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Postharvest Treatment of *Tribolium confusum* Jacquelin du Val Adults with Commercial Biopesticides

Spiridon Mantzoukas [1,2,*], Ioannis Lagogiannis [3], Ioannis Pettas [4], Georgia Korbou [2], Alexandra Magita [2], Athanasia Papadopoulou [2], Maria Trianti [2], Panagiotis A. Eliopoulos [5] and Eirini Karanastasi [2]

1 Department of Pharmacy, School of Health Sciences, University of Patras, Patras 26504, Greece
2 Department of Agricultural Technology, Technological Institute of Western Greece, Amaliada 27200, Greece; georgiakorbou@gmail.com (G.K.); alexandramag@hotmail.gr (A.M.); athanasiap610@gmail.com (A.P.); mariatrianty@yahoo.gr (M.T.); ekaran@teimes.gr (E.K.)
3 ELGO-Demeter, Plant Protection Division of Patras, NEO & L. Amerikis, Patras 26444, Greece; lagoipp@gmail.com
4 Department of Chemistry, University of Ioannina, Ioannina 45100, Greece; pettasgian@gmail.com
5 Department of Agriculture and Agricultural Technology, University of Thessaly, Larissa 45100, Greece; eliopoulos@uth.gr
* Correspondence: mantzoukas@upatras.gr

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Abstract: Within the context of the harmful side-effects of chemical pest control applications, the present study investigated the insecticidal effect of three commercial biopesticides, the fungal Metab (*Beauveria bassiana*, *Metarhizium anisopliae*) and Lecan (*Lecanicillium lecanii*), as well as raw zeolite, against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), also known as the confused flour beetle. To this end, we sprayed *Tribolium confusum* adults with suspensions of the said biopesticides, at three different dosages (250 ppm, 500 ppm, and 1000 ppm) on *Avena sativa* L. and *Linum usitatissimum* L. hull and no hull seeds. The data were analyzed in terms of three- and four-way ANOVA model, and the overall survival was determined while using the Kaplan–Meier method. The mortality of *Tribolium confusum* adults was recorded and analyzed in correlation with the following parameters: dose, product (seed), days, and treatment as factors. At the end of the experiment, all of the biopesticides were effectively pathogenic, but there was variation in their effectiveness in terms of the *T. confusum* mortality that they caused, depending on the product (seed). The type of seed can play a role in the pathogenicity or effectiveness of the biopesticides. Additionally, our results showed that the mortality percentage was dependent on the dose and treatment of the commercial biopesticides.

Keywords: *Tribolium confusum*; *Avena sativa*; *Linum usitatissimum*; Metab; Lecan; zeolite; mortality

1. Introduction

Oat (*Avena sativa* L.) and flax (*Linum usitatissimum* L.) are two of the most important crops in temperate areas and, economically, they are ranked as two of the eight most important crops in the world [1,2]. Oat seed use for human consumption has progressively increased, thanks to its dietary benefits [2]. Moreover, flax seed is used for oil production as well as in food industries due to its nutritional merits, essential Polyunsaturated fatty acids, and rich supply of soluble dietary fiber [1]. The seed weight of both species is negatively influenced by stored pest infestation.

Insects are major pests of stored products. Stored-product insects are responsible for affecting the quality, quantity, and commercial value of dried stored agricultural commodities, accounting for

significant post-harvest losses that range from 9% in developed countries to up to 20% in developing countries [3]. Coleopterans are among the most common storage pests. *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), also known as the confused flour beetle, is a cosmopolitan polyphagous species whose adults and larvae are responsible for severe economic damage in stored products, feeding on several dried foods, including flours, chocolate, fruits, and grains. Infestation results in reduced weight and quality of the product and marketability difficulties. *Tribolium* spp. also produces carcinogens, the quinones, which can cause allergies and dermatitis, among other disorders [4]. Although *T. confusum* cannot penetrate intact kernels, it might cause significant damage when the kernel is damp or broken [4]. The control of stored product pests is usually carried out with the application of chemicals to prevent post-harvest losses. However, chemicals are responsible for various problems, including environmental pollution, toxicity to humans and animals, as well as the development of pest resistance [3]. There is a growing need for the exploration of biological control methods within Integrated Pest Management (IPM), to keep pest populations to safer levels while safeguarding the environment and human health [3].

