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Efficacy Determination of Commercial Deltamethrin-Treated Storage Bags on *Trogoderma granarium* Everts Adults and Larvae

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Abstract: *Trogoderma granarium* Everts, the khapra beetle, is a serious stored product pest known to feed on >100 different products worldwide and is a major threat to global food security. Deltamethrin-treated storage bags are a resource that could be used to limit infestations during storage of grain in bags. We investigated the efficacy of deltamethrin-treated bags against *T. granarium* adults and larvae. Deltamethrin-treated and untreated packaging materials were affixed into the bottom of plastic Petri dishes (62 or 137 cm^2) to create a bioassay arena. Adult *T. granarium* were exposed and observed to determine the time to knockdown and the subsequent mortality rate within 24 h. Adult *T. granarium* were knocked down in <60 min, and 100% of adults were knocked down or dead after 24 h. *Trogoderma granarium* larvae were exposed for 0.33, 1, 2, 3, or 4 d or continually exposed and monitored for larval death and adult emergence. Larvae exposed for 4 d had 50% mortality versus 97% if continually exposed. Utilizing this deltamethrin-treated packaging could cause disruptions in natural populations of *T. granarium* found in storage facilities, and the treated packaging is an effective tool that could be implemented into an integrated pest management program for bagged grain.

Keywords: khapra beetle; treated packaging; deltamethrin; bagged storage; ZeroFly®

1. Introduction

Trogoderma granarium Everts, the khapra beetle, is a cosmopolitan stored product insect that has been classified as one of the top 100 worst invasive species in the world [1]. *Trogoderma granarium* can feed on >100 different food products found throughout the entire supply chain from raw grains to processed food products [2,3]. This species presents a major threat to global food security because of the number of products the species can feed on and the ability to survive for extended periods of time (months to years) under extreme conditions, such as extreme high and low temperatures, low relative humidity (r.h.) levels, lack of food resources, and low moisture content (m.c.) products [3,4]. For the top cereal grain producers in the world, including the United States (US), Canada, Australia, and Russia, *T. granarium* is one of the most important quarantine pests. The potential establishment of *T. granarium* in these countries would cause significant economic damage from commodity losses. Over a 25-year span in the US (1985–2010), *T. granarium* was intercepted 559 times at US ports of entry, originating from 43 different countries [5]. Often interceptions occurred in general cargo, mail, ship holds and stores, and various commodities [5]. An early *T. granarium* infestation that originated in California in 1946 took 7 years to correctly identify to species level [6]. By 1958, approximately

51,000 locations in 27 different states had been inspected for *T. granarium*, and it took until 1966 to fully eradicate it at a cost of nearly $15 million to the US federal government and private property owners [7]. Additional sources of *T. granarium* interceptions in the US occur from unsuspecting travelers bringing dried food products back to the US in their baggage after trips to countries with established *T. granarium* populations. Products include dry fava beans, dry coriander seeds, and dried dates from Sudan, India, and Turkey [8]. *Trogoderma granarium* larvae have also been found feeding on non-grain based sources such as glue on decorative feathers [9]. Therefore, importing countries without established *T. granarium* populations need to be particularly vigilant on their importation regulations and survey/trapping protocols to prevent future infestations.

Outside of the US and other major cereal grain producers, *T. granarium* populations have become established in 34 different countries in Europe, Asia, and Africa [4]. *Trogoderma granarium* causes serious damage to stored products in these countries. Larvae will cause damage to stored grains due to consumption and contaminations, but also because they will leave behind cast skins, body parts, and frass, which sometimes causes serious allergic reactions in sensitive individuals. In many countries in the world where *T. granarium* is established, grain is often stored in bulk bags instead of bulk bins, silos, or elevators [10] where grain can be fumigated, or grain protectants can be used to control stored product insect infestations. However, due to larval *T. granarium*'s ability to enter diapause, often long exposure times or high insecticidal doses are required to achieve control. As a result, non-traditional methods of controlling *T. granarium* infestations in stored grain are needed.

