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Caffeic Acid and Biopesticides Interactions for the Control of Storage Beetles

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Abstract: Infestations of stored-product pests cause significant losses of agricultural produce every year. Despite various environmental and health risks, chemical insecticides are now a ready-to-use solution for pest control. Against this background and in the context of Integrated Pest Management research, the present study focuses on the potential insecticidal effect of caffeic acid at five different concentrations (250, 500, 750, 1500 and 3000 ppm), and their combination with *Cydia pomonella* Granulovirus (CpGV), *Bacillus thuringiensis* subsp. tenebrionis and *Beauveria bassiana* strain GHA on three major insect stored-product beetle species, *Tribolium confusum* (Coleoptera: Tenebrionidae), *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) and *Trogoderma granarium* Everts (Coleoptera: Dermestidae). Treatment efficacy was expressed as mortality in relation to exposure time and adult species number. Compared to the control, the results showed a clear dose-dependent pesticidal activity, expressed as significant adult mortality at a high-dose application, although some of the combinations of caffeic acid concentrations with the other substances acted positively (synergistically and additively) and some negatively. Based on our results, bioinsecticides can be combined with plant compounds such as caffeic acid and be integrated with other modern IPM tools in storage facilities.

Keywords: caffeic acid; biopesticides; *Cydia pomonella* Granulovirus; *Bacillus thuringiensis* subsp. tenebrionis; *Beauveria bassiana*; interactions; stored pests

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1. Introduction

Storage pests can cause significant economic losses by contaminating stored products, resulting in both quantitative and qualitative deterioration. The deterioration of stored commodities is caused not only by the consumption of the product, but also by the contamination of dead skin, excreta and dead insects, that can be dangerous for human health because they cause allergic reactions [1,2]. Moreover, the presence of insect populations in stored products can considerably increase relative humidity, which promotes secondary fungal infestations [3]. Most agricultural products can be affected by such infestations, resulting in annual losses of 9–20% [4].

Practices such as sanitation, aeration cooling, drying and controlled atmospheres are implemented, but are not sufficient to effectively control insect infestations in storage facilities [3]. Until now, fumigation with synthetic insecticides such as phosphine was primarily applied in storage facilities for disinfestation, but the increasing hazards to human health and the environment restricted their use [5,6]. Needless to say, the overreliance on these substances all these years has led to resistance development, [7] and the neglect of research into alternative control methods [6].

Due to the above facts, new investigations have recently emerged aimed at finding more ecological methods for the management of storage pests, by utilizing natural plant compounds or more specific products of plants' secondary metabolism such as essential oils. Apart from the fact that they do not pollute the environment, they are very effective against insects due to their volatility [8]. Substances derived from metabolic reactions of plants can be bioactive towards insects, as they are part of their natural defense mechanisms and include compounds such as terpenes, flavonoids, alkaloids, polyphenols, quinones, and others [9]. Plant extracts and essential oils can exert a wide range of actions against insects, such as toxicity, repellency, inhibition of respiration, oviposition, growth or feeding and a reduction in adult emergence and abnormalities in larvicidal transitions [10–12].

Phenolic acids such as salicylic, coumaric, caffeic and chlorogenic acids are ubiquitously present in plants and mostly participate in plant defense mechanisms [13]. Some of these substances have already been investigated to utilize the natural immunity of plants in the concept of biological control in agriculture. Caffeic acid (CA) is an early intermediate of phenylpropanoid metabolism, and a precursor for structural polyphenols and many biologically active secondary compounds that are important in the plant defense mechanisms [14,15]. This specific phenolic compound has been attributed to antifungal, antibacterial and insecticidal properties [15].

Another promising aspect of insect biological control is the use of entomopathogens. This approach has been thoroughly investigated lately as they offer a great alternative in the context of integrated pest management (IPM). Viruses, bacteria and fungi have been described as effective against various insect species [16–18]. These insect pathogens are not hazardous as they already exist in nature and so have a very low environmental impact and low mammalian toxicity [19,20]. There have been some studies that investigated the synergistic effect of insect pathogens with biopesticides, and the results have varied between a lesser, zero or enhanced efficacy against arthropods.

In this context, the present study aimed to investigate the efficacy of CA, in combination with commercially available biopesticides (fungal, viral and bacterial) on three major insect stored-product beetle species. All tested species are globally distributed stored-product pests and cause serious quantitative and qualitative losses in a vast range of commodities. Our results are discussed in the context of enhancing the use of insect pathogens as a key component of integrated pest management against stored-product pests.

