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A Preliminary Analysis on the Insecticidal Effect of Cyantraniliprole against Stored-Product Pests

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Abstract: While existing insecticides are becoming obsolete, the need for research and application of alternative substances is growing. Cyantraniliprole is a second-generation ryanodine receptor with the ability to bind and activate the ryanodine receptors in insect striated muscle cells, causing continuous muscular contraction, paralysis, and death. Many studies indicate its effectiveness on a variety of insects, but its action on storage pests has not yet been reported. We successfully conducted experiments for the first time on adult *Tenebrio molitor*, *Tribolium confusum*, *Alphitobius diaperinus*, *Rhyzopertha dominica*, and *Trogoderma granarium* with application of cyantraniliprole, causing dose-dependent mortality. Bioassays were carried out in the laboratory, where experimental adults were sprayed with six concentrations of cyantraniliprole. Mortality was recorded at 7, 14, 21, and 28 days after exposure. Mean mortality, survival concentration, and survival time were estimated for each species. The concentrations with both the highest mortality and the lowest survival rate were 2500 and 3000 ppm. Our results indicate that the tested insecticide was effective against *T. confusum* adults and is a promising pesticide for use in storage facilities.

Keywords: storage pests; coleoptera; mortality; chemical control

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1. Introduction

The protection of stored agricultural products (cereals, legumes, flours, dried fruits, etc.) from insect pests is based on the use of two main categories of insecticides: fumigants and residual insecticides [1]. After the phase-out of methyl bromide, its main alternative aerial insecticide, phosphine, has been the most important fumigant for use on stored-grains and other durable products [2]. However, the continuous use of phosphine has certain disadvantages, such as corrosiveness and resistance development [2–5]. Moreover, new fumigants that can be used with success against stored-product pests (SPP) do not appear in the market due to reduced effectiveness or the high cost of the application and registration process [6]. In order to delay the development of resistance, new insecticides with different action mechanisms could be rotated with existing insecticides.

Cyantraniliprole is a second-generation ryanodine-receptor insecticide discovered after chlorantraniliprole by DuPont Crop Protection and belongs to the diamide family, group 28 [7–11]. New insecticides with different modes of action are necessary today to replace pyrethrins and other commonly used but less effective alternatives [8]. This new insecticide group acts by binding and activating the ryanodine receptors in insect striated muscle cells, provoking calcium release from internal stores and causing continuous muscular contraction, paralysis, and death [6–8]. Cyantraniliprole has been reported to be effective against a broad spectrum of insect pests, including lepidoptera, dipteran leafminers, aphids, leafhoppers, psyllids, beetles, whiteflies, thrips, and weevils [8,12–16]. Owing to its structural specificity to insect ryanodine receptors over mammalian counterparts, cyantraniliprole has also been shown to be safe for non-target vertebrates [8,16]. No studies on its efficiency against SPP have been carried out until today.

The aim of the present study is to investigate, for the first time, the insecticidal effect of cyantraniliprole against common SPP beetle species, such as the mealworm beetle (*Tenebrio molitor* L.), the confused flour beetle (*Tribolium confusum* Jacquelin du Val), the lesser mealworm (*Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)), the lesser grain borer (*Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae)), and the khapra beetle (*Trogoderma granarium* Everts (Coleoptera: Dermestidae)). Our results are discussed in the context of exploring new strategies of pest management in storage facilities and minimizing the damage caused by SPP.

2. Materials and Methods

2.1. Insects

Five major insect stored-product beetle species were selected for experimentation. The tested insect species were *T. molitor*, *T. confusum*, *A. diaperinus*, *R. dominica* and *T. granarium*. All these species are globally distributed stored-product pests and cause serious quantitative and qualitative losses in a vast range of commodities. Insects were reared at 27.5 °C and 75% relative humidity (r.h.). *T. molitor* and *A. diaperinus* were reared on wheat bran, *T. confusum* on whole wheat flour with 10% dried yeast, and *R. dominica* and *T. granarium* on hard wheat. A half kilogram of clean commodity was infested with ~50 adult beetles. Parental adults were left for one week for oviposition and removed. Infested commodities were kept in ambient conditions in order to obtain adult individuals of standardized age. Young adults (<2 weeks old) of mixed sex were used for experiments.

2.2. Formulation

The insecticidal formulation used for the experimentation was Benevia® which contains Cyazypyr® active—10.26% OD active ingredient (a.i.)

