



Article Five Surfaces Treated with d-Tetramethrin plus Acetamiprid for the Management of Tenebrio molitor and Alphitobius diaperinus: Which Is the Best?

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Simple Summary: This study evaluated the minimum as well as the maximum label dose of dtetramethrin plus acetamiprid applied on plastic, glass, metal, wood, and ceramic surfaces, against adults of both species, in terms of immediate and delayed mortality. All T. molitor died after a 7-day exposure to the maximum dose at all tested surfaces when food was absent. In the presence of food, only glass-, metal-, and ceramic-treated surfaces killed all adults at the same dose. Even the minimum dose provided 100% mortality on glass, metal, and ceramic surfaces in both food scenarios. In the case of A. diaperinus, only the maximum dose caused the death of all exposed individuals on glass (with food) and on glass and plastic (no food). Overall, the maximum dose of d-tetramethrin plus acetamiprid is effective against the tested species on certain types of surfaces.

Abstract: Tenebrio molitor L. (Coleoptera: Tenebrionidae) and Alphitobius diaperinus Panzer (Coleoptera: Tenebrionidae) are two common tenebrionids occurring in grain storages. In this study, we assessed the immediate and delayed mortalities caused by d-tetramethrin plus acetamiprid on five different surfaces, i.e., plastic, glass, metal, wood, and ceramic, against adults of the two species. The tests included two label doses of the insecticide (minimum and maximum) and two food scenarios (food and no food). Generally, the maximum dose was more efficient than the minimum dose, and the presence of food resulted in lower observed mortalities than when food was absent. Tenebrio molitor was more susceptible than A. diaperinus, at all dose, food, and surface scenarios. At delayed bioassays, both doses killed all T. molitor on plastic, while on wood, mortality ranged between 80.6 and 100.0%, regardless of the food scenario. Concerning A. diaperinus, delayed mortalities ranged among treated surfaces, food scenarios, and dose from 58.3 to 100.0%. The insecticide killed the most individuals when it was treated on glass, while when it was applied on wood caused the death of the least individuals. Concerning plastic, metal, and ceramic surfaces, no general trend was observed. The maximum dose of the tested insecticide provides elevated mortalities for both species when food is absent.

Keywords: plastic; glass; metal; wood; ceramic; surface treatment; yellow mealworm; lesser mealworm

1. Introduction

Tenebrio molitor L. (Coleoptera: Tenebrionidae) is an important and noxious storedproduct insect related to poor storage conditions and hygiene [1]. Apart from the direct consumption of the grains during their storage, T. molitor contaminates the products with its excrements and dead body particles, decreasing food quality [2]. This species can provoke allergic reactions, such as respiration problems and eczema, commonly appearing in people handling storage foods [3–8]. Lately, T. molitor has been in the spotlight since it is used by the food and recycling industry [9-19]. Its insect powder enhances food properties such as nutritional value, crispness, taste, and digestibility [9,12,16,18]. Apart from human nutrition, this species is used as feed for a plethora of organisms such as fish,



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reptiles, birds, and crustaceans [20–24]. In addition, recent studies revealed that *T. molitor* extracts display antiproliferative, antimicrobial, antifreeze, antithrombotic, preservative, and healing properties [25–29].

One other harmful insect worldwide is *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) [20,30]. It infests more than 70 different stored products [30], while at the same time is really prevalent in farms where livestock is raised feeding on dead animals such as mice and birds [1]. This species causes a huge problem at poultry facilities [20,30–32]. *Alphitobius diaperinus* has a short developmental period at optimal conditions and is long-lived [1]. Due to the continuous food presence and the prevailing temperature range at poultry houses, *A. diaperinus* completes its life circle in short periods of time, supporting large populations [33]. Furthermore, chickens consume *A. diaperinus* individuals that may be infected by viruses, bacteria, protozoa, and fungi, since this species is a vector of multiple pathogens [34–39]. These microorganisms can also harm humans, causing symptoms such as vomiting, fever, diarrhea, and cramps in the abdomen [40–42]. Employees exposed to these insects can display allergic reactions such as asthma, rhinitis, urticaria, conjunctivitis, and angioedema [43–45]. Apart from the health problems it provokes, it damages the structure of facilities by consuming the insulation of walls and ceilings [46].

Given the fact that both pests are enemies of major importance, several insecticides, mainly synthetic chemicals, i.e., neonicotinoids, organophosphates, pyrethroid compounds, and synergized pyrethrins, as well as insecticides of natural origin, i.e., essential oils, diatomaceous earths, and nanoemulsions, have been utilized over the years in order to handle possible invasions [2,47–58]. Among all these studies, only a few of them examined the effectiveness of the insecticides applied on surfaces. In particular, Athanassiou et al. [48] studied the efficacy of α -cypermethrin and thiamethoxam applied on concrete against *T. molitor* adults, while Kavallieratos et al. [56,57] examined chlorfenapyr and deltamethrin sprayed on concrete surfaces against *A. diaperinus* adults. In the case of *A. diaperinus*, the tests investigated the impact of the presence/absence of food on the treated surfaces [56,57], whereas, regarding *T. molitor*, all tests included food [48]. Furthermore, Lyons et al. [49] experimented with permethrin and β -cyfluthrin treated on pressure-treated wood, particle board (wood-chip-type), and concrete to control *A. diaperinus* adults.

The formulation consisting of the two active ingredients (a.i.s) (i) d-tetramethrin (pyrethroid insecticide) and (ii) acetamiprid (neonicotinoid insecticide), and the insecticide synergist piperonyl butoxide, is used for surface treatments [55,59–61]. Previously, the efficacy of this formulation was assessed against small/large larvae of *A. diaperinus*, with/without food, on concrete surfaces [55]. This mixture of a.i.s was more effective against small larvae than large larvae, but its effectiveness against adults of the species is yet to be discovered.

Given that insect farms, storage units, and poultry houses consist of numerous surfaces [20,62], there are no data available considering the impact of plastic, glass, metal, wood, and ceramic surfaces on the effectiveness of d-tetramethrin plus acetamiprid against adult individuals of *T. molitor* and *A. diaperinus*, to reveal the optimal surface for treatment. Therefore, in this study, the immediate and delayed mortality of the minimum and maximum label dose of the aforementioned formulation was assessed. To delve deeper, the parameter of food presence/absence was taken into consideration.

2. Materials and Methods

2.1. Insects and Food

The two tested tenebrionids were bred at the Laboratory of Agricultural Zoology and Entomology (Agricultural University of Athens, Athens, Greece). Both were laboratory cultures derived from Laboratory of Agricultural Entomology (Benaki Phytopathological Institute, Kifissia, Attica, Greece). The rearing mediums were wheat bran with potato, or apple cuts for extra moisture, for *T. molitor* and *A. diaperinus*, respectively. Both species were kept under the following conditions: 30 °C, 65% relative humidity (RH), and complete light absence [53,63,64]. The participating tenebrionids were taken randomly from the colonies.

The adults were younger than 14 or 7 days old (days since their emergence from pupae) for *T. molitor* and *A. diaperinus,* respectively.

2.2. Insecticide

Dobol[®] EC was used to treat the tested surfaces. This insecticide consists of d-tetramethrin [3,4,5,6-tetrahydrophthalimidomethyl (1RS)-cis-trans-chrysanthemate] (2.5% w/v) plus acetamiprid [N-[(6-chloropyridin-3-yl)methyl]-N'-cyano-N-methylethanimidamide] (5% w/v), and piperonyl butoxide (3,4-methylenedioxy-6-propylbenxyl n-butyl diethyleneglycol ether) (10% w/v) a.i.s (Société Kwizda France, Marly le Roi, France). There are two label doses tested for surface applications: (i) minimum (min), i.e., 0.0001 mL formulation/cm², and (ii) maximum (max), i.e., 0.0002 mL formulation/cm².

