2022) 'Review on major storage insect pests of cereals and pulses', *Asian Journal of Advances in Research*. Edited by G. Balint et al., 7(1), pp. 41–56. Available at: https://doi.org/10.2/JQUERY.MIN.JS.

Magby, J.P. and Richardson, J.R. (2017) 'Developmental pyrethroid exposure causes long-term decreases of neuronal sodium channel expression', *Neurotoxicology*, 60, pp. 274–279. Available at: https://doi.org/10.1016/J.NEURO.2016.04.002.

Merzendorfer, H. and Zimoch, L. (2003) 'Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases', *Journal of Experimental Biology*, 206(24), pp. 4393–4412. Available at: https://doi.org/10.1242/JEB.00709.

Okram, S. and Hath, T.K. (2019) 'Biology of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) on stored rice grains during different seasons in Terai Agro-Ecology of West Bengal', *International Journal of Current Microbiology and Applied Sciences*, 8(04), pp. 1955–1963. Available at: https://doi.org/10.20546/ijcmas.2019.804.229.

Phan, N.T. *et al.* (2020) 'A new ingestion bioassay protocol for assessing pesticide toxicity to the adult Japanese orchard bee (*Osmia cornifrons*)', *Scientific Reports 2020 10:1*, 10(1), pp. 1–9. Available at: https://doi.org/10.1038/s41598-020-66118-2.

Pitzer, E.M., Williams, M.T. and Vorhees, C. V (2021) 'Effects of pyrethroids on brain development and behavior: Deltamethrin', *Neurotoxicology and Teratology*, 87. Available at: https://doi.org/10.1016/j.ntt.2021.106983.

Pope, C.N. (2005) 'Central Nervous System effects and neurotoxicity', *Toxicology of Organophosphate and Carbamate Compounds*, pp. 271–291. Available at: https://doi.org/10.1016/B978-0120885237/500211.

Rajendran, S. (2001) 'Alternatives to methyl bromide as fumigants for stored food commodities', *Pesticide Outlook*, pp. 249–253. Available at: https://doi.org/10.1039/b110550g.

Robertson, J.L. *et al.* (2007) *Bioassays with Arthropods*. 2nd edn. Boca Raton, FL, USA: CRC Press.

Robertson, J.L., Savin, N.E. and Preisler, H.K. (2007) 'Bioassays with arthropods', *Bioassays with Arthropods* [Preprint]. Available at: https://doi.org/10.1201/9781420004045.

Rumbos, C.I., Dutton, A.C. and Athanassiou, C.G. (2013) 'Comparison of two pirimiphos-methyl formulations against major stored-product insect species', *Journal of Stored Products Research*, 55, pp. 106–115. Available at: https://doi.org/10.1016/j.jspr.2013.08.003.

Serna-Saldivar, S.O. and Carrillo, E.P. (2019) 'Food Uses of whole Corn and dry-milled fractions', *Corn: Chemistry and Technology, 3rd Edition*, pp. 435–467. Available at: https://doi.org/10.1016/B978-0-12-811971-6.00016-4.

Shankar, U. and Abrol, D.P. (2012) 'Integrated pest management in stored grains.', *Integrated pest management: principles and practice*, pp. 386–407. Available at: https://doi.org/10.1079/9781845938086.0386.

Singh, U. and Singh, B. (1992) ‘Tropical grain legumes as important human foods’, *Economic Botany*, 46(3), pp. 310–321. Available at: https://doi.org/10.1007/BF02866630/METRICS.

Soltan, H.R. *et al.* (2020) ‘Comparative acute toxicity of five insecticide against rice weevil’, *Modern Concepts & Developments in Agronomy*, 7(3). Available at: https://doi.org/10.31031/mcda.2020.07.000665.

Srivastava, C. and Subramanian, S. (2016) ‘Storage insect pests and their damage symptoms: an overview’, *Indian Journal of Entomology*, 78(special), p.53. Available at: https://doi.org/10.5958/0974-8172.2016.00025.0.

Tapia, C.M. *et al.* (2020) ‘Effects of Deltamethrin acute exposure on Nav1.6 Channels and medium spiny neurons of the nucleus accumbens’, *Toxicology*, 440. Available at: https://doi.org/10.1016/j.tox.2020.152488.

Wijayaratne, L.K.W., Arthur, F.H. and Whyard, S. (2018) ‘Methoprene and control of stored-product insects’, *Journal of Stored Products Research*, 76, pp.161–169. Available at: https://doi.org/10.1016/J.JSPR.2016.09.001.

Winfield Solutions LLC (2015) *Actellic 5E label, US EPA Pesticide Product Label*. St. Paul, MN, USA. Available at: https://www3.epa.gov/pesticides/chem_search/ppls/001381-00170-20150126.pdf (Accessed: 8 March 2023).

Zahniser, S. *et al.* (2019) *Feed Outlook No. (FDS-19F-01): The growing corn economies of Mexico and the United States*. Available at: https://www.ers.usda.gov/publications/pub-details/?pubid=93541 (Accessed: 18 June 2023).

Zhang, L. *et al.* (2021) ‘Identification of rice-weevil (*Sitophilus oryzae* L.) damaged wheat kernels using multi-angle NIR hyperspectral data’, *Journal of Cereal Science*, 101, p. 103313. Available at: https://doi.org/10.1016/J.JCS.2021.103313.