Recent investigations into the use of insecticide-treated packaging for control of stored product insect infestations showed that both deltamethrin-treated and methoprene-treated packaging materials could be used to control populations of multiple stored product insect species [10–15]. The knockdown time was 79 min for 99% of *Tribolium castaneum* Herbst, red flour beetle, adults exposed on a long-lasting deltamethrin-treated (3 g/kg) packaging material (ZeroFly® Storage Bag, Vestergaard Frandsen Inc., Lausanne, Switzerland) [12]. Continually exposing *T. castaneum* adults for 48 h or longer on the deltamethrin-treated packaging material resulted in no progeny production [14] or 100% mortality after 5 d of exposure [12]. Furthermore, 100% knockdown of *Trogoderma variabile* (Ballion), warehouse beetle, *Prostephanus truncatus* (Horn), larger grain borer, and *Rhyzopertha dominica* (F.), lesser grain borer, occurred after 1 h of exposure to treated packaging material [10]. Recent field studies investigating the use of deltamethrin-treated packaging to store maize in Ghana showed the deltamethrin-treated bags were effective at suppressing insect levels of *Sitophilus* spp., *Tribolium* spp., and *Cryptolestes* spp. to less than 10, 2, and 15 live insects/250 g of maize, respectively, after up to 6 months of storage [11]. The use of the insect growth regulator (IGR) methoprene in packaging has been shown to significantly reduce adult emergence of *T. granarium* when applied to Kraft paper or incorporated into layers of polyethylene-polyethylene, polyethylene terephthalate-polyethylene, and laminated woven packaging material [15]. The combination of these recent studies on treated packaging material has demonstrated great progress in non-traditional methods of controlling stored product insect infestations of bagged grain. However, further studies investigating the effect of long-lasting deltamethrin-treated packaging material are needed, especially on invasive *T. granarium* larvae and adults. Therefore, the objective of this study is to determine the efficacy of a commercially available deltamethrin-treated packaging material on *T. granarium* adults and larvae.

2. Materials and Methods

2.1. Insects

This research study was conducted at the United States Department of Agriculture Animal and Plant Health Inspection Service, Science and Technology, Otis Laboratory, Buzzards Bay, MA, USA. *Trogoderma granarium* adults and larvae used in this study were a field strain collected from Pakistan in 2011. Colonies were reared on a diet consisting of 160 g ground dog food, 20 g wheat germ, and 20 g of rolled oats sprinkled on the surface (4:1:1 ratio). *Trogoderma granarium* colonies were reared at 30 °C in continuous darkness in the Otis laboratory quarantine facility.

2.2. Packaging Material

ZeroFly® Storage Bags, which consist of long-lasting deltamethrin-treated woven polypropylene, were used in all the tests. Deltamethrin was incorporated into the polypropylene strands during the extrusion process at a concentration of approximately 3000 ppm. Untreated ZeroFly® Storage Bags are not manufactured by Vestergaard, due to cross-contamination issues. Therefore, an untreated laminated woven polypropylene used in previous packaging studies [16] were used as a control packaging for all experiments.

Individual arenas, 100 × 20 mm standard (62 cm^2) plastic Petri dishes, were constructed out of the deltamethrin-treated and untreated packaging as previously described [14]. Briefly, round discs were cut and secured to the bottom portions of these dishes by gluing down the disc edges with adhesive caulking (DAP Kwik Seal®, DAP Products Inc., Baltimore, MD, USA), and the internal sides of the Petri plate were coated with Fluon® (polytetrafluoroethylene, Sigma-Aldrich Co., St. Louis, MO, USA) to create the final testing arenas.

2.3. Effect of Deltamethrin on Adult *T. granarium*

The effect of contact exposure on *T. granarium* adults was studied as previously described [12]. Ten *T. granarium* adults were placed into arenas (five treated or three untreated) and monitored every 15 min under a stereo microscope until 100% of adults were knocked down. Knocked down individuals were those observed on their backs, with twitching legs and antennae, or otherwise unable to sustain coordinated movement [17]. After knockdown was determined, arenas were placed into an environmental chamber at 30 °C for 24 h, when final observations were conducted to determine if insects were live vs. affected. Adults classified as affected at 24 h were knocked down and/or dead (no movement when gently probed) at the time of observation. This sequence of observations was repeated three times for a total of 15 treatment replicates and 10 untreated replicates. The data with respect to live and affected adult *T. granarium* were analyzed for significant differences under the general linear model (GLM) procedure in SAS (Version 9.4, 2012, SAS Institute Inc., Cary, NC, USA) at $p < 0.05$.