2.3. Bioassays

For the experiment, surfaces of plastic, glass, metal, wood, and ceramic were treated with the aforementioned doses of insecticide. For this purpose, three replicates of Petri dishes, consisting of three subreplicates each, were prepared. The dishes were plastic (in the cases of plastic, metal, wood, and ceramic surfaces) or glass (in the case of glass surfaces) and had the following dimensions: 1.5 cm height; 8 cm diameter. Metal, wood, and ceramic surfaces were cut from galvanized metal sheets (1 mm thick), pieces of plywood (4 mm thick), and ceramic tiles (6 mm thick), respectively, purchased from local stores. The type of plastic that dishes were made from was polystyrene. Concerning plastic and glass, insecticides were applied directly to the dishes, while metal, wood, and ceramic pieces were cut to fit perfectly (<50.27 cm² area) into plastic dishes. The internal vertical dish surface of each dish (plastic or glass) was coated with polytetrafluoroethylene, acquired from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). This coat prevented tenebrionids from escaping. Lids had a circular cut (1.5 cm diameter); thus, dishes were fully aerated. This cut was covered with cloth. Each surface/dish was sprayed with 1 mL volume that contained the desired quantity of each tested dose. The spraying was conducted with an airbrush (AG-4 Mecafer S.A., Valence, France) in a fine mist. Afterward, a balance (Precisa XB3200D, Alpha Analytical Instruments, Gerakas, Greece) was used to weigh quantities of 0.5 g, derived from the diet of the tested tenebrionids, which was finally spread into the treated dishes. The above procedure was repeated, but this time no diet was introduced into the dishes. As controls, additional series of dishes (of each type of surface) were sprayed with distilled water. The control series were repeated twice, once with and once without diet. Two hours after spraying, 10 individuals were inserted in each dish and transferred into 30 °C/65% RH incubators, for the entire duration of the experiments. After 1, 3, 5, and 7 days, mortality of both tenebrionid species was counted with a stereomicroscope (Olympus SZX9, Bacacos S.A., Athens, Greece). For the insect inspection, a brush was used to gently poke the individuals. No movement detection meant that insects were dead. After a 7-day exposure to the treated surfaces, all living insects were transferred to non-treated surfaces for 7 additional days to evaluate delayed mortality. Adults that were exposed to a certain type of treated surface were put into dishes with the same type of non-treated surface. Furthermore, adults exposed to the insecticide with diet into the dishes were conveyed to non-treated dishes with new 0.5 g diet. Similarly, adults exposed to the insecticide with no diet on the dishes were conveyed to non-treated dishes with no diet. This procedure was followed to evaluate delayed mortality for controls. Mortalities were counted as above, at the end of the experiment.

2.4. Data Analysis

Both mortality (immediate and delayed) control values were lower than 5% for the two tenebrionid species. Therefore, no corrections were made to the mortality data, but they were log (x + 1) converted in advance of the analysis to ensure the variance normalization [65,66]. Each species was submitted to a separate repeated-measures multivariate analysis of variance (MANOVA) [67]. The repeated factor was exposure, response variable

was mortality, and main effects were dose, surface, and presence/absence of food. Associated interactions (of main effects) were incorporated into the analysis. For each species, delayed mortality data were separately analyzed with a two-way ANOVA, separately for each tested dose. The response variable was mortality, whereas surface and food presence/absence were the main effects. The Tukey–Kramer test (HSD) or the two-tailed *t* test assorted means at 0.05 level of significance were used [68]. The JMP 16.2 software was used for all analyses [69].

3. Results

3.1. Immediate Mortality of Tenebrio molitor

Between exposure intervals, all main effects and food \times surface interaction were significant (Table 1). Concerning the intervals within exposure, all main effects were significant. Exposure \times food \times surface and exposure \times dose \times food \times surface interactions were also significant. On the first day of the experiment, mortalities of the minimum dose of d-tetramethrin plus acetamiprid ranged from 0.0% (applied on metal) to 8.9% (applied on glass) when food was present (Table 2). By the end of the week, this dose killed all T. molitor adults when applied on glass, metal, and ceramic surfaces. Meanwhile, plastic and wood surfaces treated with this dose provided 96.7 and 87.8% mortality, respectively, in the same food scenario. The minimum dose caused significantly variable mortalities on the first day of exposure (1.1-37.8%) on surfaces without food. After 5 days, the treated glass and ceramic surfaces caused the death of all exposed adults. By the end of the experiments, plastic and metal killed all T. molitor individuals, while wood provided 92.2% mortality at the same exposure interval. Glass and ceramic surfaces treated with the maximum dose of the insecticide caused the death to all exposed adults after a 5-day exposure in the presence of food. The same dose and food scenario killed all adults exposed to metal after 7 days, while plastic and wood-treated surfaces reached 97.8 and 94.4% mortality, respectively. Similarly, the maximum dose, when food was absent, killed all individuals exposed to treated glass and ceramic surfaces after 5 days. All T. molitor adults were dead on treated plastic, metal, and wood 7 days post-exposure in the same food and dose scenario.

Table 1. MANOVA parameters for main effects and associated interactions for the immediate mortality of *Tenebrio molitor* and *Alphitobius diaperinus* adults between and within exposure intervals (error DF = 160).

Effect	Te	nebrio moli	itor	Alphitobius diaperinus						
Between exposure intervals										
Source	DF	F	р	F	р					
Intercept	1	23,297.2	< 0.01	18,841.7	< 0.01					
Dose	1	18.5	< 0.01	64.9	< 0.01					
Food	1	38.6	< 0.01	28.4	< 0.01					
Surface	4	57.0	< 0.01	78.2	< 0.01					
$Dose \times food$	1	0.48	0.49	0.1	0.72					
Dose \times surface	4	1.0	0.43	3.6	0.01					
Food \times surface	4	2.7	0.04	1.1	0.37					
$Dose \times food \times surface$	4	2.1	0.09	1.1	0.37					
Within exposure intervals										
Exposure	3	616.5	< 0.01	790.7	< 0.01					
Exposure \times dose	3	12.3	< 0.01	11.1	< 0.01					
Exposure \times food	3	13.7	< 0.01	6.8	< 0.01					
Exposure \times surface	12	25.1	< 0.01	26.6	< 0.01					
Exposure \times dose \times food	3	0.4	0.74	1.9	0.14					
Exposure \times dose \times surface	12	1.6	0.09	1.8	0.05					
Exposure \times food \times surface	12	2.1	0.02	0.5	0.89					
$Exposure \times dose \times food \times surface$	12	2.4	0.01	2.4	0.01					

Table 2. Mean immediate mortality ($\% \pm SE$) of *Tenebrio molitor* adults exposed to surfaces treated with d-tetramethrin plus acetamiprid for 1 day, 3 days, 5 days, and 7 days. Within each row, means followed by the same uppercase letter are not significantly different (in all cases, DF = 3, 35, Tukey–Kramer HSD test at p = 0.05). Within each column, means that are followed by the same lower-case letter are not significantly different (in all cases, DF = 4, 44, Tukey–Kramer HSD test at p = 0.05).

Surfaces	1 Day	3 Days	5 Days	7 Days	F	р
Min/Food						
Plastic	$3.3\pm1.7~\mathrm{Cabc}$	$28.9\pm3.1~\mathrm{Bab}$	$76.7\pm4.4~\mathrm{Ab}$	$96.7\pm1.7~\mathrm{Aa}$	65.8	< 0.01
Glass	$8.9\pm2.0~\mathrm{Ba}$	54.4 ± 4.4 Aa	95.6 ± 2.4 Aa	$100.0\pm0.0~\mathrm{Aa}$	43.6	< 0.01
Metal	$0.0\pm0.0~{ m Cc}$	$26.7\pm4.1~\mathrm{Bb}$	94.4 ± 2.4 Aa	100.0 ± 0.0 Aa	798.0	< 0.01
Wood	$1.1\pm1.1~{ m Cbc}$	$14.4\pm2.4~\mathrm{Bc}$	$64.4\pm4.1~\mathrm{Ab}$	$87.8\pm4.0~\mathrm{Ab}$	80.7	< 0.01
Ceramic	$6.7\pm2.4~\mathrm{Bab}$	$47.8\pm4.0~\mathrm{Aab}$	94.4 ± 1.8 Aa	$100.0\pm0.0~\mathrm{Aa}$	43.6	< 0.01
F	5.6	11.0	17.6	7.1		
р	< 0.01	< 0.01	< 0.01	< 0.01		
Min/No food						
Plastic	$17.8\pm4.7~\mathrm{Cab}$	$47.8\pm4.9~\mathrm{Bab}$	$96.7\pm1.7~\mathrm{Aa}$	100.0 ± 0.0 Aa	25.0	< 0.01
Glass	$37.8\pm5.2~\mathrm{Ca}$	67.8 ± 4.3 Ba	$100.0\pm0.0~\mathrm{Aa}$	100.0 ± 0.0 Aa	41.6	< 0.01
Metal	$1.1\pm1.1~{ m Cc}$	$41.1\pm3.9~\mathrm{Bb}$	95.6 ± 2.4 Aa	100.0 ± 0.0 Aa	213.6	< 0.01
Wood	$3.3\pm2.4~\mathrm{Cc}$	$18.9\pm3.1~\mathrm{Bc}$	$66.7\pm5.0~\mathrm{Ab}$	$92.2\pm2.2~\mathrm{Ab}$	65.0	< 0.01
Ceramic	$7.8\pm2.8~\mathrm{Bbc}$	65.6 ± 5.8 Aa	$100.0\pm0.0~\mathrm{Aa}$	100.0 ± 0.0 Aa	39.3	< 0.01
F	15.5	25.5	26.6	11.7		
р	< 0.01	< 0.01	< 0.01	< 0.01		
Max/Food						
Plastic	13.3 ± 3.3 Ba	$64.4\pm4.1~\mathrm{Aa}$	$96.7\pm1.7~\mathrm{Aa}$	97.8 ± 1.5 Aab	27.1	< 0.01
Glass	$10.0\pm2.9~\mathrm{Bab}$	56.7 ± 2.9 Aa	$100.0\pm0.0~\mathrm{Aa}$	$100.0\pm0.0~\mathrm{Aa}$	32.7	< 0.01
Metal	$2.2\pm1.5~\mathrm{Cb}$	$43.3\pm3.7~\mathrm{Ba}$	$96.7\pm1.7~\mathrm{Aa}$	100.0 ± 0.0 Aa	111.0	< 0.01
Wood	$2.2\pm1.5~\mathrm{Cb}$	$18.9\pm3.9~\mathrm{Bb}$	$73.3\pm3.3~\mathrm{Ab}$	$94.4\pm2.4~\mathrm{Ab}$	52.2	< 0.01
Ceramic	7.8 ± 2.2 Bab	56.7 ± 3.7 Aa	$100.0\pm0.0~\mathrm{Aa}$	100.0 ± 0.0 Aa	43.0	< 0.01
F	3.6	12.0	34.0	3.6		
р	0.01	< 0.01	< 0.01	0.01		
Max/No food						
Plastic	$18.9\pm4.2~\mathrm{Bab}$	$66.7\pm3.7~\mathrm{Aa}$	$97.8\pm1.5~\mathrm{Aa}$	$100.0\pm0.0~\mathrm{A}$	17.8	< 0.01
Glass	$47.8\pm2.8~\mathrm{Ca}$	81.1 ± 3.5 Ba	$100.0\pm0.0~\mathrm{Aa}$	$100.0\pm0.0~\mathrm{A}$	99.7	< 0.01
Metal	$2.2\pm1.5~\mathrm{Cc}$	$45.6\pm5.0~\text{Bb}$	98.9 ± 1.1 Aa	$100.0\pm0.0~\mathrm{A}$	110.7	< 0.01
Wood	$3.3\pm1.7~\mathrm{Cc}$	$33.3\pm3.3~\mathrm{Bb}$	$90.0\pm3.3~\mathrm{Ab}$	$100.0\pm0.0~\mathrm{A}$	73.5	< 0.01
Ceramic	$13.3\pm2.4~\mathrm{Bb}$	71.1 ± 3.1 Aa	$100.0\pm0.0~\mathrm{Aa}$	$100.0\pm0.0~\mathrm{A}$	42.3	< 0.01
F	15.2	24.7	5.8	-		
р	< 0.01	< 0.01	< 0.01	-		