2. Materials and Methods

2.1. Insect Rearing

Three important stored-product beetle species were selected for experimentation. The insect species tested were *T. confusum*, *C. ferrugineus* and *T. granarium*. Insects were reared in incubators (PHC Europe/Sanyo/Panasonic Biomedical MLR-352-PE) at 27.5 °C and 75% relative humidity (r.h.). *T. granarium* was kept on whole wheat, *C. ferrugineus* on rolled oats with 5% brewer's yeast, and *T. confusum* on whole wheat flour with 10% brewer's yeast. Adults of uniform age (<2 weeks old) and mixed sex were used for experimentation.

2.2. Caffeic Acid Solution and Biopesticides

The solution was obtained for Karvelas AVEE with lot number 15038821. The composition of the tested solution was natural caffeic acid at 1120 mg/kg, conductivity 97.9 mS/cm, pH 4.62 and density 1.215 g/cm^3.

Biopesticides tested during the present study were commercial formulations obtained from the market. Specifically, we used Madex® (*Cydia pomonella* granulovirus (CpGV) (Hellafarm, Athens, Greece), Novodor® FC (*Bacillus thuringiensis* subsp. *Tenebrionis* 3%) (BIOFA Germany, Bad Boll, Germany) and Botanigard® 10.7SC (*Beauveria bassiana* strain GHA 10.735%) (K&N Efthymiadis Single Member S.A., Thessaloniki, Greece).

2.3. Experimental Procedure

500 g of wheat (var. Mexa) were divided into separate lots and filled into 0.45 L cylinder jars. Since it is difficult for these species to reproduce on intact grains, the wheat used had 5% broken kernels. The wheat was stored for 28 days under ambient conditions to adjust the moisture content (m.c.) to 12%.

Experimentation included five concentrations of CA solution (Karvellas AVEE, Thiva, Greece) (250 ppm, 500 ppm, 750, ppm, 1500 ppm and 3000 ppm) and one (3000 ppm) for commercial biopesticides. The solvent used to prepare all solutions was distilled water. Twenty 10 g wheat samples were taken from the jars and placed in 9 cm Petri dishes. Following this, ten adult beetles of each species, of uniform age (<2 weeks old) and mixed sex, were transferred to each Petri dish. The inner "neck" of the Petri dish was covered with fluon to prevent insect escape (Northern Products, Woonsocket, RI, USA). A Potter spray tower (Burkard Manufacturing Co., Ltd., Rickmansworth, Hertfordshire, UK) was used to apply the solutions to the products at a rate of 1 kgf cm^2. For separate doses testing, the experimental adults were sprayed once with 2 mL of the CA or biopesticide. Conversely, for the combined treatments, spraying was performed twice, once with 2 mL of the CA solution and once with 2 mL of the biopesticide solution, each 2 s apart. The Petri dishes were then transferred to Toshiba incubators (PHC Europe/Sanyo/Panasonic Biomedical MLR-352-PE) and set at 27.5 °C and 75% relative humidity. The beetles were observed daily, and mortality was recorded 7, 14, 21, and 28 days after treatment.

The entire procedure was repeated twenty times by preparing new batches of treated and untreated grains at each replicate (separate treatments: 9 × 3 × 20 = 540 Petri dishes for each dose × insect species × replicate, combined treatments: 15 × 3 × 20 = 900 Petri dishes for each dose × insect species × replicate).

2.4. Mathematical Estimation and Statistical Analysis

The interaction between the CA and the biopesticides was estimated using the formula of Robertson and Preisler:

$$P_E = P_0 + (1 - P_0) \times (P_1) + (1 - P_0) \times (1 - P_1) \times (P_2),$$

where: P_E is the expected mortality induced by the combined treatment; P_0 is the mortality of the control; P_1 is the mortality caused by the CA; P_2 is the mortality caused by the biopesticide.

Distribution was determined by the chi-square formula: $x^2 = (L_0 - L_E)^2/L_E + (D_0 - D_E)^2/D_E$ where L_0 is the number of living adults, D_0 is the number of dead larvae, L_E is the expected number of live larvae, and D_E is the expected number of dead larvae. The formula was used to test the hypothesis independent–simultaneous relationship (1 df, $p = 0.05$). If $x^2 < 3.84$, the ratio is defined as additive (A); if $x^2 > 3.84$ and the observed mortality is higher than expected, the relationship is defined as synergistic (S). On the contrary, if $x^2 > 3.84$ and the observed mortality is less than expected, the relationship is defined as competitive (C).