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_{50} values, the toxicity of the four insecticides to *T. castaneum* was as follows: deltamethrin plus (S)-methoprene ≥ deltamethrin > pirimiphos-methyl > malathion. This means deltamethrin plus (S)-methoprene and deltamethrin displayed the highest toxicity against *T. castaneum* with LC_{50} 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_{50} 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 ≥ malathion. However, considering the LC_{50} 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.

Product	Formulation	Chemical Group (IRAC Code)	Mode of Action
Actellic 5E *(WinField United, St. Paul, MN, USA)*	pirimiphos-methyl 57%	1B - organophosphate	Acetylcholinesterase Inhibitors
Malathion Big-6 Dust *(Balcom Chemicals, Inc., Greeley, CO, USA)*	malathion 6%	1B - organophosphate	Acetylcholinesterase Inhibitors
Centynal EC *(Central Life Sciences, Schaumburg, IL, USA)*	deltamethrin 4.75%	3A - pyrethroids, pyrethrins	Sodium Channel Modulators
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

The products are listed based on chemical group order.

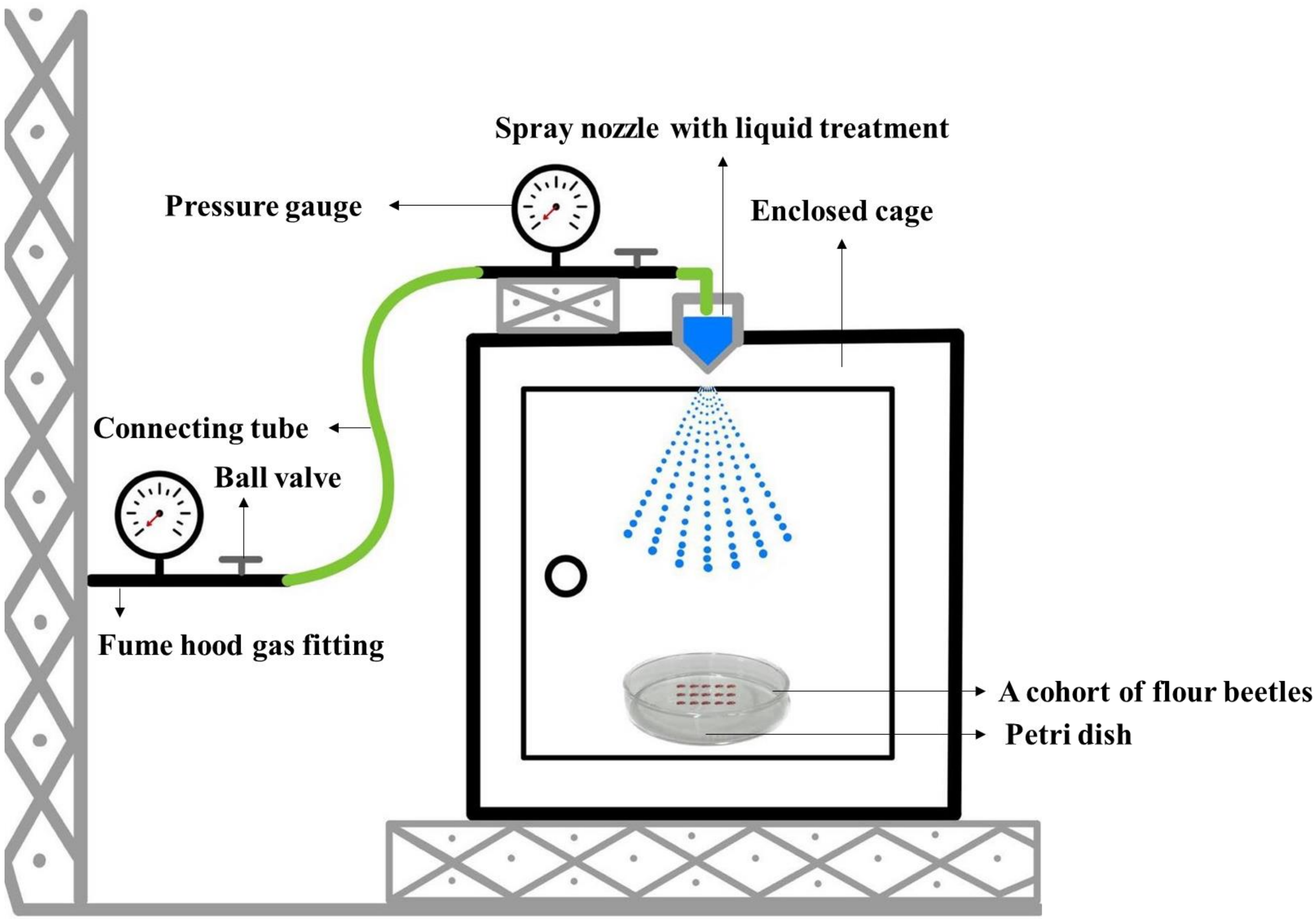

Figure 1. Design of the customized lab spray tower used for the application of insecticides in liquid formulations.