3.2. Immediate Mortality of Alphitobius diaperinus

Between and within exposure, all main effects were significant (Table 1). Dose \times surface (between), exposure \times dose \times surface, and exposure \times dose \times food \times surface interactions (within) were also significant. When A. diaperinus adults were exposed to the minimum dose of d-tetramethrin plus acetamiprid in the presence of food, no dead individuals were detected on the 1st day of the experiments on metal and wood, while treated ceramic, plastic, and glass surfaces provided mortality ranging from 1.1 to 17.8% (Table 3). After 7 days of exposure, this dose killed 53.3% (applied on wood) to 97.8% (applied on glass) in the same food scenario. When the minimum dose of this insecticide was applied in the absence of food, mortalities ranged between 0.0% (applied on metal) and 18.9% (applied on glass) after 1 day of exposure and between 63.3% (applied on wood) and 98.9% (applied on glass) 7 days post-exposure. The maximum dose of d-tetramethrin plus acetamiprid with food provided complete mortality (100.0%) when it was applied on glass, 97.8% on plastic, 87.8% on ceramic, 84.4% on metal, and 80.0% on wood surfaces 7 days post-exposure. The same dose in the absence of food provoked the death of all individuals that were exposed to treated plastic and glass surfaces by the end of the experiments. When the insecticide was applied on the other three types of surfaces, it killed 87.8% (wood), 92.2% (ceramic), and 97.8% (metal) after a 7-day exposure.

Table 3. Mean immediate mortality ($\% \pm SE$) of *Alphitobius diaperinus* adults exposed to surfaces treated with d-tetramethrin plus acetamiprid for 1 day, 3 days, 5 days, and 7 days. Within each row, means followed by the same uppercase letter are not significantly different (in all cases, DF = 3, 35, Tukey–Kramer HSD test at p = 0.05). Within each column, means that are followed by the same lower-case letter are not significantly different (in all cases, DF = 4, 44, Tukey–Kramer HSD test at p = 0.05).

Surfaces	1 Day	3 Days	5 Days	7 Days	F	p
Min/Food						
Plastic	$5.6\pm2.4~\mathrm{Bb}$	$32.2\pm5.2~\mathrm{Ab}$	$51.1\pm8.6~\mathrm{Abc}$	$70.0\pm5.5~\mathrm{Ab}$	27.8	< 0.01
Glass	$17.8\pm2.2~\mathrm{Ca}$	73.3 ± 2.9 Ba	$91.1\pm2.6~\mathrm{ABa}$	$97.8\pm1.5~\mathrm{Aa}$	142.8	< 0.01
Metal	$0.0\pm0.0~{ m Dc}$	32.2 ± 2.2 Cab	$58.9\pm5.6~\mathrm{Bab}$	$77.8 \pm 4.7 \text{ Aab}$	798.1	< 0.01
Wood	$0.0\pm0.0~{ m Cc}$	$12.2\pm3.2~\mathrm{Bc}$	$36.7\pm4.7~\mathrm{Ac}$	$53.3\pm5.8~{ m Ac}$	62.3	< 0.01
Ceramic	$1.1\pm1.1~{ m Cbc}$	$28.9\pm4.2~\text{Bb}$	$52.2\pm4.3~\mathrm{Abc}$	74.4 ± 4.1 Aab	128.0	< 0.01
F	25.4	12.7	8.9	10.5		
р	< 0.01	< 0.01	< 0.01	< 0.01		
Min/No food						
Plastic	$8.9\pm3.5~\mathrm{Bb}$	$58.9\pm5.6~\mathrm{Aab}$	77.8 ± 3.2 Aab	93.3 ± 2.4 Aa	31.4	< 0.01
Glass	$18.9\pm2.0~\mathrm{Ba}$	84.4 ± 3.4 Aa	$95.6\pm1.8~\mathrm{Aa}$	$98.9\pm1.1~\mathrm{Aa}$	177.4	< 0.01
Metal	$0.0\pm0.0~{ m Cc}$	$45.6\pm5.0~\mathrm{Bab}$	$73.3\pm4.7~\mathrm{Aab}$	84.4 ± 4.8 Aa	784.8	< 0.01
Wood	$2.2\pm1.5~{ m Cbc}$	$17.8\pm3.2~\mathrm{Bc}$	$37.8\pm4.0~\mathrm{ABc}$	$63.3\pm4.4~\mathrm{Ab}$	36.1	< 0.01
Ceramic	$2.2\pm1.5~\mathrm{Cbc}$	$37.8\pm4.7~\mathrm{Bb}$	$67.8\pm3.6~\mathrm{ABb}$	83.3 ± 4.1 Aa	83.3	< 0.01
F	13.5	12.7	20.3	13.0		
р	< 0.01	< 0.01	< 0.01	< 0.01		
Max/Food						
Plastic	$14.4\pm2.9~\mathrm{Ba}$	63.3 ± 5.3 Aa	85.6 ± 4.4 Aa	$97.8\pm1.5~\mathrm{Aa}$	31.5	< 0.01
Glass	$20.0\pm3.7~\mathrm{Ca}$	75.6 ± 2.4 Ba	92.2 ± 2.2 Aa	$100.0\pm0.0~\mathrm{Aa}$	211.1	< 0.01
Metal	$1.1\pm1.1~\mathrm{Bb}$	55.6 ± 5.8 Aa	$74.4\pm4.8~\mathrm{Aa}$	$84.4 \pm 4.4~\mathrm{Ab}$	173.7	< 0.01
Wood	$1.1\pm1.1~{ m Db}$	$18.9\pm2.6~\mathrm{Cb}$	$43.3\pm3.3~\text{Bb}$	$80.0\pm2.9~\mathrm{Ab}$	133.5	< 0.01
Ceramic	$3.3\pm1.7~\mathrm{Bb}$	53.3 ± 3.7 Aa	74.4 ± 2.9 Aa	87.8 ± 3.2 Aab	72.6	< 0.01
F	17.8	32.9	27.2	8.0		
р	< 0.01	< 0.01	< 0.01	< 0.01		
Max/No food						
Plastic	$15.6\pm1.8\mathrm{Cab}$	77.8 ± 4.3 Ba	$96.7\pm1.7\mathrm{A}\ \mathrm{Bab}$	100.0 ± 0.0 Aa	195.1	< 0.01
Glass	23.3 ± 2.4 Ba	84.4 ± 3.8 Aa	$98.9\pm1.1~\mathrm{Aa}$	100.0 ± 0.0 Aa	133.4	< 0.01
Metal	$7.8\pm2.8~\mathrm{Bbc}$	$77.8\pm3.6~\mathrm{Aa}$	$92.2\pm1.5~\mathrm{Aab}$	$97.8\pm1.5~\mathrm{Aab}$	40.2	< 0.01
Wood	$5.6\pm1.8~{ m Cc}$	$25.6\pm4.1~\mathrm{Bb}$	$63.3\pm4.4~\mathrm{Ac}$	$87.8\pm2.2~\mathrm{Ac}$	36.3	< 0.01
Ceramic	$5.6\pm1.8~\mathrm{Bc}$	63.3 ± 2.9 Aa	$84.4\pm3.8~\mathrm{Ab}$	92.2 ± 2.2 Abc	51.5	< 0.01
F	6.3	31.3	22.1	11.6		
р	< 0.01	< 0.01	< 0.01	< 0.01		

3.3. Delayed Mortality of Tenebrio molitor

All remaining *T. molitor* individuals died after their exposure to the minimum dose of d-tetramethrin plus acetamiprid treated on plastic in the presence of food (Table 4). The same dose treated on wood killed 80.6% and 100.0% of the remaining adults in the presence or absence of food, respectively. Concerning the maximum dose of the insecticide, all remaining individuals died on both plastic and wood surfaces when food was present.