The general linear model of SPSS (version 23.0, IBM Corp., Armonk, NY, USA) was then used to evaluate the data using a three-way ANOVA (IBM 2014). The Bonferroni test was used to compare means in cases where there were substantial F values.

3. Results

The results of the laboratory bioassays on adults of *T. granarium*, *C. ferrugineus*, and *T. confusum* showed that separate treatments with CA and all pathogens caused varying degrees of time-, treatment- and dose-dependent mortality. Adult mortality of *T. granarium* was 57–73%, of *C. ferrugineus* was 43–67%, and of *T. confusum* was 27–67% twenty-eight days after treatment with CA solution at the highest dose (3000 ppm). After twenty-eight days, the application of *B. thuringiensis* caused 67% mortality in *T. granarium* adults, 73% in *C. ferrugineus*, and 69% in *T. confusum*. After twenty-eight days of CpGV treatment, the observed mortality of adults of *T. granarium*, *C. ferrugineus*, and *T. confusum* was 70%, 43%, and 47%, respectively. The mortality of *T. confusum*, *C. ferrugineus*, and *T. granarium* after twenty-eight days of treatment with *B. bassiana* was 93%, 77%, and 93%, respectively. In all of the tested insects, the control mortality was less than 3%.

According to results of the combined bioassays, all combinations tested induced various levels of time- and dose-dependent mortality (Table 1). The results of the combined treatments showed a distinct interaction between treatments, as follows: for *T. granarium* adults, the interaction between the pathogens was additive in nine combinations the first seven days, synergistic in two and antagonistic in five. The following fourteen days, the interactions proved to be additive in seven combinations, synergistic in one and antagonistic in six. After twenty-one days, the interaction was additive in eight combinations and competitive in seven (Table 1). Finally, twenty-eight days later, the interaction was characterized as additive in seven combinations and competitive in eight (Table 1). Adult *T. granarium* mortality was between 37 and 100% (F: 19.764; df: 654.2360; *p*: <0.001) (overall 15 treatments).

Interactions between treatments on *T. confusum* for seven days were additive in ten combinations, synergistic in four combinations and competitive in one combination. For fourteen days, the interactions between treatments were all additive. At twenty-one days, the interaction between treatments was additive in fourteen combinations and synergistic in one combination (Table 2). As for the twenty-eighth day, the interaction between treatments was additive in fourteen combinations and synergistic in one combination (Table 2). Adult *T. confusum* mortality ranged from 27 to 100% (F: 20.764; df: 654.2360; *p*: <0.001) (overall 15 treatments).

Table 1. Percentage of observed and expected mortality of *T. granarium* adults at seven, fourteen, twenty-one and twenty-eight days of the experiment, treated with treatments in several combinations, and their interactions (n = 100).

Treatment		Mortality Observed% [1]	Mortality Expected% [2]	x^2	Interaction [3]	Mortality Observed%	Mortality Expected%	x^2	Interaction	Mortality Observed%	Mortality Expected%	x^2	Interaction	Mortality Observed%	Mortality Expected%	x^2	Interaction
Entomopathogen (3000 ppm)	caffeic acid (ppm)	7 days				14 days				21 days				28 days			
Bacillus thuringiensis [4]	250	37	55	7.0100	C	37	75	18.7841	C	43	84	46.0425	C	47	89	30.6352	C
	500	37	63	7.0169	C	37	75	18.5344	C	47	86	40.2042	C	47	89	30.0133	C
	750	37	61	7.0094	C	40	75	17.4215	C	47	84	34.6456	C	53	89	29.2205	C
	1500	40	61	7.0292	C	47	74	15.5904	C	47	85	29.3120	C	57	89	23.8527	C
	3000	43	63	7.0450	C	57	79	4.3251	C	60	85	11.9527	C	67	93	18.5516	C
Cydia pomonella Granulovirus (CpGV) [5]	250	40	42	0.0435	A	57	67	0.7815	A	62	78	3.2709	A	69	90	7.6806	C
	500	57	53	0.3601	A	62	67	0.0967	A	70	80	1.9105	A	77	90	2.7879	A
	750	60	50	1.6065	A	65	67	0.1029	A	70	78	0.8179	A	77	90	2.7879	A
	1500	67	50	9.3082	S	77	72	0.5404	A	71	79	0.8291	A	81	90	3.2932	A
	3000	67	53	2.7871	A	79	65	3.1823	A	83	79	0.6247	A	89	94	0.5655	A
Beauveria bassiana strain GHA [6]	250	47	53	0.5122	A	57	87	15.8274	C	69	97	66.4130	C	84	98	16.5679	C
	500	50	62	1.4289	A	83	87	0.0438	A	87	97	9.3253	C	90	98	4.1772	C
	750	52	59	0.3538	A	97	87	3.5782	A	93	97	1.0476	A	93	98	1.0648	A
	1500	57	59	0.0292	A	100	89	4.4242	S	100	97	1.0261	A	100	98	1.0154	A
	3000	60	62	0.0162	A	100	86	5.6963	S	100	97	1.0261	A	100	98	1.0070	A

