Efficacy of Hygienic Insecticides in Controlling Tobacco Beetles (*Lasioderma serricorne*)

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Abstract: Tobacco beetles (*Lasioderma serricorne* Fabricius) are significant pests in tobacco warehouses. To control tobacco beetles in environments such as tobacco warehouses, surrounding areas, and tobacco processing areas, we compared the biological toxicity of seven insecticides: K-Obiol®, Crackdown®, Aqua-K-Othrine®, Temprid®, alphacypermethrin, Actellic, and Demand. We evaluated their median lethal doses (LD_{50}) using the drop method on tobacco beetle larvae, which allowed us to select the most effective insecticide for controlling tobacco beetles in warehouses through ultra-low-volume disinfection. The results showed that (1) the LD_{50} values for these pesticides were as follows: 3.55×10^{-3} μg for K-Obiol®, 1.47×10^{-2} μg for Crackdown®, 1.66×10^{-2} μg for Aqua-K-Othrine®, 1.51×10^{-2} μg for Temprid®, 5.40×10^{-3} μg for alphacypermethrin, 2.02×10^{-1} μg for Actellic, and 1.64×10^{-3} μg for Demand. Among these insecticides, Demand, K-Obiol®, and alphacypermethirn exhibited the highest toxicity to tobacco beetle larvae, while the toxicity of Actellic to tobacco beetle larvae was the lowest. (2) Demand, K-Obiol®, and alphacypermethrin were selected to control tobacco beetles in warehouses. Their control efficiencies were as follows: 97.54% for Demand, 95.31% for K-Obiol®, and 93.71% for alphacypermethrin.

Keywords: Hygienic Insecticides; Median Lethal Dose; Tobacco Beetles (*Lasioderma serricorne* Fabricius); Storage Pests; Toxicity

1. Introduction

Warehouse pests are widely distributed in warehouses, processing plants, residential environments, and other locations, where they inflict damage on grains, dried fruits, medicinal materials, tobacco, etc. In the grain industry, these pests not only consume grains directly but also lay eggs and excrete waste in the grains, leading to a decline in grain quality [1]. It is estimated that annual grain losses attributable to storage pests account for approximately 10% of total grain storage, amounting to as much as 2 billion yuan [2]. In the tobacco industry, principal warehouse pests include the tobacco beetle (*Lasioderma serricorne* Fabricius) and the tobacco powder moth (*Ephestia elutella*), and the larvae of these pests bore into tobacco leaves. The tobacco beetle is a pervasive threat globally to tobacco [3,4]. These pests produce a large amount of fecal matter, leave behind insect corpses, and impart an unpleasant flavor, thereby compromising the integrity and quality of tobacco leaves and products. This poses a serious threat to the availability and quality of tobacco leaf raw materials and cigarettes, resulting in substantial economic losses in China [5-8].

In tobacco warehouses, the predominant method of control is aluminum phosphide fumigation. However, its high toxicity poses significant environmental and safety concerns for humans and livestock [9,10]. In recent years, botanical insecticides have garnered considerable attention due to their low toxicity and eco-friendly attributes [11-18]. Plant extracts primarily function through contact killing, repelling, suppressing, and fumigating effects on storage pests. Botanical insecticides are environmentally friendly; however, they are more costly, more volatile, less stable, and involve complex production processes. Scaling up for commercial use presents challenges, hindering widespread application and adoption [19-21].

In general, the primary measure for preventing storage pests is prior prevention, combined with control strategies. Among these, cleanliness and sanitation, as well as disinfection during warehouse vacancies, are important preventive measures. These practices can notably reduce the density of storage pests and prevent secondary cross-infection of pests. Using hygienic pesticides for disinfection in empty warehouses, alongside maintaining cleanliness and sanitation, are fundamental methods for controlling warehouse pests and play a positive role in preventing their occurrence and damage. This study compared the toxicity of seven hygienic insecticides against tobacco beetles, aiming to identify low-toxicity and high-toxicity hygienic pesticides. Our findings serve as a guide for the selection and use of pesticides to control tobacco beetle damage.

2. Materials and Methods

2.1 Materials

Tested reagents included 25 g/L deltamethrin (K-Obiol®), 25 g/L deltamethrin (Crackdown®), 2% deltamethrin (Aqua-K-Othrine®), 31% lambda-cyhalothrin and imidacloprid (Temprid®), 10% of 1000 g/L alphacypermethrin, 500 g/L methylpyrimidinophosphore (Actellic), and 25 g/L cyfluthrin (Demand).