3.4. Delayed Mortality of Alphitobius diaperinus

All the remaining *A. diaperinus* adults died after their exposure to plastic and glass surfaces treated with the minimum dose of d-tetramethrin plus acetamiprid for both food and no-food scenarios (Table 5). Concerning metal, wood, and ceramic surfaces, the mortality of remaining adults reached 91.7, 84.3, and 84.4% in the presence of food, and 81.9, 91.3, and 89.3% in the absence of food, respectively. Regarding the maximum dose of the insecticide, all remaining *A. diaperinus* died on plastic and metal surfaces in the presence of food, as well as on wood surfaces in the absence of food. The scenarios metal/no food, wood/food, and ceramic/food caused the death of 75.0, 92.9, and 92.9% of the remaining

adults. The lowest delayed mortality was noted in the case of ceramic surfaces, when food was absent, not exceeding 58.3% at the end of the assays.

Table 4. Mean delayed mortality ($\% \pm$ SE) of *Tenebrio molitor* adults exposed for 7 days on untreated dishes, with or without food, after 7 days of exposure on dishes treated with d-tetramethrin plus acetamiprid. Within each row, asterisks indicate significant differences (two-tailed *t*-test at *p* = 0.05). Within each column, means that are followed by the same lower-case letter are not significantly different (two-tailed *t*-test at *p* = 0.05). Where no letters or no asterisks exist, no significant differences were recorded. Where dashes exist, no analysis was performed. Numbers in parentheses denote the number of remaining individuals.

Min			Max								
Surfaces	Food	No Food	DF	t	p	Food	No Food	DF	t	р	
Plastic	100.0 ± 0.0 (0)	-	-	-	-	100.0 ± 0.0 (0)	-	-	-	-	
Wood	80.6 ± 12.5 (11)	100.0 ± 0.0 (0)	11	1.5	0.16	100.0 ± 0.0 (0)	-	-	-	-	
DF	8	-				5	-				
t	-1.0	-				-	-				
р	0.33	-				-	-				

Table 5. Mean delayed mortality ($\% \pm SE$) of *Alphitobius diaperinus* adults exposed for 7 days on untreated dishes, with or without food, after 7 days of exposure on dishes treated with d-tetramethrin plus acetamiprid. Within each row, asterisks indicate significant differences (two-tailed *t*-test at p = 0.05). Within each column, means that are followed by the same lower-case letter are not significantly different (Tukey–Kramer HSD test at p = 0.05). Where no letters or no asterisks exist, no significant differences were recorded. Where dashes exist, no analysis was performed. Numbers in parentheses denote the number of remaining individuals.

Surfaces	Min Food	No Food	DF	t	v	Max Food	No Food	DF	t	v
ounaces	1004	110 1 000	21	•	P	1004		21	•	P
Plastic	100.0 ± 0.0 (0)	100.0 ± 0.0 (0)	12	-	-	100.0 ± 0.0 (0)	-	-	-	-
Glass	100.0 ± 0.0 (0)	100.0 ± 0.0 (0)	2	-	-	-	-	-	-	-
Metal	91.7 ± 4.2 (3)	81.9 ± 8.7 (3)	14	-1.2	0.24	$100.0 \pm 0.0 * (0)$	50.0 ± 50.0 (1)	7	2.3	0.05
Wood	84.3 ± 6.6 (5)	91.3 ± 4.5 (3)	17	1.0	0.35	92.9 ± 7.1 (3)	100.0 ± 0.0 (0)	14	1.1	0.30
Ceramic	84.4 ± 7.8 (4)	89.3 ± 7.4 (2)	15	0.5	0.65	92.9 ± 7.1 (1)	58.3 ± 20.1 (3)	12	-1.8	0.10
DF	36	27				22	15			
F	1.5	0.9				0.4	2.4			
р	0.24	0.47				0.75	0.13			

4. Discussion

The findings of the current study revealed that there are significant differences in the impact of d-tetramethrin plus acetamiprid depending on the type of surfaces they were applied to. In general, the insecticide applied on glass resulted in the most elevated mortality rates, while when it was applied on wood, it killed the fewest adult individuals, regardless of food/no food scenario and insect species, on both immediate and delayed bioassays. There was no general trend among the other three types of surfaces, i.e., metal, plastic, and ceramic. However, treatments on metal and ceramic caused higher mortality levels than plastic surfaces. This was evident for both immediate and delayed mortalities for *T. molitor* adults, given that there was no delayed mortality in the case of metal and ceramic. The variable structural properties of the tested surfaces, i.e., the existence of pores, may cause the observed differential insecticidal efficacy. Glass, metal, and ceramic are non-porous surfaces, while plastic, followed by wood, are more porous surfaces [70–72]. Arthur [73] understood the significance of porous and non-porous surfaces, and how they affected the efficacy of the insecticides applied to them. The author noticed that when concrete was sealed, the applied insecticides had better residual efficacy than non-sealed concrete, i.e., concrete with pores [73].

Similarly to our results, Arthur [74] reported that deltamethrin treated on ceramic tile had higher efficacy than when it was treated on wood surfaces. In a previous study, Vojoudi et al. [72] applied abamectin, chlorpyrifos, and deltamethrin on paper, plastic, ceramic, and glass surfaces. The authors found that higher doses of insecticide were needed to kill 90% of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) on paper < plastic < ceramic < glass. In a more recent study, Arthur et al. [75] tested deltamethrin and cyfluthrin against young/old larvae of *Trogoderma granarium* Everts (Coleoptera: Dermestidae). The authors applied the insecticides on metal, vinyl flooring tile, wood, painted wood, and concrete surfaces. Treated metal provided the highest mortalities for a period of three months [75]. Combining the findings of this study with the previous studies, the type of surface should be taken into consideration when insecticidal treatments will be followed for the management of *T. molitor* and *A. diaperinus* adults. Furthermore, whether more porous surfaces, other than concrete, should be sealed before treatment [73] merits investigation.

In all tested scenarios, T. molitor adults were more susceptible than A. diaperinus adults. This was documented before in several studies, since there is no general trend in tenebrionids concerning their developmental stage susceptibility to insecticides [54,76]. For example, *T. molitor's* susceptible developmental stage is the adult stage, while larvae are more tolerant [53]. However, this does not apply to A. diaperinus, since larvae are its susceptible developmental stage, and adults are more tolerant than larvae [56,57]. Susceptibility differences among species and their developmental stages occur because of the variability of their cuticle's lipid composition and/or variety, their setae, their morphology, and their behavior [77-94]. In a more recent study, Kavallieratos et al. [55] treated concrete surfaces with d-tetramethrin plus acetamiprid against A. diaperinus small and large larvae. The formulation killed more small than large larvae, while the presence of food caused a significant reduction in the efficacy of the insecticide [55]. Total mortality was observed only in the case of small larvae [55]. These are rather important findings since d-tetramethrin plus acetamiprid applied on porous surfaces is not able to suppress the susceptible developmental stage of A. diaperinus, i.e., larvae [55], while the same insecticide treated on non-porous surfaces (glass) killed all the tolerant individuals, i.e., adults. These results prove the significance of the comprehensive organization of pest management strategies to choose the best surface for insecticidal application, depending on the target pest and/or developmental stage.

One other important factor concerning the efficacy of d-tetramethrin plus acetamiprid was the presence/absence of food on treated surfaces, in terms of immediate and delayed mortality. Generally, food presence resulted in lower observed mortality rates at both doses, regardless of the insect species. Similarly, Kavallieratos et al. [55–57] noticed that food on treated concrete reduced mortality levels caused by chlorfenapyr, deltamethrin, etofenprox, and d-tetramethrin plus acetamiprid, against small/large larvae and adults of *A. diaperinus*. Food is considered an obstacle since it can prevent the contact of the insecticide [95–103]. As a result, intensive cleaning is suggested to eliminate the possibility of the insecticidal efficacy reduction caused by food prior to the application, as well as during the storage period [99,104,105].