2.4. Effect of Short-Term Exposure of *T. granarium* Larvae

The effect of short-term exposure of *T. granarium* larvae on the treated packaging builds upon previous studies with methoprene-treated packaging [13]. Six treated packaging arenas and six untreated packaging arenas were constructed using a large 150 × 20 mm (137 cm^2) plastic Petri dish. Treated and untreated packaging materials were cut and affixed to the bottom portion of the large Petri dish to create bioassay arenas using adhesive caulking and Fluon®. Sixty untreated transfer arenas were also created by affixing filter paper to the bottom of a 100 × 20 mm (62 cm^2) plastic Petri dish using adhesive caulking and Fluon®.

Fifty *T. granarium* larvae, 4–5 mm in length, were added to the six treated arenas and six untreated arenas (137 cm^2), along with ~1 g of the standard rearing diet. Arenas were placed inside an environmental chamber at 30 °C. After 0.33 d (8 h), ten larvae from each arena (treated and untreated packaging) were selected at random, removed from the arena, and transferred to new untreated Petri dishes, in which ~500 mg of fresh diet was placed. All treated and untreated arenas were then placed back inside the environmental chambers. Similar transfers were repeated for 1, 2, 3, and 4 d of exposure on the treated or untreated packaging. At each time period, ten larvae were removed and placed into untreated arenas with a fresh diet. Larvae were held for 3–4 wks and monitored weekly for adult emergence.

Data for adult emergence and dead larvae were transformed to angular values for statistical analysis [18]. Data on the percentage of adults and dead larvae were analyzed for significant differences by employing the GLM procedure in SAS (SAS Institute, version 9.4, 2012) and were subjected to a two-way analysis of variance (ANOVA) with treatment and length of exposure as the main effects. Significance was determined at $p < 0.05$.

2.5. Effect of Continual Exposure of T. granarium Larvae

Twenty-four arenas (62 cm^2) were constructed, half out of deltamethrin-treated (12 arenas) and half out of untreated (12 arenas) packaging materials as previously described. *Trogoderma granarium* larvae were classified into two categories: (a) medium larvae (2–3 mm in length) and (b) large larvae (4–5 mm in length). Ten medium larvae were added to six treated and six untreated arenas, along with ~1 g of the diet and held in an environmental chamber at 30 °C for 8 wks. Ten large larvae were similarly added to six treated and six untreated arenas and given ~1 g of the diet and held in an environmental chamber for 8 wks. Larval mortality and adult emergence were monitored weekly. Larvae that appeared black and shriveled (Figure 1) were classified as larval mortality.

Figure 1. Dead *Trogoderma granarium* larvae exposed on deltamethrin-treated packaging material for ~4 weeks. Dead larvae appear dark brown/black in color and shriveled in appearance near the edge of the treatment arena.

The mean percentage and standard errors (SE) for adult emergence and dead larvae were transformed to angular values for statistical analysis [16]. Data on the percentage of adult and dead larvae were analyzed for significant differences under the general linear model (GLM) procedure in SAS (SAS Institute, version 9.4, 2012) at $p < 0.05$.

3. Results

3.1. Effect on Adult Beetles

Trogoderma granarium adults were highly susceptible to the deltamethrin-treated packaging material, with 92% knockdown before 15 min and 100% knockdown within 60 min. After 24 h of exposure on the treated packaging, 100% of adult *T. granarium* were affected compared to 10% of adults on the untreated packaging ($F = 1043.59$; $df = 1, 23$; $p < 0.001$). Specifically, *T. granarium* knockdown was 52% and mortality was 48% on deltamethrin-treated packaging after 24 h. The 10% of affected adults on untreated packaging was expected from natural mortality. Adult beetles are short-lived [4] and being selected from colony jars, the age range of those adults varied.

3.2. Effect of Short-Term Exposure of T. granarium Larvae

As the length of exposure on the deltamethrin-treated packaging increased, the percentage of adult emergence decreased. Adult emergence on larvae exposed on the treated packaging material ranged from 34–61%, with the lowest percent adult emergence occurring after 3 d of exposure (Figure 2). However, the main effects of treatment ($F = 1.51$; df = 1.50; $p = 0.2247$) and exposure length ($F = 1.09$; df = 4, 50; $p = 0.3716$) were not significant. The interaction between treatment and exposure length was also non-significant ($F = 0.56$; df = 4, 50; $p = 0.6916$).