2.3. Commodities and Insecticides

Bioassays were carried out on hard wheat (*Triticum durum* var. Mexa). These grains were adjusted to 12% moisture content (m.c.), determined by a Farmex Mt-Pro Plus Moisture Meter (AgraTronix LLC, Streetsboro, OH, USA) via storage in ambient conditions for 28 d.

2.4. Bioassays

Individual lots of 500 g of wheat were placed in 0.45lt cylindrical glass jars. The product was directly sprayed with 1 mL of pesticide suspension using a Potter spray tower (Burkard Manufacturing Co., Ltd., Rickmansworth, Hertfordshire, UK) at 1 kgf/cm^2. The utilized pesticide concentrations were 250, 500, 1000, 1250, 1500, 2000, 2250, 2500, and 3000 ppm. Following the application of the pesticide, each wheat lot was placed back in the jar and shaken manually for 30 s to achieve equal distribution of the dose. A separate series of lots was sprayed with distilled water alone to serve as control. Twenty 10-g samples were taken from each jar and placed in 9 cm Petri dishes. The internal "neck side" of the Petri dishes was covered by fluon (Northern Products, Woonsocket, RI, USA) to prevent insect individuals from escaping. Next, ten adult individuals of similar age (2–3 weeks old) were placed within each Petri dish and then in plastic boxes with saturated solutions of sodium chloride to maintain 75% r.h. The Petri dishes were then transferred to incubators set at 27.5 °C and 75% r.h. All Petri dishes were opened, and adult mortality was recorded after 7 d, 14 d, 21 d, and 28 d. Each treatment was replicated twenty times ($20 \times 10 \times 5 = 1000$ Petri dishes for each replicate x dose x insect species).

2.5. Data Analysis

Mortality values were arcsine transformed to stabilize variance. Data were then analyzed by means of univariate ANOVA involving a multi-factor analysis, using the general linear model of the SPSS (SPSS Inc., Armonk, NY, USA, ver. 25). In the case of significant F values, means were compared using the Bonferroni test. LC50 values were calculated by probit analysis with 95% confidence interval (CI) of the SPSS (SPSS Inc., Armonk, NY, USA, ver. 25). We also applied the Kaplan-Meier method to determine the mean survival time of adults. Comparison of median survival time was performed using one-way ANOVA (dose as factor).

3. Results

All tested doses of cyantraniliprole were effective against beetle adults, with variation in virulence. As expected, the mortality percentage depended on the dose of cyantraniliprole. The final mortality percentages of *T. molitor* adults, which were recorded on day 28 after exposure, ranged from 13.3% (250 ppm) to 93.3% (3000 ppm) in the treatments with cyantraniliprole ($F = 3.181$, $df = 36.996$, $p < 0.001$) (Figure 1). The final mortality percentages of *T. confusum* adults ranged from 33.3% (250 ppm) to 100% (3000 ppm) ($F = 5.226$, $df = 36.996$, $p < 0.001$) (Figure 2). The medial survival time of tested adults after exposure to cyantraniliprole was dose-dependent. On the other hand, the medial survival time of control adults was longer in comparison to the medial survival time of adults that had been sprayed with cyantraniliprole. More specifically, the medial survival time of *T. molitor* adults, after treatment with different doses of cyantraniliprole, ranged from 26.7 (250 ppm) to 13.4 days (3000 ppm) ($F = 5.887$, $df = 9$, $p = 0.034$) (Table 1); for *T. confusum* adults, it ranged from 24.3 (250 ppm) to 7.7 days (3000 ppm) ($F = 2.113$, $df = 9$, $p < 0.001$) (Table 1). Control mortality was low for *T. molitor* adults, with a high median survival time (28 days). Control mortality of *T. confusum* with a high median survival time was 27.3 days (Table 1).