Concerning d-tetramethrin plus acetamiprid, this is a mixture of a.i.s that has been scarcely examined. After an extensive search into the international bibliography, only Kavallieratos et al. [55] and Kavallieratos and Boukouvala [106] have examined this mixture on *A. diaperinus* and *T. granarium*, respectively. The authors revealed the elevated efficacy on both adults and larvae of *T. granarium*, reaching 100.0% and 84.4% mortality, respectively [106]. This was an important finding since *T. granarium* larvae are very tolerant. Taking into account our findings and those of the aforementioned studies, we can conclude that this mixture of a.i.s has the potential concerning stored-product pest management in storages. It can also be assumed that piperonyl butoxide, as an insecticide synergist that enhances the insecticidal properties of d-tetramethrin and acetamiprid [59,107], plays a significant role in the elevated efficacy of this mixture in this study, compared with the

insecticidal properties of these a.i.s in former studies [108–115]. Previous research has revealed that the incorporation of piperonyl butoxide into pyrethroid insecticides did not alter the susceptibility of the susceptible strain of *A. diaperinus* [113]. It should be noted that former studies revealed the antifeedant and repellent effects of tetramethrin [109,110]. This can partially explain the lower delayed mortalities observed in no-food scenarios.

5. Conclusions

To conclude, *A. diaperinus* adults were more tolerant than *T. molitor* adults, regardless of insecticidal dose, surface, and food/no food scenarios. When both doses were applied on wood resulted in the lowest mortality levels against both species, while treated glass provided the most elevated mortality, in all tested food/no food scenarios. No trend was revealed during this study concerning the suitability of treated plastic, metal, and ceramic surfaces against these tenebrionids in the absence or presence of food. Food absence resulted in elevated efficacy, contrary to the presence of food. Overall, d-tetramethrin plus acetamiprid is effective against both tested species when they are treated on several types of surfaces. Whether there are ways to enhance the efficacy of insecticides on porous surfaces merits further investigation.

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References

- 1. Rees, D. Insects of Stored Products; Manson Publishing: London, UK, 2004.
- Plata-Rueda, A.; Martínez, L.; Santos, M.; Dos Santos, M.H.; Fernandes, F.L.; Wilcken, C.F.; Soares, M.A.; Serrão, J.E.; Zanuncio, J.C. Insecticidal activity of garlic essential oil and their constituents against the mealworm beetle, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae). Sci. Rep. 2017, 7, 46406. [CrossRef] [PubMed]
- Bernstein, D.I.; Gallagher, J.S.; Bernstein, I.L. Mealworm asthma: Clinical and immunologic studies. J. Allergy Clin. Immunol. 1983, 72, 475–480. [CrossRef] [PubMed]
- Schroeckenstein, M.D.D.C.; Meier-Davis, D.V.M.S.; Bush, M.D.R.K. Occupational sensitivity to *Tenebrio molitor* Linnaeus (yellow mealworm). J. Allergy Clin. Immunol. 1990, 86, 182–188. [CrossRef] [PubMed]
- 5. Beaumont, P.; Courtois, J.; Van der Brempt, X.; Tollenaere, S. Food-induced anaphylaxis to *Tenebrio molitor* and allergens implicated. *Rev. Fr. Allergol.* **2019**, *59*, 389–393. [CrossRef]
- Nebbia, S.; Lamberti, C.; Giorgis, V.; Giuffrida, M.G.; Manfredi, M.; Marengo, E.; Pessione, E.; Schiavone, A.; Boita, M.; Brussino, L.; et al. The cockroach allergen-like protein is involved in primary respiratory and food allergy to yellow mealworm (*Tenebrio molitor*). *Clin. Exp. Allergy* 2019, 49, 1379–1382. [CrossRef]
- Ribeiro, J.C.; Sousa-Pinto, B.; Fonseca, J.; Caldas Fonseca, S.; Cunha, L.M. Edible insects and food safety: Allergy. J. Insects Food Feed 2021, 7, 833–847. [CrossRef]
- Premrov Bajuk, B.; Zrimšek, P.; Kotnik, T.; Leonardi, A.; Križaj, I.; Jakovac Strajn, B. Insect protein-based diet as potential risk of allergy in dogs. *Animals* 2021, 11, 1942. [CrossRef]
- Bußler, S.; Rumpold, B.A.; Jander, E.; Rawel, H.M.; Schlüter, O.K. Recovery and techno-functionality of flours and proteins from two edible insect species: Mealworm (*Tenebrio molitor*) and black soldier fly (*Hermetia illucens*) larvae. *Heliyon* 2016, 2, e00218. [CrossRef]
- Azzollini, D.; Derossi, A.; Fogliano, V.; Lakemond, C.M.M.; Severini, C. Effects of formulation and process conditions on microstructure, texture and digestibility of extruded insect-riched snacks. *Innov. Food Sci. Emerg. Technol.* 2018, 45, 344–353. [CrossRef]
- Costa, S.; Pedro, S.; Lourenço, H.; Batista, I.; Teixeira, B.; Bandarra, N.M.; Murta, D.; Nunes, R.; Pires, C. Evaluation of *Tenebrio* molitor larvae as an alternative food source. NFS J. 2020, 21, 57–64. [CrossRef]