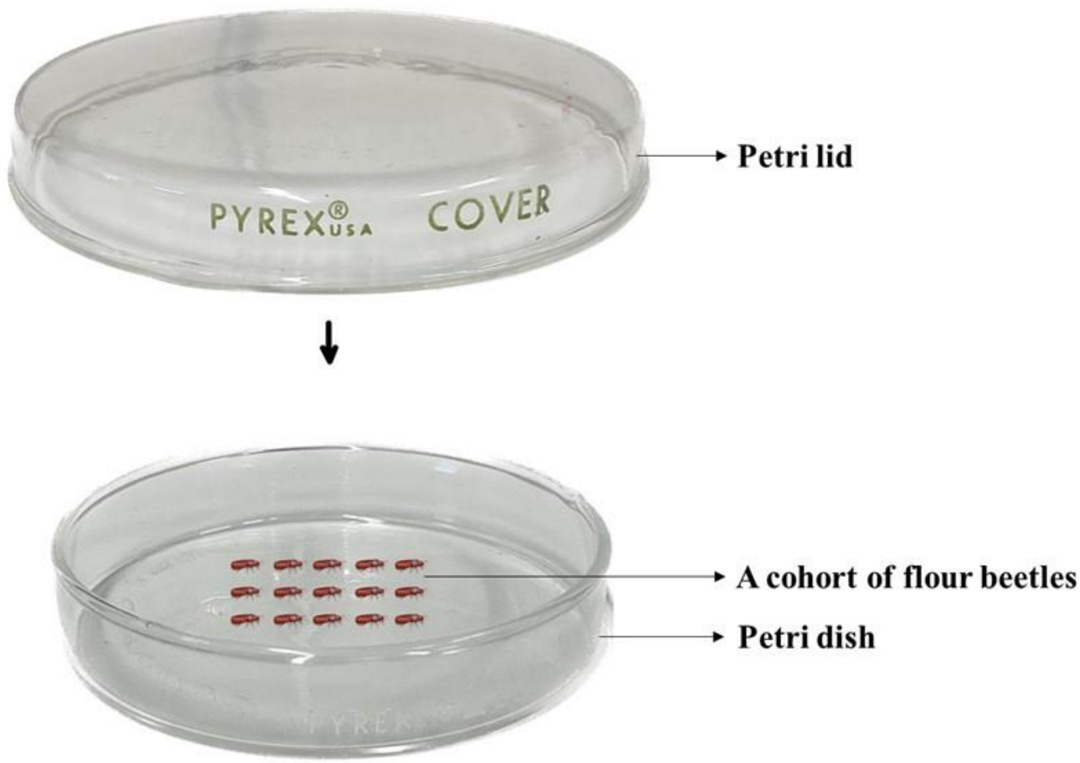

Figure 2. Experimental container used in the study.

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 ± SE, and LC_{50} (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 ≥ deltamethrin > pirimiphos-methyl > malathion. Among them, deltamethrin plus (S)-methoprene exhibited the highest toxicity to *T. castaneum*, with an LC_{50} value of 0.025ppm (95% CL 0.021–0.029) followed by deltamethrin and pirimiphos-methyl with LC_{50} 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_{50} 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 ≥ malathion **(Table 2, Figure 3)**. However, based on the LC_{50} values and the 95% confidence limits found in this study, pirimiphos-methyl exhibited the lowest LC_{50} 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_{50} ratio, or ratio of concentrations causing 50% mortality, through dividing the LC_{50} of *Tribolium castaneum* by LC_{50} of *Tribolium confusum*. Species was considered significantly more susceptible to a pesticide when LC_{50} ratio was less than 1.0, and where the 95% confident limit of LC_{50} 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_{50} ratios ranging from 1/10 to ½ **(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. casteneum* 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_{50} 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_{50} 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_{50} 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_{50} 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_{50} ratios ranging from 1/10 to ½ 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_{50} 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_{50} value for both species of flour beetles, and the rates for deltamethrin, deltamethrin plus (S)-methoprene, and malathion were also higher than the LC_{50} 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[1]	Species	N[‡]	Slope ± SE	LC_{50} (ppm) (95% CL)	LC_{50} ratio[2] (95% CL)	Recommended application rate (ppm)[#]
Pirimiphos-methyl	*Tribolium castaneum*	270	4.55 ± 0.60	0.785 (0.609 – 1.049)	7.142 (5.203 – 9.805)	6 – 8
	Tribolium confusum	270	2.32 ± 0.28	0.148 (0.095 – 0.200)		
Deltamethrin	*Tribolium castaneum*	225	1.55 ± 0.25	0.026 (0.002 – 0.052)	0.010 (0.007 – 0.015)	0.5 – 1.0
	Tribolium confusum	225	1.91 ± 0.20	4.247 (2.422 – 7.800)		
Deltamethrin + (S)-methoprene	*Tribolium castaneum*	225	2.88 ± 0.28	0.025 (0.021 – 0.029)	0.049 (0.039 – 0.062)	0.5 – 1.0
	Tribolium confusum	225	2.74 ± 0.33	0.512 (0.430 – 0.605)		
Malathion	*Tribolium castaneum*	225	1.30 ± 0.16	4.090 (2.908 – 5.785)	0.546 (0.353 – 0.842)	10
	Tribolium confusum	225	1.54 ± 0.19	7 .842 (5.838 – 10.462)		

[1] The products are listed based on chemical group order.