[1]: Percentage of dead adults recorded during experiments. [2]: Mortality calculated according to Robertson and Preisler. [3]: A = Additive, C = Competitive, S = Synergistic. [4]: Novodor® FC (BIOFA Germany). [5]: Madex® (Hellafarm, Athens. Greece). [6]: Botanigard® 10.7SC (K&N Efthymiadis Single Member S.A., Thessaloniki, Greece).

Table 2. Percentage of observed and expected mortality of *T. confusum* adults at seven, fourteen, twenty-one and twenty-eight days of the experiment, treated with treatments in several combinations, and their interactions (A = Additive, C = Competitive, S = Synergistic) (n = 100). Expected mortality calculated according to Robertson and Preisler [20].

Treatment		Mortality Observed% [1]	Mortality Expected% [2]	x^2	Interaction [3]	Mortality Observed%	Mortality Expected%	x^2	Interaction	Mortality Observed%	Mortality Expected%	x^2	Interaction	Mortality Observed%	Mortality Expected%	x^2	Interaction
Entomopathogen (3000 ppm)	caffeic acid (ppm)	7 days				14 days				21 days				28 days			
Bacillus thuringiensis [4]	250	20	19	0.0558	A	37	50	1.4805	A	43	61	3.7692	A	63	78	3.2719	A
	500	30	22	1.3274	A	37	51	2.2317	A	50	64	2.4033	A	80	83	0.0296	A
	750	35	25	1.6483	A	50	56	0.2562	A	63	68	0.2309	A	90	85	0.8895	A
	1500	47	19	15.6913	S	50	58	0.6202	A	77	70	1.1977	A	93	88	0.9887	A
	3000	47	25	7.7754	S	57	60	0.1210	A	83	70	3.1762	A	97	90	1.6296	A
Cydia pomonella Granulovirus (CpGV) [5]	250	23	28	0.3229	A	40	48	0.6507	A	50	53	0.0373	A	63	62	0.0688	A
	500	33	30	0.1053	A	57	50	0.8984	A	60	56	0.3524	A	80	71	1.3673	A
	750	33	34	0.0037	A	57	55	0.1206	A	60	61	0.0050	A	87	74	2.7530	A
	1500	47	28	5.5592	S	60	56	0.3524	A	67	63	0.4454	A	90	79	2.7072	A
	3000	47	34	2.4437	S	63	59	0.3567	A	77	63	3.1074	A	97	83	5.0396	S

Table 2. *Cont.*

Treatment		Mortality Observed% [1]	Mortality Expected% [2]	x^2	Interaction [3]	Mortality Observed%	Mortality Expected%	x^2	Interaction	Mortality Observed%	Mortality Expected%	x^2	Interaction	Mortality Observed%	Mortality Expected%	x^2	Interaction
Beauveria bassiana strain GHA [6]	250	10	31	5.9015	C	43	51	0.6802	A	77	85	0.9549	A	87	95	2.0092	A
	500	23	33	1.1206	A	50	53	0.0492	A	87	86	0.2435	A	90	96	0.6173	A
	750	23	36	1.9048	A	67	57	1.2404	A	87	88	0.0046	A	90	97	0.6355	A
	1500	37	31	0.5979	A	67	59	1.0598	A	97	88	2.6542	A	93	97	1.2666	A
	3000	40	36	0.3271	A	70	62	1.1024	A	100	88	4.4576	S	100	98	1.0154	A

[1]: Percentage of dead adults recorded during experiments. [2]: Mortality calculated according to Robertson and Preisler. [3]: A = Additive, C = Competitive, S = Synergistic. [4]: Novodor® FC (BIOFA Germany). [5]: Madex® (Hellafarm, Athens, Greece). [6]: Botanigard® 10.7SC (K&N Efthymiadis Single Member S.A., Thessaloniki, Greece). The interaction between treatments for *C. ferrugineus* was additive in ten combinations and competitive in five combinations over the first seven days. After fourteen and twenty-one days, the interactions between the treatments were all additive. At last, for twenty-eight days, the interaction between the treatments was additive in fourteen combinations and synergistic in one combination (Table 3). Adult *C. ferrugineus* mortality was 10–100% (F: 15.164; df: 654.2360; *p*: <0.001) (overall 15 treatments).