Instruments and equipment: glass bottles, pipettes, Petri dishes, an incubator, test tubes, and ultra-low volume sprays. Tobacco beetle (L. serricorne) larvae, aged 3–4 instars and of the same size, were used as test insects. The feed consisted of 90% whole wheat flour and 10% yeast. The incubation temperature was maintained at 28 ℃, with a humidity level of 70% ± 5%.

2.2 Methods

Seven Petri dishes were taken as a group, and 30 larvae of the same size were placed into each Petri dish for standby.

For the preparation of the reagent concentration gradient, a group of seven test tubes labeled 7, 6, 5, 4, 3, 2, and 1, respectively, were used. A pipette was used to transfer 0.4 mL of the reagent into test tube 7, and distilled water was added to make a constant volume of 12 mL. Subsequently, 6 mL from test tube 7 was transferred into test tube 6, and the volume in test tube 6 was adjusted to 12 mL with distilled water. This process was repeated, transferring 6 mL from test tube 6 into test tube 5 and adjusting the volume to 12 mL, continuing this pattern until test tube 1. This procedure resulted in reagent dilutions of 1,920, 960, 480, 240, 120, 60, and 30-fold. Distilled water treatment was used as control.

The tested tobacco beetle larvae were subjected to the drop method. The culture dishes were numbered according to the gradient of dilution ratios. A pipette gun was used to draw the reagent corresponding to the dilution ratio (1 μL for K-Obiol®, Crackdown®, Aqua-K-Othrine®, and Temprid®, and 0.5 μL for others). The heads of each larva were inoculated in the culture dish, which was then placed in an incubator for 24 h. After the incubation period, the culture dishes were removed, and the death of the tobacco beetle larvae was observed. The larvae were gently touched with a brush; those showing no or slight response were considered dead. The death rate and corrected death rate were calculated using the following formulas:

$$\text{Death rate (96)} = \frac{\text{Number of dead tobacco beetles}}{\text{Total number of tobacco beetles}} \times 100 \quad (1)$$

Corrected death rate (%) = (survival rate of the control group - survival rate of the experimental group)/survival rate of the control group × 100

Three warehouses, each as large as 2000 m^3, were chosen. Tobacco beetle lures (SERRICO®, FUJI FLAVOR Co., Ltd.) were hung in each warehouse at a density of one per 400 m^3 before and after ultra-low volume spraying disinfection. The number of tobacco beetle imagoes captured by lures was counted 7 days before and after disinfection. For disinfection, each insecticide was sprayed at a dose of 0.05 mL/m^3, diluted by a factor of 10 with water, in an ultra-low volume. Subsequently, the controlling efficiency was calculated using the following formula:

Controlling efficiency (%) = (number of captured individuals before disinfection - number of captured individuals after disinfection)/number of captured individuals before disinfection × 100

2.3 Toxicity Calculation

Using the logarithm of the dose-probit of death curve method, we derived the negative logarithm of the dose and the mortality rate through a linear regression equation. The corresponding value was determined when the probability of death reached 5.0%. Subsequently, the median lethal dose (LD_{50}) could be calculated. A lower LD_{50} value indicated greater toxicity.

3. Results

3.1 Toxicity of Hygienic Insecticides

We recorded the mortality rates of tobacco beetle larvae in each experimental group and calculated the probit death rates using tabulated data. Please refer to Tables 1–7 for the toxicity test results of these reagents.

Table 1. Biological Toxicity Assay of K-Obiol® Against Tobacco Beetle Larvae.

Treatment	Dose (10^{-3}μg)	Number of inoculated larvae	Number of dead larvae	Corrected death rate	Death rate probit	LD negative logarithm
Control	0.00	60	1		-	-
1	1.302	33	11	32.20%	4.5379	2.885
2	2.604	30	12	38.98%	4.7207	2.584
3	5.208	30	17	55.93%	5.1484	2.283
4	10.417	30	21	69.49%	5.5101	1.982
5	20.833	30	26	86.44%	6.0985	1.681
6	41.667	30	29	96.61%	6.8250	1.380
7	83.333	31	30	96.72%	6.8384	1.079

Table 2. Biological Toxicity Assay of Crackdown® Against Tobacco Beetle Larvae.

Treatment	Dose (10^{-3}μg)	Number of inoculated larvae	Number of dead larvae	Corrected death rate	Death rate probit	LD negative logarithm
1	1.302	30	3	8.47%	3.6278	2.885
2	2.604	30	5	15.25%	3.9763	2.584
3	5.208	30	12	38.98%	4.7207	2.283
4	10.417	30	14	45.76%	4.8945	1.982
5	20.833	30	20	66.10%	5.4152	1.681
6	41.667	31	22	70.48%	5.5388	1.380
7	83.333	30	22	72.88%	5.6098	1.079

Table 3. Biological Toxicity Assay of Aqua-K-Othrine® Against Tobacco Beetle Larvae.