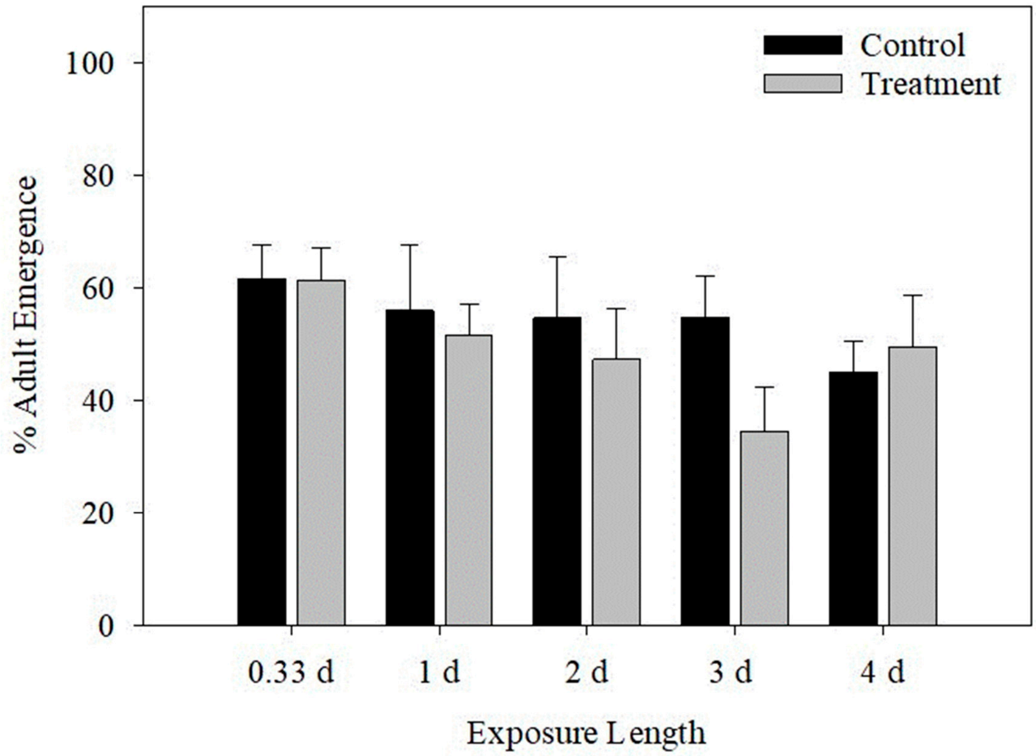

Figure 2. Percent (mean ± SE) adult emergences from *T. granarium* larvae exposed on control (black bars) or deltamethrin-treated packaging (gray bars) for 0.33–4 days.

Though there was no significant reduction in adult emergence for *T. granarium* larvae exposed on deltamethrin-treated packaging for short-term intervals, there was a significant increase in the percentage of dead larvae after brief exposures to the treated packaging (Figure 3). As the length of exposure on the treated packaging increased, the percentage of dead larvae also increased. Both main effects of treatment ($F = 92.35$; df = 1, 50; $p < 0.0001$) and exposure time ($F = 4.29$; df = 4, 50; $p = 0.0046$) were significant, but their interaction was not significant ($F = 1.17$; df = 4, 50; $p = 0.3341$).

Treatment comparisons at each exposure length, 0.33 d to 4 d, were all significantly ($F_{range} = 12.37–32.28$; df = 1, 10; $P_{range} = 0.0002–0.0056$). Dead larvae exposed on the deltamethrin-treated packaging ranged from 15% to 50% but were not statistically different among the exposure times ($F = 2.39$; df = 4, 25; $p = 0.0778$). Similarly, the longer exposure periods of larvae on the untreated material resulted in an increase in dead larvae but did not exceed 10%. Ultimately, the deltamethrin-treated netting had a substantial impact on larval development during a short-term exposure.