[‡]N is the number of individuals tested for each product. Response regression lines are presented by Slope ± SE, and LC_{50} (in ppm). Control mortality was 0% during the study period.

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

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

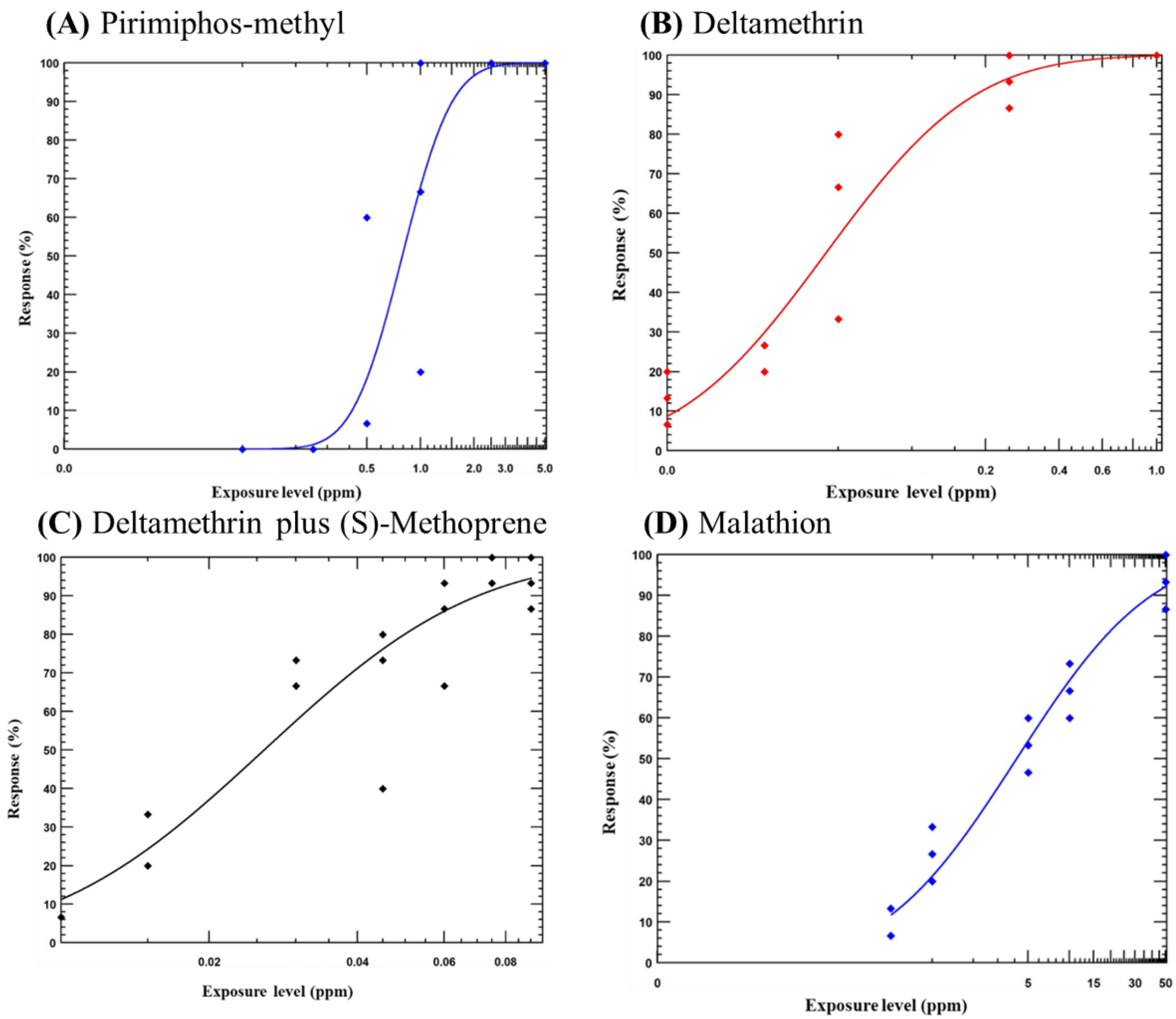

Figure 3. Toxicity response of *Tribolium castaneum* to selected pesticides: (A) pirimiphos-methyl, (B) deltamethrin, (C) deltamethrin plus (S)-methoprene, and (D) malathion (at 48h after treatment).