- 12. Cho, S.Y.; Ryu, G.H. Effects of mealworm larva composition and selected process parameters on the physicochemical properties of extruded meat analog. *Food Sci. Nutr.* **2021**, *9*, 4408–4419. [PubMed]
- Peng, B.Y.; Chen, Z.; Chen, J.; Zhou, X.; Wu, W.M.; Zhang, Y. Biodegradation of polylactic acid by yellow mealworms (larvae of *Tenebrio molitor*) via resource recovery: A sustainable approach for waste management. *J. Hazard. Mater.* 2021, 416, 125803. [CrossRef] [PubMed]
- 14. Yang, S.S.; Brandon, A.M.; Flanagan, J.C.A.; Yang, J.; Ning, D.; Cai, S.Y.; Fan, H.Q.; Wang, Z.Y.; Ren, J.; Benbow, E.; et al. Biodegradation of polystyrene wastes in yellow mealworms (larvae of *Tenebrio molitor* Linnaeus): Factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle. *Chemosphere* **2018**, *191*, 979–989. [CrossRef] [PubMed]
- Yang, S.S.; Ding, M.Q.; Zhang, Z.R.; Ding, J.; Bai, S.W.; Cao, G.L.; Zhao, L.; Pang, J.W.; Xing, D.F.; Ren, N.Q.; et al. Confirmation of biodegradation of low-density polyethylene in dark- versus yellow- mealworms (larvae of *Tenebrio obscurus* versus *Tenebrio molitor*) via. gut microbe-independent depolymerization. *Sci. Total Environ.* 2021, 789, 147915. [CrossRef]
- 16. Djouadi, A.; Sales, J.R.; Carvalho, M.O.; Raymundo, A. Development of healthy protein-rich crackers using *Tenebrio molitor* flour. *Foods* **2022**, *11*, 702. [CrossRef]
- 17. Espinoza Pinchi, J.; Ordonez Galvez, J.; Castaneda-Olivera, C.A.; Benites Alfaro, E.G. Environmental biotechnology: Biodegradation of microplastics with larvae of *Tenebrio molitor* and *Galleria mellonella*. *Chem. Eng. Trans.* **2022**, *93*, 187–192.
- Sriprablom, J.; Kitthawee, S.; Suphantharika, M. Functional and physicochemical properties of cookies enriched with edible insect (*Tenebrio molitor* and *Zophobas atratus*) powders. *Food Meas.* 2022, *16*, 2181–2190. [CrossRef]
- Sugumar, P.; Sha, D.S.M.; Gowda, S.; Vijay, T.; Keerthana, S. An assessment on the potential of *Tenebrio molitor* used for biodepolymerization of plastics and polystyrene: Influencing factors, various feeding cases and gut microbiota. *IOP Conf. Ser. Earth Environ. Sci.* 2022, 1074, 012029. [CrossRef]
- 20. Hill, D.S. Pests of Stored Foodstuffs and Their Control; Kluwer Academic Publishers: New York, NY, USA, 2003.
- Mazlum, Y.; Turan, F.; Yildirim, Y.B. Evaluation of mealworms (*Tenebrio molitor*) meal as an alternative protein source for narrow-clawed crayfish (*Pontastacus leptodactylus*) juveniles. *Aquat. Res.* 2021, 52, 4145–4153. [CrossRef]
- Shafique, L.; Abdel-Latif, H.M.R.; Hassan, F.-u.; Alagawany, M.; Naiel, M.A.E.; Dawood, M.A.O.; Yilmaz, S.; Liu, Q. The feasibility of using yellow mealworms (*Tenebrio molitor*): Towards a sustainable aquafeed industry. *Animals* 2021, 11, 811. [CrossRef]
- Shaviklo, A.R.; Alizadeh-Ghamsari, A.H.; Hosseini, S.A. Sensory attributes and meat quality of broiler chickens fed with mealworm (*Tenebrio molitor*). J. Food Sci. Technol. 2021, 58, 4587–4597. [CrossRef] [PubMed]
- Mouadi, J.; Pafilis, P.; Elbahi, A.; Okba, Z.; Elouizgani, H.; Mouden, E.H.E.; Aourir, M. The effect of weight and prey species on gut passage time in an endemic gecko *Quedenfeldtia moerens* (Chabanaud, 1916) from Morocco. *Acta Herpetol.* 2022, 17, 21–26. [CrossRef]
- Chen, F.; Jiang, H.; Gan, Y.; Chen, W.; Huang, G. Optimization of hydrolysis conditions for obtaining antithrombotic peptides from *Tenebrio molitor* larvae. *Am. J. Biochem. Biotechnol.* 2019, 15, 52–60.
- 26. Song, D.H.; Kim, M.; Jin, E.S.; Sim, D.W.; Won, H.S.; Kim, E.K.; Jang, S.; Choi, Y.S.; Chung, K.H.; An, J.H. Cryoprotective effect of an antifreeze protein purified from *Tenebrio molitor* larvae on vegetable. *Food Hydrocoll.* **2019**, *94*, 585–591. [CrossRef]
- Ding, Q.; Wu, R.A.; Shi, T.; Yu, Y.; Yan, Y.; Sun, N.; Sheikh, A.R.; Luo, L.; He, R.; Ma, H. Antiproliferative effects of mealworm larvae (*Tenebrio molitor*) aqueous extract on human colorectal adenocarcinoma (Caco-2) and hepatocellular carcinoma (HepG2) cancer cell lines. *Food Biochem.* 2021, 45, e13778. [CrossRef]
- 28. Hwang, D.; Lee, S.H.; Goo, T.-W.; Yun, E.-Y. Potential of antimicrobial peptide-overexpressed *Tenebrio molitor* larvae extract as a natural preservative for Korean traditional sauces. *Insects* **2022**, *13*, 381. [CrossRef] [PubMed]
- 29. Klost, M.; Ramirez-Huerta, M.I.; Drusch, S. Heat-induced gelation of protein from mealworm (*Tenebrio molitor*): Influence of pH and zinc concentration. *Food Hydrocoll. Health* **2022**, *2*, 100105. [CrossRef]
- Hagstrum, D.W.; Klejdysz, T.; Subramanyam, B.; Nawrot, J. Atlas of Stored-Product Insects and Mites; AACC International: St. Paul, MN, USA, 2013.
- Donoso, A.; Paredes, N.; Retamal, P. Detection of antimicrobial resistant *Salmonella enterica* strains in larval and adult forms of lesser mealworm (*Alphitobius diaperinus*) from industrial poultry farms. *Front. Vet. Sci.* 2020, 7, 577848. [CrossRef]
- 32. Smith, R.; Hauck, R.; Macklin, K.; Price, S.; Dormitorio, T.; Wang, C. A review of the lesser mealworm beetle (*Alphitobius diaperinus*) as a reservoir for poultry bacterial pathogens and antimicrobial resistance. *Worlds Poult. Sci. J.* **2021**, *78*, 197–214. [CrossRef]
- 33. Chernaki, A.M.; Almeida, L.M. Exigências térmicas, período de desenvolvimento e sobrevivência de imaturos de *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). *Neotrop. Entomol.* **2001**, *30*, 365–368. [CrossRef]
- 34. Agabou, A.; Alloui, N. Importance of *Alphitobius diaperinus* (Panzer) as a reservoir for pathogenic bacteria in Algerian broiler houses. *Vet. World* **2010**, *3*, 71–73.
- 35. Dinev, I. The darkling beetle (Alphitobius diazecines)—A health hazard for broiler chicken production. Trakia J. Sci. 2013, 11, 1.
- Li, Z.; Huang, S.; Huand, W.F.; Geng, H.; Zhao, Y.; Li, M.; Chen, Y.; Su, S. A scientific note on detection of honeybee viruses in the darkling beetle (*Alphitobius diaperinus*, Coleoptera: Tenebrionidae), a new pest in *Apis cerana cerana* colonies. *Apidologie* 2016, 47, 759–761. [CrossRef]
- Wynants, E.; Crauwels, S.; Verreth, C.; Gianotten, N.; Lievens, B.; Claes, J.; Van Campenhout, L. Microbial dynamics during production of lesser mealworms (*Alphitobius diaperinus*) for human consumption at industrial scale. *Food Microbiol.* 2018, 70, 181–191. [CrossRef] [PubMed]
- Krinsky, W.L. Beetles (Coleoptera). In *Medical and Veterinary Entomology*; Mullen, G.R., Durden, L.A., Eds.; Academic Press: London, UK, 2019; pp. 129–143.

- Zbrun, M.V.; Rossler, E.; Olivero, C.R.; Soto, L.P.; Zimmermann, J.A.; Frizzo, L.S.; Signorini, M.L. Possible reservoirs of thermotolerant *Campylobacter* at the farm between rearing periods and after the use of enrofloxacin as a therapeutic treatment. *Int. J. Food Microbiol.* 2021, 340, 109046. [CrossRef] [PubMed]
- 40. Jertborn, M.; Haglind, P.; Iwarson, S.; Svennerholm, A.M. Estimation of symptomatic and asymptomatic *Salmonella* infections. *Scand. J. Infect. Dis.* **1990**, 22, 451–455. [CrossRef]
- 41. Blaser, M.J. Epidemiologic and clinical features of Campylobacter jejuni infections. J. Infect. Dis. 1997, 176, S103–S105. [CrossRef]
- 42. Litrup, E.; Torpdahl, M.; Malorny, B.; Huehn, S.; Helms, M.; Christensen, H.; Nielsen, E.M. DNA microarray analysis of Salmonella serotype *Typhimurium* strains causing different symptoms of disease. *BMC Microbiol.* **2010**, *10*, 96. [CrossRef]
- 43. Wirtz, R.A. Allergic and toxic reactions to non-stinging arthropods. Annu. Rev. Entomol. 1984, 29, 47–69. [CrossRef]
- 44. Schroeckenstein, D.C. Occupational sensitivity to *Alphitobius diaperinus* (lesser mealworm). *J. Allergy Clin. Immunol.* **1988**, *82*, 1081–1088. [CrossRef]
- 45. Van Broekhoven, S.; Bastiaan-Net, S.; de Jong, N.W.; Witchers, H.J. Influence of processing and in vitro digestion on the allergic cross-reactivity of three mealworm species. *Food Chem.* **2016**, *196*, 1075–1083. [CrossRef] [PubMed]
- Kaufman, P.E.; Burgess, M.; Rutz, D.A. Population dynamics of manure inhabiting arthropods under an integrated pest management (IPM) program in New York poultry facilities-3 case studies. J. Appl. Poult. Res. 2002, 11, 90–103. [CrossRef]
- Tomberlin, J.K.; Richman, D.; Myers, H.M. Susceptibility of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) from broiler facilities in Texas to four insecticides. *J. Econ. Entomol.* 2008, 101, 480–483. [PubMed]
- Athanassiou, C.G.; Kavallieratos, N.G.; Boukouvala, M.C.; Mavroforos, M.E.; Kontodimas, D.C. Efficacy of alpha-cypermethrin and thiamethoxan against *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and *Tenebrio molitor* L. J. Stored Prod. Res. 2015, 62, 101–107. [CrossRef]
- 49. Lyon, B.N.; Crippen, T.L.; Zheng, L.; Teel, P.D.; Swiger, S.L.; Tomberlin, J.K. Susceptibility of *Alphitobius diaperinus* in Texas to permethrin and β-cyfluthrin treated surfaces. *Pest Manag. Sci.* **2017**, *73*, 562–567.
- 50. Plata-Rueda, A.; Zanuncio, J.C.; Serrão, J.E.; Martínez, L.C. *Origanum vulgare* essential oil against *Tenebrio molitor* (Coleoptera: Tenebrionidae): Composition, insecticidal activity, and behavioral response. *Plants* **2021**, *10*, 2513. [CrossRef] [PubMed]
- Arena, J.S.; Omarini, A.B.; Zunino, A.P.; Peschiutta, M.L.; Defagó, M.T.; Zygadlo, J.A. Essential oils from *Dysphania ambrosioides* and *Tagetes minuta* enhance the toxicity of a conventional insecticide against *Alphitobius diaperinus*. *Ind. Crops Prod.* 2018, 122, 190–194. [CrossRef]
- Hickmann, F.; De Morais, A.F.; Bronzatto, E.S.; Giacomelli, T.; Guedes, J.V.C.; Bernardi, O. Susceptibility of the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), from broiler farms of Southern Brazil to insecticides. J. Econ. Entomol. 2018, 111, 980–985.
- 53. Kavallieratos, N.G.; Michail, E.J.; Boukouvala, M.C.; Nika, E.P.; Skourti, A. Efficacy of pirimiphos-methyl, deltamethrin, spinosad and silicoSec against adults and larvae of *Tenebrio molitor* L. on wheat, barley and maize. *J. Stored Prod. Res.* 2019, 83, 161–167.
- 54. Kavallieratos, N.G.; Nika, E.P.; Skourti, A.; Ntalli, N.; Boukouvala, M.C.; Ntalaka, C.T.; Maggi, F.; Rakotosaona, R.; Cespi, M.; Perinelli, D.R.; et al. Developing a *Hazomalania voyronii* essential oil nanoemulsion for the eco-friendly management of *Tribolium confusum*, *Tribolium castaneum* and *Tenebrio molitor* larvae and adults on stored wheat. *Molecules* 2021, 26, 1812. [CrossRef]
- Kavallieratos, N.G.; Nika, E.P.; Skourti, A.; Filintas, C.S.; Goumenou, T.D. Short and long-term mortalities of small and large larvae of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) on concrete surfaces treated with three insecticides: Impact of food. *Insects* 2022, 13, 366. [CrossRef] [PubMed]
- 56. Kavallieratos, N.G.; Nika, E.P.; Skourti, A.; Goumenou, T.D.; Filintas, C.S. Immediate and delayed mortality of different *Alphitobius diaperinus* developmental stages on chlorfenapyr-treated concrete. *J. Stored Prod. Res.* **2022**, *98*, 101998. [CrossRef]
- 57. Kavallieratos, N.G.; Nika, E.P.; Skourti, A.; Virvidaki, A.J.V. Deltamethrin residual mission against *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) on concrete for six weeks. *J. Stored Prod. Res.* **2022**, *99*, 102036. [CrossRef]
- 58. Kavallieratos, N.G.; Nika, E.P.; Skourti, A.; Spinozzi, E.; Ferrati, M.; Petrelli, R.; Benelli, G. *Carlina acaulis* essential oil: A candidate product for agrochemical industry due to its pesticidal capacity. *Ind. Crops Prod.* **2022**, *188*, 115572. [CrossRef]
- 59. Di Blasi, G. A Review of the chemistry of piperonyl butoxide. In *Piperonyl Butoxide. The Insecticide Synergist;* Jones, D.G., Ed.; Academic Press: London, UK, 1999; pp. 55–70.
- 60. EPA (Environmental Protection Agency). *Registration Eligibility Decision (RED) Document for Tetramethrin;* EPA: Washington, DC, USA, 2008; pp. 1–50.
- 61. Ghanim, M.; Ishaaya, I. Insecticides with novel modes of action: Mechanism and resistance management. In *Insecticides with Novel Modes of Action: Mechanism and Application*; Ishaaya, I., Degheele, D., Eds.; Springer: Berlin/Heidelberg, Germany, 2010; pp. 385–407.
- 62. FAO, Food and Agriculture Organization of the United Nations. Poultry Development Review. 2013. Available online: https://www.fao.org/publications/card/en/c/90c86c8d-96e8-5db6-914f-2bd9fa24e421/ (accessed on 31 March 2023).
- 63. Rice, S.J.; Lambkin, T.A. A new culture method for lesser mealworm, Alphitobius diaperinus. J. Appl. Entomol. 2009, 133, 67–72. [CrossRef]
- 64. Sagheer, M.; Aman, Y.; Mansoor-ul-Hasan; Ahmed, F.; Ranjha, M.H.; Ali, Q.; Ali, K.; Sidra-tul-Muntaha. Fumigant bioactivity of extracts of *Citrulus colocynthes, Moringa oleifera* and *Azadirachta indica* against *Tribolium castaneum* and *Alphitobius diaperinus* under laboratory conditions. In Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products, New Delhi, India, 6–11 November 2016; Navarro, S., Jayas, D.S., Alagusundaram, K., Eds.; CAF Permanent Committee Secretariat: Winnipeg, MB, Canada, 2016; pp. 459–464.
- 65. Zar, J.H. Biostatistical Analysis; Pearson Education Limited: Essex, UK, 2014.