Treatment	Dose(10^{-3}μg)	Number of inoculated larvae	Number of dead larvae	Corrected death rate	Death rate probit	LD negative logarithm
1	2.08	30	4	11.86%	3.82	2.681
2	4.17	30	6	18.64%	4.1073	2.380
3	8.33	30	10	32.20%	4.5379	2.079
4	16.67	30	16	52.54%	5.0627	1.778
5	33.33	31	21	67.20%	5.4454	1.477
6	66.67	30	22	72.88%	5.6098	1.176
7	100.00	30	27	89.83%	6.2702	1.000

Table 4. Biological Toxicity Assay of Temprid® Against Tobacco Beetle Larvae.

Treatment	Dose(10^{-3}μg)	Number of inoculated larvae	Number of dead larvae	Corrected death rate	Death rate probit	LD negative logarithm
1	5.05	30	6	18.64%	4.1073	2.297
2	10.09	28	11	38.26%	4.7024	1.996
3	20.18	30	17	55.93%	5.1484	1.695
4	40.36	30	24	79.66%	5.831	1.394
5	80.73	30	28	93.22%	6.4909	1.093
6	161.46	30	29	96.61%	6.825	0.792
7	322.92	31	26	83.60%	5.9782	0.491

Note: The control group for Crackdown®, Aqua-K-Othrine®, and Temprid® is the same as that for K-Obiol®.

Table 5. Biological Toxicity Assay of Alphacypermethrin Against Tobacco Beetle Larvae.

Treatment	Dose(10^{-3}μg)	Number of inoculated larvae	Number of ead larvae	Corrected death rate	Death rate probit	LD negative logarithm
Control	0.00	30	0	0%	-	-
1	0.87	30	8	26.67%	4.3781	3.06
2	1.74	30	10	33.33%	4.5684	2.76
3	3.47	30	13	43.33%	4.8313	2.46
4	6.94	30	16	53.33%	5.0853	2.16

5	13.89	30	20	66.67%	5.4316	1.86
6	27.78	30	21	70.00%	5.5244	1.56
7	55.56	30	24	80.00%	5.8436	1.26

Table 6. Biological Toxicity Assay of Actellic Against Tobacco Beetle Larvae.

Treatment	Dose(10^{-3}μg)	Number of inoculated larvae	Number of dead larvae	Corrected death rate	Death rate probit	LD negative logarithm
Control	0.00	30	0	0%	-	-
1	4.34	30	1	3.33%	3.1616	2.36
2	8.68	30	1	3.33%	3.1616	2.06
3	17.36	30	2	6.67%	3.5015	1.76
4	34.72	30	7	23.33%	4.2710	1.46
5	69.44	30	12	40.00%	4.7467	1.16
6	138.89	30	13	43.33%	4.8313	0.86
7	277.78	30	17	56.67%	5.1687	0.56

Table 7. Biological Toxicity Assay of Demand to Tobacco Beetle Larvae.

Treatment	Dose(10^{-3}μg)	Number of inoculated larvae	Number of dead larvae	Corrected death rate	Death rate probit	LD negative logarithm
Control	0.00	30	0	0%	-	-
1	0.22	30	9	30.00%	4.4756	3.66
2	0.43	30	12	40.00%	4.7467	3.36
3	0.87	30	14	46.67%	4.9172	3.06
4	1.74	30	16	53.33%	5.0828	2.76
5	3.47	30	17	56.67%	5.1687	2.46
6	6.94	30	17	56.67%	5.1687	2.16
7	13.89	30	21	70.00%	5.5244	1.86

Subsequently, we calculated the linear regression equation between the negative logarithm of dose and the probability of death, resulting in curves with their respective regression formulas shown in Figures 1–7. According to Tables 1–7 and Figures 1–7, we calculated the LD_{50}, as shown in Table 8.

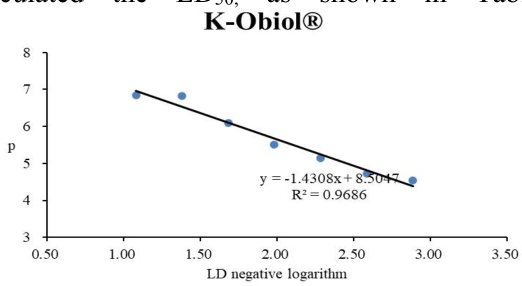

Figure 1. Regression Line of the Toxicity of K-Obiol® Against Tobacco Beetle Larvae.