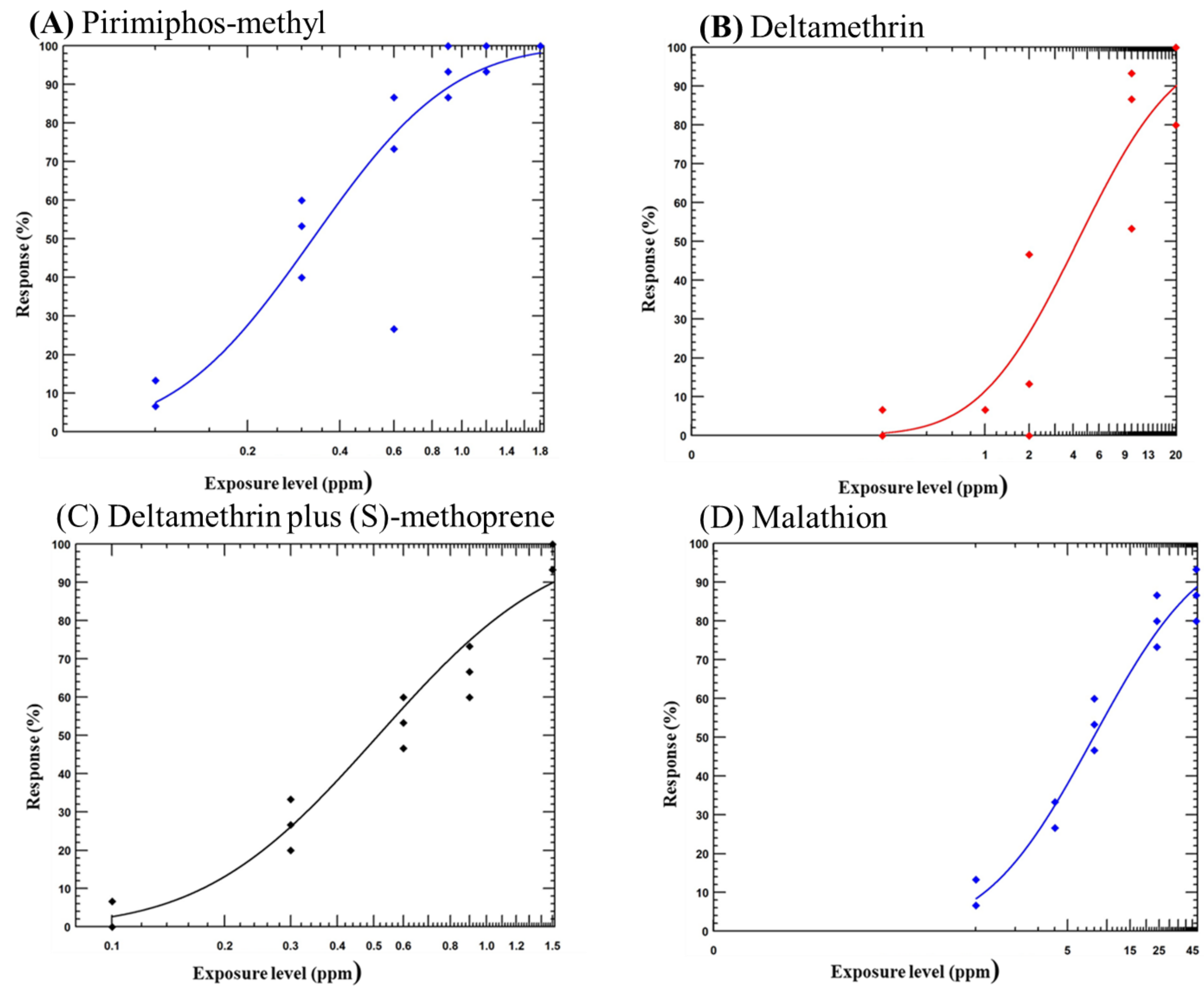

Figure 4. Toxicity response of *Tribolium confusum* to selected pesticides: (A) pirimiphos-methyl (after 24h treatment), (B) deltamethrin, (C) deltamethrin plus (S)-methoprene, and (D) malathion (at 48h after treatment).

References

Agarwal, Aparna, and Deepa Agashe. 2020. "The Red Flour Beetle *Tribolium castaneum*: A Model for Host-Microbiome Interactions." *PloS One* 15 (10). https://doi.org/10.1371/JOURNAL.PONE.0239051.

Aktar, Wasim, Dwaipayan Sengupta, and Ashim Chowdhury. 2009. "Impact of Pesticides Use in Agriculture: Their Benefits and Hazards." *Interdisciplinary Toxicology* 2 (1): 1. https://doi.org/10.2478/V10102-009-0001-7.

Andrić, Goran. 2006. "Effects of Several Contact Insecticides on Adults of Three Sitophilus Species Development of Integrated Management of Harmful Organisms in Plant Production in Order to Overcome Resistance and Improve Food Quality and Safety". Petar Kljajić Pesticide and Environment Research Institute." https://www.researchgate.net/publication/255607450.

Anusree R P, Berin Pathrose, and Mani Chellappan. 2023. "Malathion Resistance in Red Flour Beetle (*Tribolium castaneum*) (Herbst) (Coleoptera: Tenebrionidae) from FCI Godowns of Kerala, India." *Journal of Tropical Agriculture* 57 (2): 201–5. https://www.researchgate.net/publication/370198242_Malathion_resistance_in_red_flour_beetle_Tribolium_castaneum_Herbst_Coleoptera_Tenebrionidae_from_FCI_godowns_of_Kerala_India.

Athanassiou, Christos G, Nickolas G Kavallieratos, Frank H Arthur, and Christos T Nakas. 2021. "Rating Knockdown of Flour Beetles after Exposure to Two Insecticides as an Indicator of Mortality." *Scientific Reports 2021 11:1* 11 (1): 1–8. https://doi.org/10.1038/s41598-020-78982-z.