- Scheff, D.S.; Arthur, F.H. Fecundity of *Tribolium castaneum* and *Tribolium confusum* adults after exposure to deltamethrin packaging. J. Pest Sci. 2018, 91, 717–725. [CrossRef]
- 67. Sall, J.; Lehman, A.; Creighton, L. JMP start statistics. In *A Guide to Statistics and Data Analysis Using JMP and JMP in Software;* Duxbury Press: Belmont, ON, Canada, 2001.
- 68. Sokal, R.R.; Rohlf, F.J. Biometry; Freeman & Company: New York, NY, USA, 1995.
- 69. SAS Institute Inc. Using JMP 16.2; SAS Institute Inc.: Cary, NC, USA, 2021.
- 70. Arthur, F.H. Aerosols and contact insecticides as alternatives to methyl bromide in flour mills, food production facilities, and food warehouses. *J. Pest Sci.* 2012, *85*, 323–329. [CrossRef]
- 71. Arthur, F.H.; Liu, S.; Zhao, B.; Phillips, T.W. Residual efficacy of pyriproxyfen and hydroprene applied to wood, metal and concrete for control of stored-product insects. *Pest Manag. Sci.* **2009**, *65*, 791–797. [CrossRef]
- Vojoudi, S.; Saber, M.; Mahdavi, V.; Golshan, H.; Abedi, Z. Efficacy of some insecticides against red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) adults exposed on glass, ceramic tile, plastic and paper disc surfaces. *J. Life Sci.* 2012, *6*, 405–410.
- 73. Arthur, F.H. Residual efficacy of cyfluthrin emulsifiable concentrate and wettable powder formulations on porous concrete and on concrete sealed with commercial products prior to insecticide application. J. Stored Prod. Res. **1994**, 30, 79–86. [CrossRef]
- 74. Arthur, F.H. Differential effectiveness of deltamethrin dust on plywood, concrete, and tile surfaces against three stored-product beetles. *J. Stored Prod. Res.* **1997**, *33*, 167–173. [CrossRef]
- Arthur, F.H.; Ghimire, M.N.; Myers, S.W.; Phillips, T.W. Evaluation of pyrethroid insecticides and insect growth regulators applied to different surfaces for control of *Trogoderma granarium* (Coleoptera: Dermestidae) the khapra beetle. *J. Econ. Entomol.* 2018, 111, 612–619. [CrossRef] [PubMed]
- 76. Kavallieratos, N.G.; Skourti, A.; Nika, E.P.; Ntalaka, C.T.; Boukouvala, M.C.; Bonacucina, G.; Cespi, M.; Petrelli, R.; Cappellacci, L.; Maggi, F.; et al. Isofuranodiene-based nanoemulsion: Larvicidal and adulticidal activity against tenebrionid beetles attacking stored wheat. *J. Stored Prod. Res.* 2021, *93*, 101859. [CrossRef]
- 77. Peterson, A. Larvae of Insects. Part I Lepidoptera and Hymenoptera; Edwards Bothers Inc.: Ann Arbor, MI, USA, 1948.
- 78. Peterson, A. Larvae of Insects. Part II Coleoptera, Diptera, Neuroptera, Siphonaptera, Mecoptera, Trichoptera; Edwards Bothers Inc.: Ann Arbor, MI, USA, 1951.
- Gast, R.T. The relationship of weight of lepidopterous larvae to effectiveness of topically applied insecticides. *J. Econ. Entomol.* 1959, 52, 1115–1117. [CrossRef]
- Armold, M.T.; Blomquist, G.J.; Jackson, L.L. Cuticular lipids of insects III. The surface lipids of aquatic and terrestrial life forms of the big stonefly *Pteronarcys california* (Newport). *Comp. Biochem. Physiol.* **1969**, *31*, 685–692. [CrossRef]
- Andersen, S.O.; Thompson, P.R.; Hepburn, H.R. Cuticular sclerotization in the honeybee (*Apis mellifera adansonii*). J. Comp. Physiol. 1981, 145, 17–20. [CrossRef]
- 82. Cox, P.D.; Bell, C.H.; Pearson, J.; Beirne, M.A. The effect of diapause on the tolerance of larvae of *Ephestia kuehniella* to methyl bromide and phosphine. *J. Stored Prod. Res.* **1984**, *20*, 215–219. [CrossRef]
- 83. Andersen, S.O. Sclerotization and tanning of the cuticle. In *Comprehensive Insect Physiology Biochemistry and Pharmacology;* Kerkut, G.A., Gilbert, L.I., Eds.; Pergamon Press: Oxford, UK, 1985; pp. 59–74.
- 84. Mewis, I.; Ulrichs, C. Action of amorphous diatomaceous earth against different stages of the stored product pests *Tribolium confusum*, *Tenebrio molitor*, *Sitophilus granarius* and *Plodia interpunctella*. *J. Stored Prod. Res.* **2001**, *37*, 153–164. [CrossRef]
- 85. Yu, S.J. The Toxicology and Biochemistry of Insecticide; CRC Press: Boca Raton, FL, USA, 2008.
- 86. Andersen, S.O. Insect cuticular sclerotization: A review. Insect Biochem. Mol. Biol. 2010, 40, 166–178. [CrossRef]
- 87. Moussian, B. Recent advances in understanding mechanisms of insect cuticle differentiation. *Insect Biochem. Mol. Biol.* 2010, 40, 363–375. [CrossRef]
- Wink, M. Medicinal plants: A source of anti-parasitic secondary metabolites. *Molecules* 2012, 17, 12771–12791. [CrossRef] [PubMed]
- Gatton, M.L.; Chitnis, N.; Churcher, T.; Donnelly, M.J.; Ghani, A.C.; Godfray, H.C.J.; Gould, F.; Hastings, I.; Marshall, J.; Ranson, H.; et al. The importance of mosquito behavioural adaptations to malaria control in Africa. *Evolution* 2013, 67, 1218–1230. [CrossRef] [PubMed]
- Lilly, D.G.; Latham, S.L.; Webb, C.E.; Doggett, S.L. Cuticle thickening in a pyrethroid-resistant strain of the common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae). *PLoS ONE* 2016, 11, e0153302. [CrossRef] [PubMed]
- 91. Panini, M.; Manicardi, G.C.; Moores, G.D.; Mazzoni, E. An overview of the main pathways of metabolic resistance in insects. *Invertebr. Surviv. J.* **2016**, *13*, 326–335.
- Giordano, G.; Carbone, M.; Ciavatta, M.L.; Silvano, E.; Gavagnin, M.; Garson, M.J.; Cheney, K.L.; Mudianta, I.W.; Russo, G.F.; Villani, G.; et al. Volatile secondary metabolites as aposematic olfactory signals and defensive weapons in aquatic environments. *Proc. Natl. Acad. Sci. USA* 2017, 114, 3451–3456. [CrossRef]
- 93. Pavela, R.; Pavoni, L.; Bonacucina, G.; Cespi, M.; Kavallieratos, N.G.; Cappellacci, L.; Petrelli, R.; Maggi, F.; Benelli, G. Rationale for developing novel mosquito larvicides based on isofuranodiene microemulsions. *J. Pest Sci.* **2019**, *92*, 909–921. [CrossRef]
- Pavela, R.; Maggi, F.; Petrelli, R.; Cappellacci, L.; Buccioni, M.; Palmieri, A.; Canale, A.; Benelli, G. Outstanding insecticidal activity and sublethal effects of *Carlina acaulis* root essential oil on the housefly, *Musca domestica*, with insights on its toxicity on human cells. *Food Chem. Toxicol.* 2020, 136, 111037. [CrossRef]