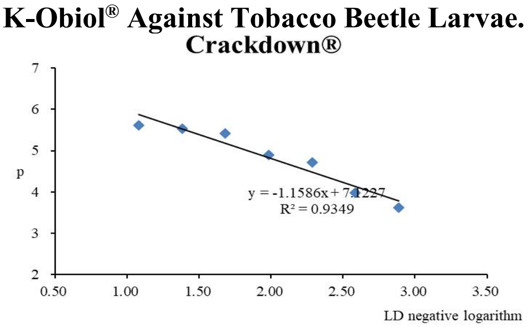

Figure 2. Regression Line of the Toxicity of Crackdown® Against Tobacco Beetle Larvae.

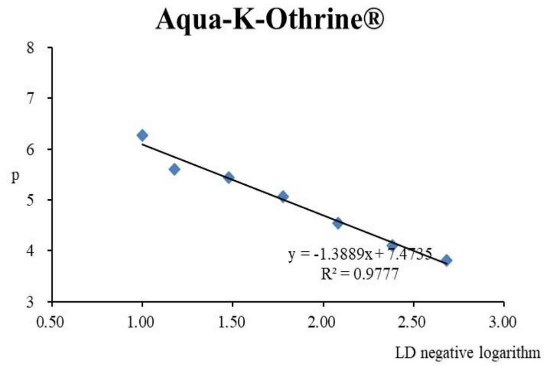

Figure 3. Regression Line of the Toxicity of Aqua-K-Othrine® Against Tobacco Beetle Larvae.

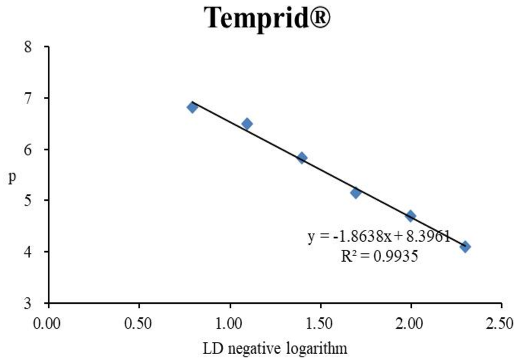

Figure 4. Regression Line of the Toxicity of Temprid® Against Tobacco Beetle Larvae.

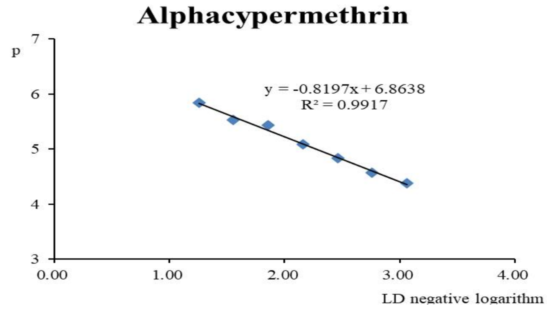

Figure 5. Regression Line of the Toxicity of Alphacypermethrin Against Tobacco Beetle Larvae.

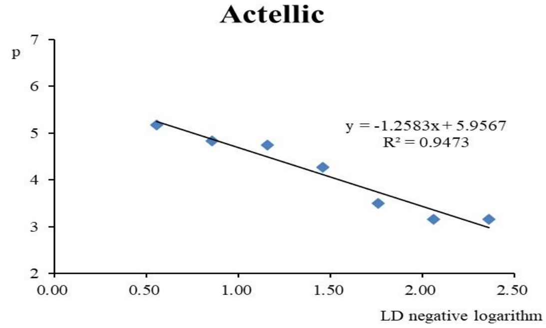

Figure 6. Regression Line of the Toxicity of Actellic Against Tobacco Beetle Larvae.

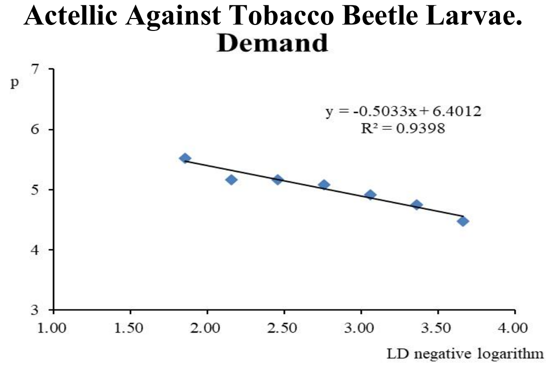

Figure 7. Regression Line of the Toxicity of Demand Against Tobacco Beetle Larvae.