Athanassiou, Christos G., Nickolas G. Kavallieratos, and James F. Campbell. 2016. "Capture of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) in Floor Traps: The Effect of Previous Captures." *Journal of Economic Entomology* 109 (1): 461–66. https://doi.org/10.1093/JEE/TOV307.

Athanassiou, Christos G, Nickolas G Kavallieratos, and George J Chintzoglou. 2008. "Effectiveness of Spinosad Dust against Different European Populations of the Confused Flour Beetle, *Tribolium confusum* Jacquelin Du Val." *Journal of Stored Products Research* 44 (1): 47–51. https://doi.org/10.1016/J.JSPR.2007.05.001.

Attia, Manal A., Trandil F. Wahba, Nehal Shaarawy, Fathia I. Moustafa, Raul Narciso C. Guedes, and Youssef Dewer. 2020. "Stored Grain Pest Prevalence and Insecticide Resistance in Egyptian Populations of the Red Flour Beetle *Tribolium castaneum* (Herbst) and the Rice Weevil *Sitophilus oryzae* (L.)." *Journal of Stored Products Research* 87 (May). https://doi.org/10.1016/J.JSPR.2020.101611.

Balcom Chemicals Inc. 1975. "Malathion Big-6 Dust Label." US EPA Pesticide Product Label. Greeley, CO, USA. June 10, 1975. https://www3.epa.gov/pesticides/chem_search/ppls/000960-00202-19750610.pdf.

Baldwin, Rebecca, and Thomas R Fasulo. 2020. "Confused Flour Beetle, *Tribolium confusum* Jacquelin Du Val (Insecta: Coleoptera: Tenebrionidae) and Red Flour Beetle, *Tribolium* *castaneum* (Herbst) (Insecta: Coleoptera: Tenebrionidae) 1." *UF/ IFAS Extension #EENY289*. https://edis.ifas.ufl.edu.

Biddinger, D J, L A Hull, and E G Rajotte. 1998. "Stage Specificity of Various Insecticides to Tufted Apple Bud Moth Larvae (Lepidoptera: Tortricidae)." *Journal of Economic Entomology* 91 (1): 200–208. https://doi.org/10.1093/JEE/91.1.200.

Brown, Susan J, Teresa D Shippy, Sherry Miller, Renata Bolognesi, Richard W Beeman, Marcé D Lorenzen, Gregor Bucher, Ernst A Wimmer, and Martin Klingler. 2009. "The Red Flour Beetle, *Tribolium castaneum* (Coleoptera): A Model for Studies of Development and Pest Biology." *Cold Spring Harbor Protocols* 2009 (8): pdb.emo126. https://doi.org/10.1101/PDB.EMO126.

Buckman, Karrie A, James F Campbell, and Bhadriraju Subramanyam. 2013. "*Tribolium castaneum* (Coleoptera: Tenebrionidae) Associated with Rice Mills: Fumigation Efficacy and Population Rebound." *Journal of Economic Entomology* 106 (1): 499–512. https://doi.org/10.1603/EC12276.

Campbell, J F, and C Runnion. 2003. "Patch Exploitation by Female Red Flour Beetles, *Tribolium castaneum*." *Journal of Insect Science* 3 (1). https://doi.org/10.1093/JIS/3.1.20.

Central Garden & Pet Company. 2016a. "Diacon IGR Plus Label." Schaumburg, IL. November 2016. https://www.bugfreegrains.com/products/diacon-igr-plus.

———. 2016b. "Centynal EC Label." Schaumburg, IL. December 2016. https://www.bugfreegrains.com/products/centynal-ec-insecticide?gclid=Cj0KCQjw7aqkBhDPARIsAKGa0oIfSYlXJ4r4NkdmlUJvCxCAUHmOiC7QJEltx-gho0lRoRp8FFuIGwUaAvboEALw_wcB.

Chaubey, Mukesh Kumar. 2023. "Corresponding Red Flour Beetle, *Tribolium castaneum* (Herbst): Biology and Management." *International Journal of Zoology and Applied Biosciences* 8 (2): 11–21. https://doi.org/10.55126/ijzab.2023.v08.i02.002.

Damalas, Christos A, and Spyridon D Koutroubas. 2016. "Farmers' Exposure to Pesticides: Toxicity Types and Ways of Prevention." *Toxics* 4 (1). https://doi.org/10.3390/TOXICS4010001.

Davyt-Colo, Belén, Juan R Girotti, Andrés González, and Nicolás Pedrini. 2022. "Secretion and Detection of Defensive Compounds by the Red Flour Beetle *Tribolium castaneum* Interacting with the Insect Pathogenic Fungus *Beauveria Bassiana*." *Pathogens* 11 (5). https://doi.org/10.3390/PATHOGENS11050487.

Dowdy, Alan K., and Paul G. Fields. 2002. "Heat Combined with Diatomaceous Earth to Control the Confused Flour Beetle (Coleoptera: Tenebrionidae) in a Flour Mill." *Journal of Stored Products Research* 38 (1): 11–22. https://doi.org/10.1016/S0022-474X(00)00037-0.