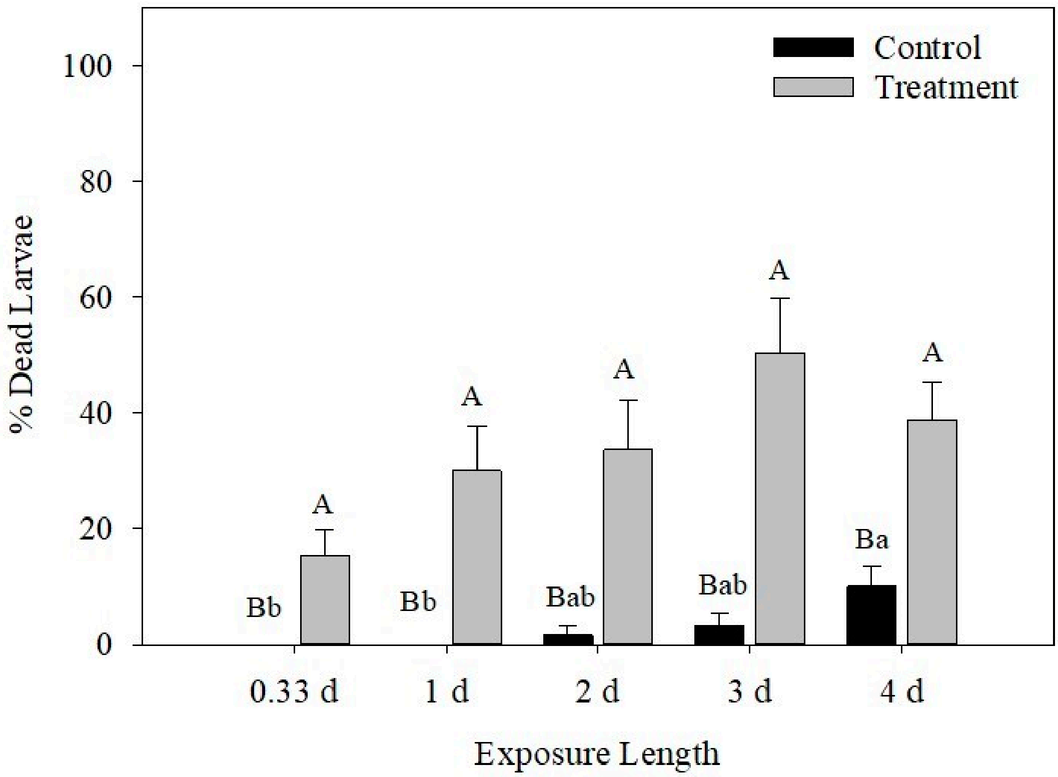

Figure 3. Percent (mean ± SE) dead larvae after exposure to deltamethrin-treated (gray bars) or untreated (black bars) packaging material for 0.33–4 days. Different uppercase letters among control and treatment means are significantly different at each exposure length ($p < 0.05$; by Ryan–Einot–Gabriel–Welsch (REGWQ) multiple range test). Different lowercase letters among all exposure lengths are significantly different among control packaging ($p < 0.05$; by RGEWQ).

3.3. Continual Exposure of Larvae

The continual exposure of *T. granarium* larvae on deltamethrin-treated packaging had a significant effect on adult emergence for both medium and large larvae. Medium larvae held on the treated packaging had <2% adult emergence compared with 25% adult emergence on the untreated packaging ($F = 30.31$; df = 1, 10; $p = 0.0003$). There was <4% adult emergence from large larvae exposed on the treated packaging compared to 55% on the control packaging ($F = 60.93$; df = 1, 10; $p < 0.0001$). The most impactful differences were in the percent of dead larvae, medium and large, observed on the deltamethrin-treated packaging material versus the control. For both species, 97% of larvae died when exposed to the treated material, compared with <5% on the control packaging.

Many medium and large larvae in the untreated packaging arenas, 72% and 40% respectively, did not emerge as adults, indicating diapause. For treated packaging, this percentage was <2% for both larval sizes. Conducting bioassays indicative of developmental progression with *T. granarium* larvae proves to be difficult because of the tendency of larval diapause to occur [19]. However, the larvae that were in diapause on the untreated packaging material were alive and healthy compared to the dead larvae found in the treatment arenas. Additionally, since the percentage of diapaused larvae on the treatment arenas was <2% among both larval stages, the effect of the deltamethrin- treated packaging was clearly evident by the larval mortality on the treated packaging.

4. Discussion

Adult *T. granarium* were highly susceptible to the deltamethrin-treated packaging material, which is similar to the reported susceptibility of *T. variabile* Ballon, warehouse beetle, exposed on the same deltamethrin-treated material [10]. In that study, *T. variabile* adults were knocked down after