Table 8. Median Lethal Dose of the Reagents.

Reagent name	Median lethal dose
K-Obiol®	3.55×10^{-3}μg
Crackdown®	1.47×10^{-2}μg
Aqua-K-Othrine®	1.66×10^{-2}μg
Temprid®	1.51×10^{-2}μg
Alphacypermethrin	5.40×10^{-3}μg
Actellic	2.02×10^{-1}μg
Demand	1.64×10^{-3}μg

The LD_{50} of the insecticides against tobacco beetle larvae were as follows: K-Obiol®, 3.55×10^{-3} μg; Crackdown®, 1.47×10^{-2} μg; Aqua-K-Othrine®, 1.66×10^{-2} μg; Temprid®, 1.51×10^{-2} μg; alphacypermethrin, 5.40×10^{-3} μg; Actellic, 2.02×10^{-1} μg; Demand, 1.64×10^{-3} μg. The toxicity ranking from highest to lowest according to LD_{50} was: Actellic > Aqua-K-Othrine® > Temprid® > Crackdown® > alphacypermethrin > K-Obiol® > Demand. Therefore, Demand exhibited the highest toxicity against tobacco beetle larvae, while Actellic exhibited the lowest toxicity against tobacco beetle larvae.

3.2 Efficacy of Hygienic Insecticides in Controlling Tobacco Beetle

According to the toxicity results of the various hygienic insecticides, Demand, K-Obiol®, and alphacypermethrin were selected as warehouse insecticides against tobacco beetles. Subsequently, we monitored the number of tobacco beetle imagoes captured by lures 7 days before and after disinfection. The results are presented in Table 9.

Table 9. Efficacy of Hygienic Insecticides in Controlling Tobacco Beetles.

Hygienic insecticide	Captured individuals 7d before disinfection	Captured individuals 7d after disinfection	Controlling efficiency
K-Obiol®	128	6	95.31%
Alphacypermethrin	175	11	93.71%
Demand	203	5	97.54%

According to Table 9, we found that these three insecticides exhibited high efficacy in controlling tobacco beetles, with Demand showing the highest effectiveness.

4. Discussion

The tobacco beetle is a common pest found in tobacco storage and processing facilities. While aluminum phosphide fumigation is primarily used to control infestations in tobacco bales within warehouses, challenges persist in processing workshops. Therefore, basic methods such as cleaning and disinfection are essential for controlling infestations in warehouses. This study assessed seven hygienic insecticides for their LD_{50}. Actellic exhibited the lowest LD_{50} at 2.02×10^{-1} μg, while Demand exhibited the highest LD_{50} at 1.64×10^{-3} μg, indicating a 10-fold difference from Actellic. Different insecticides vary notably in their effectiveness at controlling tobacco beetles, likely due to differences in their active ingredients and formulations.

Employing K-Obiol®, alphacypermethrin, and Demand for warehouse disinfection according to the results of the toxicity tests of hygienic insecticides, where control efficiency was measured based on changes in captured tobacco beetles before and after disinfection, may exhibit some drawbacks. Traps cannot capture all tobacco beetle imagoes in warehouse facilities, and their numbers are influenced by the development periods of tobacco beetles. However, traps can serve to monitor tobacco beetles, indicating their density and the extent of infestation.

Moreover, the long-term use of a single insecticide increases the risk of resistance development [22]. This study identified various hygienic insecticides with different active ingredients that can be alternatively employed for the control of tobacco beetles in warehouses, thereby reducing the risk of resistance.

5. Conclusion

We evaluated the biological toxicity of seven insecticides to tobacco beetles using the drop method, measured by LD_{50}: 3.55×10^{-3} μg for K-Obiol®, 1.47×10^{-2} μg for Crackdown®, 1.66×10^{-2} μg for Aqua-K-Othrine®, 1.51×10^{-2} μg for Temprid®, 5.40×10^{-3} μg for alphacypermethrin, 2.02×10^{-1} μg for Actellic, and 1.64×10^{-3} μg for Demand. Among these reagents, Demand, K-Obiol®, and alphacypermethirn exhibited the highest toxicity to tobacco beetle larvae, while Actellic showed the lowest toxicity.

The results of ultra-low volume disinfection showed that Demand, K-Obiol®, and alphacypermethrin can effectively control tobacco beetles in warehouses, with control efficiencies of 97.54% for Demand, 95.31% for K-Obiol®, and 93.71% for alphacypermethrin, with Demand achieving the highest efficiency.

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