Gao, Fuqiang, Yajing Qi, Alkassoumi Hassane Hamadou, Jiyao Zhang, Muhammad Faisal Manzoor, Qin Guo, and Bin Xu. 2022. "Enhancing Wheat-Flour Safety by Detecting and Controlling Red Flour Beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)." *Journal of Consumer Protection and Food Safety 2022 17:2* 17 (2): 113–26. https://doi.org/10.1007/S00003-022-01371-3.

Hawkins, Nichola J, Chris Bass, Andrea Dixon, and Paul Neve. 2019. "The Evolutionary Origins of Pesticide Resistance." *Biological Reviews of the Cambridge Philosophical Society* 94 (1): 135. https://doi.org/10.1111/BRV.12440.

Hussain, Aftab. 1993. "Chemical Ecology of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae): Factors Affecting Biology and Application of Pheromone." Oregon State University.

Jilani, G, R C Saxena, and B P Rueda. 1988. "Repellent and Growth-Inhibiting Effects of Turmeric Oil, Sweet Flag Oil, Neem Oil, and 'Margosan-o' on Red Flour Beetle (Coleoptera: Tenebrionidae)." *Journal of Economic Entomology* 81 (4): 1226–30. https://doi.org/10.1093/JEE/81.4.1226.

Klingler, Martin, and Gregor Bucher. 2022. "The Red Flour Beetle *T. castaneum*: Elaborate Genetic Toolkit and Unbiased Large-Scale RNAi Screening to Study Insect Biology and Evolution." *EvoDevo 2022 13:1* 13 (1): 1–11. https://doi.org/10.1186/S13227-022-00201-9.

Kumar, Harshit, Manjit Panigrahi, Supriya Chhotaray, V Bhanuprakash, Rahul Shandilya, Arvind Sonwane, and Bharat Bhushan. 2018. "Red Flour Beetle (*Tribolium castaneum*): From Population Genetics to Functional Genomics." *Veterinary World* 11 (8): 1043. https://doi.org/10.14202/VETWORLD.2018.1043-1046.

LaHue, Delmon W. 1975. "Pirimiphos Methyl as a Short Term Protectant of Grain Against Stored-Product Insects." *Journal of Economic Entomology* 68 (2): 235–36. https://doi.org/10.1093/JEE/68.2.235.

LeOra Software LLC. 2005. "PoloPlus 2.0." 2005. https://leora-software-company-poloplus.software.informer.com/2.0/.

Linz, David M, Alan W Hu, Michael I Sitvarin, and Yoshinori Tomoyasu. 2016. "Functional Value of Elytra under Various Stresses in the Red Flour Beetle, *Tribolium castaneum*." *Scientific Reports* 6 (October). https://doi.org/10.1038/srep34813.

Lis, L, T Bakula, M Baranowski, and A Czarnewicz. 2011. "The Carcinogenic Effects of Benzoquinones Produced by the Flour Beetle." *Polish Journal of Veterinary Sciences* 14 (1). https://doi.org/10.2478/v10181-011-0025-8.

Liu, Nannan. 2015. "Insecticide Resistance in Mosquitoes: Impact, Mechanisms, and Research Directions." *Annual Review of Entomology* 60 (January): 537–59. https://doi.org/10.1146/ANNUREV-ENTO-010814-020828.

Lü, Jianhua, Yafei Guo, and Zongwen Huang. 2022. "Influence of *Tribolium castaneum* (Herbst) Infestation on Farinographical Properties of Wheat Flour Dough." *Journal of Food and Nutrition Research, Vol. 10, 2022, Pages 175-179* 10 (3): 175–79. https://doi.org/10.12691/JFNR-10-3-1.

Mason, Linda J. 2018. "Red and Confused Flour Beetles *Tribolium castaneum* (Bhst.) and *Tribolium confusum* Duval Stored Product Pests Department of Entomology." www.extension.purdue.edu.

McGregor, Harrison E, and Karl J Kramer. 1975. “Activity of Insect Growth Regulators, Hydroprene and Methoprene, on Wheat and Corn against Several Stored-Grain Insects.” *Journal of Economic Entomology* 68 (5): 668–70. https://doi.org/10.1093/JEE/68.5.668.

Mensah, G. W. K., and F. L. Watters. 1979. “Comparison of Four Organophosphorus Insecticides on Stored Wheat for Control of Susceptible and Malathion-Resistant Strains of the Red Flour Beetle.” *Journal of Economic Entomology* 72 (3): 456–61. https://doi.org/10.1093/JEE/72.3.456.

Muhamad, Rita, and Hassan Sule. 2019. “Biology, Host Range and Management of Red Flour Beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionadae): A Review.” https://www.researchgate.net/publication/337824598.

Nicolopoulou-Stamati, Polyxeni, Sotirios Maipas, Chrysanthi Kotampasi, Panagiotis Stamatis, and Luc Hens. 2016. “Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture.” *Frontiers in Public Health* 4 (July): 1. https://doi.org/10.3389/FPUBH.2016.00148.

Paudyal, S, G P Opit, F H Arthur, G V Bingham, and S G Gautam. 2016. “Contact Toxicity of Deltamethrin against *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Sitophilus oryzae* (Coleoptera: Curculionidae), and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) Adults.” *Journal of Economic Entomology* 109 (4): 1936–42. https://doi.org/10.1093/JEE/TOW107.