Table 3. Percentage of observed and expected mortality of *C. ferrugineus* adults at seven, fourteen, twenty-one and twenty eight days of the experiment, treated with treatments in several combinations, and their interactions (A = Additive, C = Competitive, S = Synergistic) (n = 100). Expected mortality calculated according to Robertson and Preisler [20].

Treatment		Mortality Observed% [1]	Mortality Expected% [2]	x^2	Interaction [3]	Mortality Observed%	Mortality Expected%	x^2	Interaction	Mortality Observed%	Mortality Expected%	x^2	Interaction	Mortality Observed%	Mortality Expected%	x^2	Interaction
Entomopathogen (3000 ppm)	caffeic acid (ppm)	7 days				14 days				21 days				28 days			
Bacillus thuringiensis [4]	250	10	46	14.4489	C	57	60	0.1155	A	70	79	0.8927	A	90	86	0.8651	A
	500	20	48	9.0018	C	67	64	0.1496	A	73	79	0.3189	A	93	86	1.8692	A
	750	27	51	6.5817	C	70	64	0.5271	A	81	81	0.0151	A	93	86	1.8692	A
	1500	33	55	5.3190	C	70	68	0.1276	A	83	84	0.0029	A	97	88	2.6660	A
	3000	33	55	5.3190	C	77	72	0.6169	A	91	84	1.2601	A	100	92	3.2246	A
Cydia pomonella Granulovirus (CpGV) [5]	250	27	20	0.9188	A	53	60	0.6191	A	67	64	0.1496	A	73	70	0.4055	A
	500	27	23	0.3924	A	60	64	0.1692	A	73	64	1.1362	A	80	70	2.0486	A
	750	30	27	0.1455	A	60	64	0.1692	A	77	68	1.3612	A	87	70	4.9951	S
	1500	37	33	0.3555	A	63	68	0.2114	A	83	72	2.2449	A	87	75	2.6684	A
	3000	40	33	0.8740	A	67	72	0.1750	A	83	72	2.2449	A	90	83	1.7042	A
Beauveria bassiana strain GHA [6]	250	20	20	0.0000	A	53	67	2.5699	A	73	75	0.0142	A	83	88	0.3815	A
	500	23	23	0.0337	A	56	70	2.8425	A	73	75	0.0142	A	93	88	0.9946	A
	750	30	27	0.1455	A	67	70	0.1390	A	80	77	0.1733	A	93	88	0.9946	A
	1500	37	33	0.3555	A	73	73	0.0902	A	87	80	0.9188	A	97	90	2.5684	A
	3000	37	33	0.3555	A	77	77	0.1522	A	87	80	0.9188	A	97	93	1.5232	A

[1]: Percentage of dead adults recorded during experiments. [2]: Mortality calculated according to Robertson and Preisler. [3]: A = Additive, C = Competitive, S = Synergistic. [4]: Novodor® FC (BIOFA Germany). [5]: Madex® (Hellafarm, Athens. Greece). [6]: Botanigard® 10.7SC (K&N Efthymiadis Single Member S.A., Thessaloniki, Greece).

Overall, all the main effects of examined factors (insect species, exposure time, treatment) and their interactions proved to be significant as was demonstrated by a 3-way analysis of variance (Table 4).

Table 4. An analysis of variance (3-way ANOVA) for the main effects and interactions for the mortality of *T. granarium*, *T. confusum* and *C. ferrugineus* adults exposed to separate and combined treatments with CA and biopesticides.