- 95. Arthur, F.H. Impact of accumulated food on survival of *Tribolium castaneum* on concrete treated with cyfluthrin wettable powder. J. Stored Prod. Res. 2000, 36, 15–23. [CrossRef]
- 96. Arthur, F.H. Residual efficacy of aerosols to control *Tribolium castaneum* and *Tribolium confusum*. In Proceedings of the 10th International Working Conference on Stored Product Protection, Estoril, Portugal, 27 June–2 July 2010; Carvalho, M.O., Fields, P.G., Adler, C.S., Arthur, F.H., Athanassiou, C.G., Campbell, J.F., Fleurat-Lessard, F., Flinn, P.W., Hodges, R.J., Isikber, A.A., Eds.; Julius Kühn-Institut: Berlin, Germany, 2010; pp. 789–792.
- 97. Toews, M.D.; Campbell, J.F.; Arthur, F.H. The presence of flour affects the efficacy of aerosolized insecticides used to treat the red flour beetle, *Tribolium castaneum*. J. Insect Sci. 2010, 10, 196. [CrossRef]
- 98. Athanassiou, C.G.; Arthur, F.H.; Kavallieratos, N.G.; Throne, J.E. Efficacy of pyriproxyfen for control of stored-product psocids (Psocoptera) on concrete surfaces. *J. Econ. Entomol.* 2011, 104, 1765–1769. [CrossRef]
- 99. Arthur, F.H. Food source effect and residual efficacy of chlorfenapyr as a surface treatment on sealed and unsealed concrete. *J. Stored Prod. Res.* **2015**, *64*, 65–71. [CrossRef]
- Wijayaratne, L.K.; Fields, P.G.; Arthur, F.H. Residual efficacy of methoprene for control of *Tribolium castaneum* (Coleoptera: Tenebrionidae) larvae at different temperatures on varnished wood, concrete, and wheat. *J. Econ. Entomol.* 2012, 105, 718–725. [CrossRef] [PubMed]
- 101. Velki, M.; Plavšin, I.; Dragojević, J.; Hackenberger, B.K. Toxicity and repellency of dimethoate, pirimiphos-methyl and deltamethrin against *Tribolium castaneum* (Herbst) using different exposure methods. *J. Stored Prod. Res.* **2014**, *59*, 36–41. [CrossRef]
- 102. Arthur, F.H.; Domingue, M.J.; Scheff, D.S.; Myers, S.W. Bioassays and methodologies for insecticide tests with larvae of *Trogoderma* granarium (Everts), the khapra beetle. *Insects* **2019**, *10*, 145. [CrossRef]
- 103. Boukouvala, M.C.; Kavallieratos, N.G. Effect of six insecticides on egg hatching and larval mortality of *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *Insects* **2020**, *11*, 263. [CrossRef]
- 104. Lorini, I.; Filho, A.F. Integrated pest management strategies used in stored grain in Brazil to manage phosphine resistance. In Proceedings of the 7th International Conference on Controlled Atmosphere and Fumigation in Stored Products, Gold-Coast, Australia, 8–13 August 2004; Donahaye, E.J., Navarro, S., Bell, C., Jayas, D., Noyes, R., Phillips, T.W., Eds.; FTIC Ltd. Publishing: Israel, 2007; pp. 293–300.
- 105. Kharel, K.; Arthur, F.H.; Campbell, J.F.; Zhu, K.Y.; Subramanyam, B. Susceptibility of different life stages of *Tribolium confusum* to pyrethrin aerosol: Effects of flour source on insecticidal efficacy. *J. Pest Sci.* 2014, *87*, 295–300. [CrossRef]
- 106. Kavallieratos, N.G.; Boukouvala, M.C. Efficacy of d-tetramethrin and acetamiprid for control of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) adults and larvae on concrete. *J. Stored Prod. Res.* **2019**, *80*, 79–84. [CrossRef]
- 107. Farnham, A.W. The mode of action of piperonyl butoxide with reference to studying pesticide resistance. In *Piperonyl Butoxide*. *The Insecticide Synergist;* Jones, D.G., Ed.; Academic Press: London, UK, 1999; pp. 199–213.
- Carter, S.W.; Chadwick, P.R.; Wickham, J.C. Comparative observations on the activity of pyrethroids against some susceptible and resistant stored products beetles. J. Stored Prod. Res. 1975, 11, 135–142. [CrossRef]
- Noack, V.S.; Schmidt, H.U. Effects of repellents and insecticides using for impregnation of packing material on stored-product pests. Z. Angew. Entomol. 1981, 92, 202–212. [CrossRef]
- 110. Khan, V.M.A. Effectiveness of insecticides and repellents on stored product insect pests. *Anz. Schaedlingskd. Pflanzenschutz Umweltschutz* **1983**, *56*, 25–29. [CrossRef]
- 111. Mongkalangoon, P.; Grieco, J.P.; Achee, N.L.; Suwonkerd, W.; Chareonviriyaphap, T. Irritability and repellency of synthetic pyrethroids on an *Aedes aegypti* population from Thailand. *J. Vector Ecol.* **2009**, *34*, 217–224. [CrossRef] [PubMed]
- 112. Okamoto, H.; Sembo, S.; Ishiwatari, T.; Miyaguchi, J. Insecticidal activity of 8 household and hygiene insecticides against bedbug (*Cimex lectularius*). *Med. Entomol. Zool.* **2010**, *61*, 245–250. [CrossRef]
- 113. Lambkin, T.A.; Furlong, M.J. Metabolic mechanisms only partially explain resistance to pyrethroids in Australian broiler house populations of lesser mealworm (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* **2011**, *104*, 629–635. [CrossRef] [PubMed]
- 114. Jin, T.; Lin, Y.Y.; Jin, Q.A.; Wen, H.B.; Peng, Z.Q. Sublethal effect of avermectin and acetamiprid on the mortality of different life stages of *Brontispa longissima* (Gestro) (Coleoptera: Hispidae) and its larvae parasitoid *Asecodes hispinarum* Bouček (Hymenoptera: Eulophidae). *Crop Prot.* 2014, 58, 55–60. [CrossRef]
- 115. Maluta, N.K.P.; Lopes, J.R.S.; Fiallo-Olivé, E.; Navas-Castillo, J.; Lourenção, A.L. Foliar spraying of tomato plants with systemic insecticides: Effects on feeding behavior, mortality and oviposition of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and inoculation efficiency of tomato chlorosis virus. *Insects* 2020, 11, 559. [CrossRef]

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