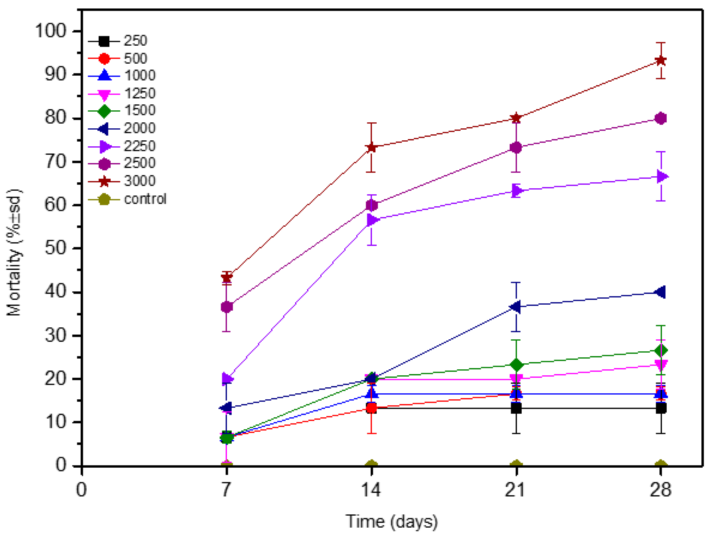

Figure 1. Mean mortality (% ± SD) of *T. molitor* adults sprayed with six concentrations of cyantraniliprole after 28 days.

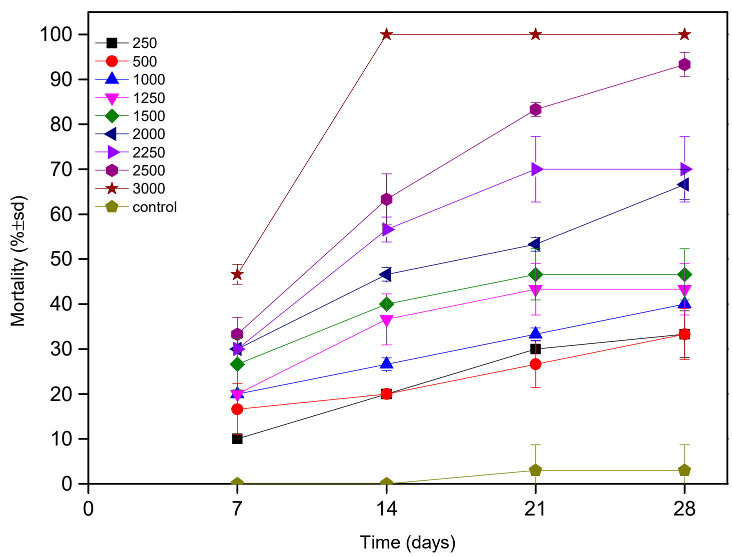

Figure 2. Mean mortality (% ± SD) of *T. confusum* adults sprayed with six concentrations of cyantraniliprole after 28 days.

Table 1. Median survival time (Days ± SD) of insect species adults. Mean ± SD values with the same letter within a column are not significantly different ($p < 0.05$).

Dose (ppm)	Insect Species				
	T. molitor	*T. confusum*	*A. diaperinus*	*R. domenica*	*T. granarium*
	Median Survival Time (Days ± SD)				
250	26.7 ± 0.8 [a]	24.3 ± 1.3 [a]	22.9 ± 1.7 [a]	25.3 ± 0.7 [a]	24.2 ± 1.7 [a]
500	25.6 ± 0.8 [a]	22.6 ± 0.8 [a]	21.6 ± 1.8 [a]	23.9 ± 1.1 [a]	23.9 ± 1.9 [a]
1000	25.3 ± 0.9 [a]	21.3 ± 1.1 [a]	20.3 ± 1.1 [a]	21.5 ± 1.3 [a]	21.5 ± 1.3 [a]
1250	24.2 ± 1.2 [a]	20.2 ± 1.0 [a]	19.2 ± 1.0 [a]	20.2 ± 1.1 [a]	20.2 ± 1.1 [a]
1500	23.8 ± 1.7 [a]	18.8 ± 1.3 [a]	18.6 ± 1.3 [a]	17.6 ± 1.1 [b]	17.7 ± 1.4 [b]
2000	20.2 ± 0.5 [b]	13.2 ± 1.5 [b]	15.3 ± 1.5 [a]	15.5 ± 2.1 [b]	16.5 ± 1.1 [b]
2250	16.5 ± 2.2 [b]	11.5 ± 1.2 [b]	13.5 ± 1.3 [a]	13.1 ± 1.4 [b]	15.1 ± 1.4 [b]
2500	15.7 ± 1.3 [c]	9.8 ± 1.3 [b]	10.8 ± 1.0 [b]	11.1 ± 1.0 [b]	13.1 ± 1.2 [b]
3000	13.4 ± 0.9 [c]	7.7 ± 0.2 [c]	9.9 ± 1.2 [b]	10.7 ± 1.2 [b]	12.2 ± 1.3 [b]
Control	28 ± 0.0 [d]	27.3 ± 0.5 [d]	26.9 ± 0.4 [c]	27.3 ± 0.4 [c]	27.3 ± 0.4 [c]