60 min of exposure and 24 h mortality was ~60% after 24 h of exposure on the treated material [10]. The time to knockdown for both *Trogoderma* species was faster than previously reported times for *Sitophilus oryzae* (L.), rice weevil, and *T. castaneum*. The KD_{99} was 143 and 79 min for *S. oryzae* and *T. castaneum*, respectively [12]. *Rhyzopertha dominica* and *P. truncatus* were observed to be knocked down after 60 min of exposure, but actual time to knockdown was not observed for either species [10]. In combination with previous results, our results demonstrate the wide range of stored product insect species that are highly susceptible to deltamethrin-treated and methoprene-treated packaging. The deltamethrintreated packaging material in this study was more lethal to *T. granarium* adults and larvae compared to woven polypropylene (WPP), biaxially oriented polypropylene (BOPP), and Kraft paper (KF) sprayed with 0.05 mg (active ingredient, AI)/cm^2 deltamethrin insecticide (K-Othrine WG with 25% deltamethrin (AI)) on one side of the packaging material [18]. Adult mortality after 24 h in the current study was 48% compared to 7%, 11% and 4% for the WPP, BOPP, and KP, respectively [20]. The incorporation of the deltamethrin directly into the packaging material is a much more effective measure than spraying an insecticide on untreated packaging material. Mortalities of *T. granarium* exposed on WPP, BOPP, and KP increased up to 66%, 67%, and 72%, respectively, after 5 d of exposure [20]. The current study only observed adult *T. granarium* after 24 h, but we propose that if the length of exposure on the treated packaging increased further, the percentage of dead adults would increase because the knocked down adults would succumb to the effects of the insecticide. However, the concentration of deltamethrin is higher in the commercial packaging (3000 ppm) compared to the amount sprayed on the packaging (0.05 mg (AI)/cm^2) in accordance with the label rate of the insecticide. Higher dosages of the deltamethrin could account for the rapid knockdown and mortality at 24 h. The results from both these studies demonstrated that the application of deltamethrin, topically applied to packaging or incorporated into the packaging material, may not be directly comparable because of the way the insecticide is applied, the release rate of the deltamethrin, and the different ways in which the insect picks up the insecticide [21]. Nevertheless, both materials cause delayed mortality in adults and larvae that would disrupt natural populations and could be implemented into an integrated pest management program for bagged grain.

During the entire storage life of bagged grain, the storage bags could be moved around within a warehouse, shipped in containers across oceans or between countries, or opened, and the contents could be removed/mixed, etc. Throughout this storage time, multiple stored product insect species, both adults and larvae, could encounter the treated storage bags in search of food or refuge. The length of contact could be short, if the bags are moved and the adults become dislodged from the packaging. The results of this study demonstrated fast knockdown of adult *T. granarium*, in <60 min, when exposed to the deltamethrin-treated material. Additionally, adult beetles exposed to deltamethrin-treated surfaces have shown significant effects on distance walked, velocity, and stationary time [21,22]. Therefore, if sublethal exposure times were to occur, it is hypothesized that delayed effects on *T. granarium* adult movement could occur. Larvae that encounter these treated storage bags may try to chew through the material and, if unable to do so, could move to a different food source and thus only be exposed to the treated material for a short time. We have shown that larvae exposed for <4 d would have ~50% delayed larval mortality and longer continual exposures will increase mortality. These delayed effects on adults and larvae of *T. granarium* are important if these insects are moved (intentionally or unintentionally) from the treated packaging material. There will still be an impact on resident populations due to the delayed mortality. Further evaluation of the deltamethrin-treated packaging in semi-field or field conditions is needed to obtain a comprehensive understanding of the impact on resident insect populations and how grain quality is maintained throughout the storage periods. We hypothesize that if implemented into an integrated pest management program, the deltamethrin-treated packaging could mimic a broader surface treatment of the storage room, suppress pest populations, and limit infestations. The current material is not hermetic, so air exchange will occur. Therefore, evaluations of grain quality parameters such as insect damaged kernels or compositional parameters of protein, lipid, and starch content over storage periods of up to 12 months are needed to fully comprehend the significance of this treated packaging on stored products.

5. Conclusions

The commercial deltamethrin-treated packaging material is highly effective on *T. granarium*, which is consistent with other results from previous studies with stored product insects. This treated packaging is a methodology that countries with *T. granarium* populations, as well as other stored product insects, could implement into their bagged stored grain program to protect the stored grains from infestations. This packaging could aid in preventing transportation of this invasive pest species between villages, cities, and countries. Implementing this type of packaging into an extensive integrated pest management (IPM) program would be another tool in the multi-hurdle or holistic approach to IPM as an important control strategy in the supply chain. These treated bags would help protect grain imports and exports and combat the global food security concerns from the spread of the invasive *T. granarium*.

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Conflicts of Interest: The authors declare no conflict of interest.

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