	Separate Treatments			**Combined Treatments**		
Source	df	F	Sig.	df	F	Sig.
Exposure time	3	11.838	<0.001	3	8.142	<0.001
Insect species	2	10.099	<0.001	2	6.499	<0.001
Treatment	3	16.476	<0.001	4	3.702	<0.001
Exposure time * Insect species	6	11.109	<0.001	6	7.288	<0.001
Exposure time * Treatment	9	11.540	<0.001	12	11.534	<0.001
Insect Species * Treatment	6	13.829	<0.001	8	5.420	<0.001
Exposure time * Insect species * Treatment	16	14.950	<0.001	24	9.946	<0.001
Error	210			380		
Total	280			400		
Corrected total	279			399		

4. Discussion

As chemical insecticides are being more and more neglected, many studies now focus on alternatives, investigating compounds derived from nature. Plant chemicals can act as insecticides by preventing insects from feeding or by demonstrating repellent and growth inhibition effects [21,22]. The insecticidal potential of phenolic plant compounds such as CA has been well documented [23–28]. In our bioassays, adult beetles treated only with CA showed noteworthy mortality (up to 70%). The lethal effect of CA on insects has been also verified for the tobacco cutworm, *Spodoptera litura* (Fabricius) [29] and the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) [30]. Apart from mortality effects, various studies have demonstrated that CA and other plant phenolic compounds may have negative effects on insect feeding, larval growth rate and reproduction [31–35]. Pacifico et al. [35] investigated the effect of CA on the larvae of *Phthorimaea operculella* and recorded sublethal effects and anti-nutrient action as it inhibited larval growth.

A possible explanation for these results may lie in the interaction of the phenolic compounds with digestive proteins of the insects leading to a decrease in nutritional quality. The way phenolic compounds affect the interaction of plants with bacteria and fungi has already been investigated even though little is known about the toxicity of phenolics against insects [36].

As expected, separate treatments with biopesticides caused high mortality in all tested species. There are several main factors that can influence the efficacy of biopesticides, such as the type of biopathogen, the dose applied, temperature, relative humidity and the type of product [20,37–43]. Moreover, the insecticidal efficacy of biopesticides can be highly influenced by a host's physiology, morphology and behavior, the population density, age, nutrition, and genetic information [39].

Our original hypothesis was that the interaction between CA and biopesticides either leads to additional efficacy or plays only a supporting role. Based on our results, the interaction was additive in *T. confusum* in most combinations. On the other hand, it was negative in four treatments in some combinations for *T. granarium* and *C. ferrugineus* adults, especially in the first 7 days of the experiment when the bacterial insecticide was applied. A negative interaction refers to the competitive relationship between CA and the pathogen. The nature of this competition is not precisely known. Entomopathogenic microorganisms have also shown increased efficacy when applied in combination treatments not only with other entomopathogens but also with synthetic insecticides [44]. Regarding their coexistence with plant extracts, entomopathogenic microorganisms have shown both an inhibitory effect [45] and a positive interaction as Neem seed cake improved the pathogenicity of the fungus *Metarhizium anisopliae* against the Black Vine Weevil [46]. The entomopathogenic fungus *M. anisopliae* has been successfully combined with plant extracts for the control of ticks [47], whereas other plant extracts showed compatible capacity with entomopathogenic bacteria against aphids [48]. To the best of our knowledge, there are no data available concerning the interaction of CA or other plant phenolic metabolites with entomopathogens.

In general, combinations of feeding stimulants and deterrents affect the feeding response of phytophagous insects [49,50]. It has been suggested that the Colorado potato beetle selects its hosts among solanaceous plants based on the presence of deterrents such as alkaloid glycosides rather than on the presence of feeding stimulants [51,52]. Various types of sesquiterpene lactones are present in Asteraceae and deter numerous phytophagous insects from feeding on the plants [53]. Caffeic acid derivatives play an important role in plant defense [54]. Chlorogenic acid has been reported to inhibit larval development of some Lepidoptera, such as *H. armigera*, the corn earworm *Heliothis zea* (Boddie), and the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) [55–58] and deters feeding in leaf beetles *Lochmaea caprea* (L.) [59], and *Agelastica alni* (L.) (Coleoptera: Chrysomelidae) [60,61].

To conclude, the interactions between tested insecticidal agents could be positive or negative, acting synergistically (increasing host mortality compared to single pathogen infections) [20,62,63] or antagonistically (reducing the observed host mortality compared to single pathogen infections) [64]. Needless to say, pest mortality can be affected by genotype, dose and sequence of infection [65,66].

5. Conclusions

Based on our results, the combined application of plant extracts and entomopathogenic microorganisms may become an effective strategy for eco-friendly pest management in storage facilities. However, special attention should be paid to the selection of the combined agents as the additive or synergistic effect is not always valid. Our study has shown the significant insecticidal action of CA alone or in combination with biopesticides. Further research is needed to clarify the effects of various factors, such as pest species, storage environment, application dose, time interval, stored product type, etc., and to enhance the use of plant compounds in stored-product IPM.

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