The final mortality percentages of *A. diaperinus* adults, which were recorded on day 28 after exposure, ranged from 26.6% (250 ppm) to 93.3% (3000 ppm) in the treatments with cyantraniliprole (F = 4.112, df = 36.996, $p < 0.001$) (Figure 3). The final mortality percentages of *R. domenica* adults ranged from 16.6% (250 ppm) to 86.6% (3000 ppm) (F = 5.222, df = 36.996, $p < 0.001$) (Figure 4). Finally, the final mortality percentages of *T. granarium* adults ranged from 26.6% (250 ppm) to 80% (3000 ppm) (F = 3.911, df = 36.996, $p < 0.001$) (Figure 5). Moreover, the medial survival time of *A. diaperinus* adults, after treatment with different doses of cyantraniliprole, ranged from to 9.9 days (3000 ppm) to 22.9 days (250 ppm) (F = 7.124, df = 9, $p = 0.032$) (Table 1). For *R. domenica* adults, it ranged from 10.7 days (3000 ppm) to 25.3 days (250 ppm) (F = 5.195, df = 9, $p < 0.001$) (Table 1). Lastly, for *T. granarium*, the median survival time ranged from 12.2 days (3000 ppm) to 24.2 days (250 ppm) (F = 6.669, df = 9, $p = 0.025$) (Table 1). Control mortality was low for *A. diaperinus* adults, with a long median survival time (26.9 days). Finally, the control mortality of *R. domenica* and *T. granarium* was minimal, with a long median survival time of 27.3 days.

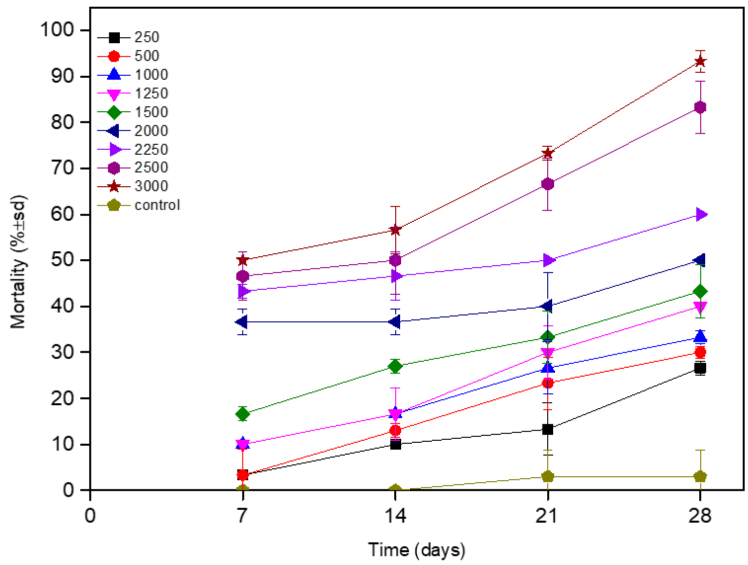

Figure 3. Mean mortality (% ± SD) of *A. diaperinus* adults sprayed with six concentrations of cyantraniliprole after 28 days.

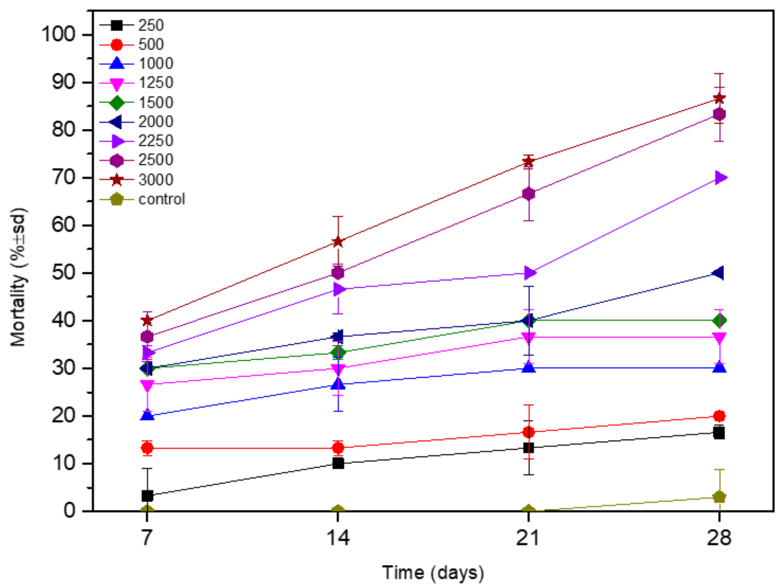

Figure 4. Mean mortality (% ± SD) of *R. domenica* adults sprayed with six concentrations of cyantraniliprole after 28 days.