Phan, Ngoc T, Neelendra K Joshi, Edwin G Rajotte, Margarita M López-Uribe, Fang Zhu, and David J Biddinger. 2020. “A New Ingestion Bioassay Protocol for Assessing Pesticide Toxicity to the Adult Japanese Orchard Bee (*Osmia cornifrons*).” *Scientific Reports 2020 10:1* 10 (1): 1–9. https://doi.org/10.1038/s41598-020-66118-2.

Pires, Evaldo Martins, Roberta Martins Nogueira, Leda Rita D Antonino Faroni, Marcus Alvarenga Soares, and Marco Antônio Oliveira. 2019. “Biological and Reproductive Parameters of *Tribolium castaneum* in Brazil Nut.” *Florida Entomologist* 102 (1): 76–78. https://doi.org/10.1653/024.102.0111.

Reina, J M, J A Duarte, L Carrilos, J D Bautista, and I Moreno. 2017. “Insecticide Reproductive Toxicity Profile: Organophosphate, Car-Bamate and Pyrethroids.”

Robertson, J L, R M Russel, H K Preisler, and N E Savin. 2007. *Bioassays with Arthropods*. 2nd ed. Boca Raton, FL, USA: CRC Press.

Robertson, J L, N E Savin, and H K Preisler. 2007. “Bioassays with Arthropods.” *Bioassays with Arthropods*, June. https://doi.org/10.1201/9781420004045.

Rumbos, Christos I, Anna Cristina Dutton, and Christos G Athanassiou. 2015. “Insecticidal Efficacy of Two Pirimiphos-Methyl Formulations for the Control of Three Stored-Product Beetle Species: Effect of Commodity.” https://doi.org/10.1016/j.cropro.2015.10.002.

Sakka, Maria, and Christos G Athanassiou. 2021. “Insecticidal Effect of Diatomaceous Earth and Pirimiphos-Methyl against Phosphine-Susceptible and Phosphine-Resistant Populations of Two Stored Product Beetle Species.” *Environmental Science and Pollution Research* 28 (25): 33181–91. https://doi.org/10.1007/S11356-021-12971-1/TABLES/9.

Singh, Shweta, and Sant Prakash. 2013. “Development of Resistance in *Tribolium castaneum*, Herbst (Coleoptera: Tenebrionidae) towards Deltamethrin in Laboratory.” *International Journal of Scientific and Research Publications* 3 (8). www.ijsrp.org.

Smith, Hugh, Adam Dale, and Julien Beuzelin. 2022. “Understanding Insecticide Modes of Action and Resistance Management in Florida Horticulture.” *EDIS* 2022 (6). https://doi.org/10.32473/EDIS-IN1379-2022.

Stack, C, ed. 2015. *Beetles: Biodiversity, Ecology and Role in the Environment*. Hauppauge, NY, USA: Nova Science Publishers Inc.

Tawfeeq Al-Ani, L K, M I Yonus, B A Mahdii, M A Omer, J K Taher, S F A Albaayi, and S B Al-Khoja. 2018. “First Record of Use *Fusarium Proliferatum* Fungi Indirect Treatment to Control the Adult of Wheat Flour *Tribolium confusum*, as well as Use the Entomopathogenic Fungi *Beauveria bassiana*.” *Eco. Env. & Cons* 24 (3): 1043–47.

Umar, A, U Shirama, and J M Turak. 2015. “Susceptibility of Two Maize Grain Flour to Infestation by the Rust-Red Flour Beetle (*Tribolium castaneum* Herbst).” *The Journal of Phytopharmacology* 4 (5): 259–62.

Velki, Mirna, Ivana Plavšin, Jelena Dragojević, and Branimir K Hackenberger. 2014. “Toxicity and Repellency of Dimethoate, Pirimiphos-Methyl and Deltamethrin against *Tribolium castaneum* (Herbst) Using Different Exposure Methods.” *Journal of Stored Products Research* 59: 36–41. https://doi.org/10.1016/j.jspr.2014.04.005.

Winfield Solutions LLC. 2015. “Actellic 5E Label.” US EPA Pesticide Product Label. St. Paul, MN, USA. January 26, 2015. https://www3.epa.gov/pesticides/chem_search/ppls/001381-00170-20150126.pdf.

Zettler, J L, and F H Arthur. 1997. “Dose-Response Tests on Red Flour Beetle and Confused Flour Beetle (Coleoptera: Tenebrionidae) Collected from Flour Mills in the United States.” *Journal of Economic Entomology* 90 (5): 1157–62. https://doi.org/10.1093/JEE/90.5.1157.

Chapter 4

Conclusion

In this study, we evaluated the toxicity of pirimiphos-methyl, deltamethrin, and deltamethin plus (S)- methoprene against *S. oryzae, T. castenum*, 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_{50} values, the recommended rate for pirimiphos-methyl exceeded its LC_{50}. 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_{50}. 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_{50} ratios ranging from

1/10 to ½ compared to *T. confusum*. We also assessed the current recommended pesticide application rates of these four insecticides based on their respective LC_{50} 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_{50} value for both species of flour beetles, and the rates for deltamethrin, deltamethrin plus (S)-methoprene, and malathion were higher than the LC_{50} value for *T. castaneum*. On the other hand, the recommended rate for deltamethrin was lower than the LC_{50} 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.