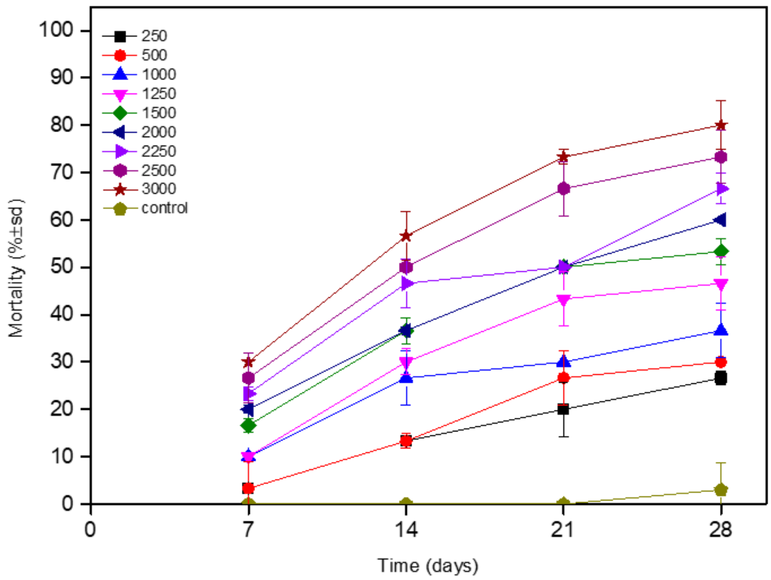

Figure 5. Mean mortality (% ± SD) of *T. granarium* adults sprayed with six concentrations of cyantraniliprole after 28 days.

The results of LC50 values of the tested adults are shown in Table 2. The estimated LC50 values were lower for *T. confusum* compared with the other species, indicating higher virulence of cyantraniliprole against *T. confusum.* Furthermore, *R. dominica* and *T. molitor* adults were more tolerant of cyantraniliprole.

Table 2. LC50 values (slope, intercept, chi-test, R^2) of the tested coleopteran adults.

Insect	Slope	Intercept	Chi-Test (x^2)	R^2	Lethal Dose (LD50) (ppm)
T. molitor	2.281	−2220	0.014	0.687	1466.54
T. confusum	1.818	−0.425	0.186	0.618	981.66
A. diaperinus	1.563	0.133	0.466	0.661	1342.45
R. domenica	1.933	−1.166	0.500	0.819	1557.80
T. granarium	1.354	0.751	0.945	0.886	1368.26

4. Discussion

Cyantraniliprole, the second-generation anthranilic diamide insecticide, has both foliar and systemic activity (for direct application to the soil) and targets a broad number of chewing and sucking pests by acting exclusively on their ryanodine receptors [17]. Insecticides such as this have a characteristic mode of action, targeting the ryanodine receptor at a site separate from ryanodine and causing the release of intracellular Ca2+ [18]. So far, cyantraniliprole has not been tested on stored-product pests, given that it has very low MRLs and has not yet been registered for application in storage facilities. However, the urgent and great need for active compounds and formulations in storage facilities has been well documented [19–21]. We hope that this study will make a valuable contribution to global research that is now in progress.

We demonstrated that when cyantraniliprole was applied to wheat, it was able to cause noteworthy mortality on all tested insect pests, especially in high doses (>2500 ppm) and long exposure-time intervals (>14 days). *T. confusum* adults exhibited the highest sensitivity in all doses compared with the other tested beetles. Experimental data from previously published bioassays of chemical insecticides against adults of the species tested in this study are provided in Table 3.

Table 3. Experimental data from previously published time–mortality bioassays of chemical insecticides against adults of the species tested in this study.

Species	Active Ingredient	Mortality	Remarks	Reference
T. molitor	pirimiphos-methyl	High	Phosphorylating the acetylcholinesterase (AChE)	[22]
T. confusum	pyrrole	High on barley and wheat	More effective on barley and wheat than on maize	[23]
A. diaperinus	cypermethrin	High	LC50 values = 68.1 to 6263 ng (AI)/cm^2	[24]
	dichlorvos	High	LC50 values = 10.3 to 1385 ng (AI)/cm^2	[24]
R. domenica	triflumuron	High	LC50 = 272 µg (AI)/mL of solution	[24]
	pirimiphos-methyl	High	Proved an efficacious method	[25]
	spinosad	High	Proved an efficacious method	[26]
T. granarium	cypermethrin	High	Higher mortalities recorded on wheat and barley than on rough rice and maize	[27]
	deltamethrin	High	Higher mortalities recorded on wheat and barley than on rough rice and maize	[27]
	pirimiphos-methyl	High	Phosphory-lating the acetylcho-linesterase (AChE)	[27]

Our results are similar to those reported in a recent study, where application of cyantraniliprole against adult *Hypothenemus hampei* Ferrari was found to be very toxic and had a strong effect upon topical application [14]. Cyantraniliprole has already been used as a seed treatment for the management of some chewing insects but not on stored pests. The toxicological effects of cyantraniliprole include rapid cessation in feeding, leading to lethargy, paralysis, and death [28]. Activity against the adult stage of stored pests has not previously been reported. Relevant effects of cyantraniliprole have been demonstrated, including reduced fecundity, fertility, feeding, oviposition, and mating of *Frankliniella occidentalis* Pergande [29]. Studies have shown that cyantraniliprole seed treatment significantly reduces infestation by the rice water weevil (*Lissorhoptrus oryzophilus* Kuschel), and rice cultivation treated with cyantraniliprole resulted in fewer larvae and pupae compared to rice treated with other insecticides [30,31]. High mortality of *Agrotis ipsilon* larvae also occurred when maize plants were treated with cyantraniliprole [12]. Cyantraniliprole is highly toxic to *Helicoverpa assulta* Guenée, and the LC05, LC15, and LC30 concentration of cyantraniliprole reduced the percentage of pupating larvae and increased the percentage of adults with deformities [32]. Tiwari and Stelinski [13] indicate that cyantraniliprole reduces feeding by *Diaphorina citri* adults. Cyantraniliprole has been shown to reduce feeding of other insect pests [33]. Moreover, cyantraniliprole provided moderate to very good reduction in leaf damage depending on larvae of tested *Plutella xylostella* L., *Trichoplusia ni* Hubner, *Spodoptera exigua* Hubner, and *Helicoverpa zea* Boddie [34]. On the other hand, the most tolerant species was *R. domenica*, demonstrating the highest LC50 value. Han et al. [35] observed that the proportion of *P. xylostella* L. adults with deformed wings in the parental generation increased when larvae were treated with chlorantraniliprole at LC10 and LC25 concentrations.

The survival time of tested adults was significantly shorter than that of the control adults. The dose effect on survival time was obvious because it was at the highest doses where the survival time was the shortest. The doses of insecticides not only affect insect physiology but also alter insect behavior [36]. Zhang et al. [37] showed that a subsurvival dose of cyantraniliprole increased the mating competitiveness of treated *Bactrocera dorsalis* (Hendel) males, as well as the number of times a cyantraniliprole-treated mating pair mated. Moreover, the significant effect of cyantraniliprole on feeding behavior was analyzed using electrical penetration graphing (EPG) and was observed in several insects, including *Myzus persicae* (Sulzer), *Bactericera cockerelli* (Sulc), *Frankliniella fusca* (Hinds), and *F. occidentalis* [38–40]. Piercing and sucking insect pests not only cause direct damage to plants by stylet probing but also indirectly by transmitting plant viruses between plants during feeding [41]. Cyantraniliprole has excellent cross-spectrum activity against a wide range of pests, including lepidoptera, dipteran leafminers, fruit flies, beetles, whiteflies, thrips, aphids, leafhoppers, psyllids, and weevils [42].

5. Conclusions

In conclusion, our preliminary results show that the tested insecticide is a promising control agent for all tested stored-product pests, demonstrating increased effectiveness, especially against *T. confusum*. However, there are some obstacles to overcome prior to its registration and application in storage facilities. Additional experimentation is required to achieve thorough evaluation of this insecticide as a control agent by introducing more abiotic and biotic factors, such as certain larval instars, commodities, temperatures, and RH levels. Apart from that, higher doses than those tested in our study may be required to achieve total mortality under real conditions, and that may cause problems with MRLs in stored products. Moreover, to obtain comprehensive information on the application of cyantraniliprole on these stored pests, a study of a possible genetic variability in response to doses should also be carried out.

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