Various biological control agents, including fungi, bacteria, as well as inert dust, are being considered for supplementing or replacing chemical insecticides, which cause toxicity to non-target organisms. *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae), which has been widely tested, has been proven to be effective against several stored product insect species in both laboratory and field tests [5–15], thus showing promise for commercial use. *Metarhizium anisopliae* (Metschinkoff) Sorokin (Hypocreales: Clavicipitaceae) too, which is a globally distributed mitosporic haploid fungus, is pathogenic to many important agricultural pests [16,17]. All of the above entomopathogenic fungi have proven efficacy against many insect pests of stored grains and grain products. The entomopathogenic fungus *Lecanicillium lecanii* (Zimmermann) Zare & W. Gams [previously known as *Cephalosporium lecanii*] (Hypocreales: Cordycipitaceae) [18,19], is capable of infecting various insect pests, has a broad geographical distribution, and, therefore, also appears to be promising for commercial development.

Another potential biological agent is zeolite, which has been relatively less studied for its control against stored product pests. Zeolite has been investigated for its potential insecticidal effect against *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) adults (also known as the rice weevil and the red flour beetle, respectively) [20]. Zeolite is a crystalline hydrated aluminosilicate of alkali or alkaline earth metals. Natural zeolite forms following the reaction of ash layers and volcanic rocks with alkaline groundwater [21,22].

This is the first paper evaluating the potential of this commercial biopesticides for the control of stored product pests. The objective of the present study was to investigate the insecticidal effect of three commercial biopesticides against adults of the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), on the hull and no hull seeds of *A. sativa* and *L. usitatissimum*, as well as the extent to which the presence of a hull, or lack thereof, in the seeds affects the insecticidal effect of these products.

2. Materials and Methods

2.1. Insects

The initial batch of *T. confusum*, counting 1000 individuals, was obtained from infested wheat in the prefecture of Achaia, Greece. Insects were mass produced in an environmentally controlled chamber (25 ± 1 ºC, 65 ± 5% Relative Humidity, Light:Night 12:12) (PHC Europe B.V /Sanyo/Panasonic Biomedical MLR-352-PE), where they were maintained in 0.25 L glass jars with 200 g of sterilized and pesticide-free corn flour. The jars were covered with a sterilized muslin cloth. After two weeks, the original adults were removed by sieving. Each jar was then observed daily to collect the progeny that were placed in separate jars, in accordance with their age.

2.2. Biopesticides

Metab is a commercial biopesticide from the companies Microspore Hellas and Sacom Hellas, (Athens, Greece), which contains *B. bassiana* and *M. anisopliae* at concentrations of 11.5×10^7 conidia/mL and 6.5×10^7 conidia/mL respectively. Lecan is a commercial biopesticide, also from the same companies, which contains *L. lecanii* at 4.22×10^7 conidia/mL. The above commercial biopesticides are registered for pest biocontrol in Europe, but they have not been tested against *T. confusum.*

A commercial zeolite formulation was also used in the bioassays (bulk/raw zeolite). The bulk zeolite that is meant for soil amendment was diluted in an aqueous solution with ddH_2O. The above-mentioned process was completed inside a laminar flow chamber (Equip Vertical Air Laminar Flow Cabinet Clean Bench, Mechanical Application LTD, Athens, Greece).

2.3. Experimental Protocol

Laboratory-reared mixed sex adult insects (25 ± 1 ºC, 65 ± 5% R.H., L:N 12:12) (PHC Europe/Sanyo/Panasonic Biomedical MLR-352-PE) (< 1 week old) were used for this study. Each batch of adults was collected from rearing jars and then placed in 9-cm diameter Petri dishes with 10 g of sterilized product, after they had been starved for 1 h. To test its pathogenicity against *T. confusum*, each biopesticide was directly sprayed on the adults and the product in the same petri dish, at three different doses, 250 ppm, 500 ppm, and 1000 ppm, with a 2.5 mL aqueous suspension, while using a Potter spray tower (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, U.K.) at 1 kgf cm^{-2}. The products were hull and no hull seeds of *A. sativa* and *L. usitatissimum.* One hundred *T. confusum* adults were used for each dose (10 adults in 10 replications) and the experiment was replicated twenty times. The control involved adults and product that had been merely treated with ddH_2O. Petri dishes were observed after 7, 14, 21, and 28 days after the spraying.

2.4. Fungal Identification Method

The Petri dishes were observed at 7, 14, 21, and 28 days for dead individuals that were collected while using sterilized forceps. The collected dead individuals were immediately submerged in 95% ethanol for 1 min., washed in sterile distilled water for 5 min., allowed to dry, and then placed on moistened filter paper. Cadavers were kept at 25 °C for 5–7 days in the dark, and those that showed signs of fungal infection were noted as infected. These infected individuals were transferred to a Petri dish containing a piece of moistened cotton to promote the outgrowth and sporulation of the respective fungi. External mycelial growth on cadavers was identified while using a stereomicroscope ZEISS Stemi 508 (Carl Zeiss Microscopy GmbH, Jena, Germany) at 2× magnification, and conidia that were retrieved from the cadavers were recognized using a microscope ZEISS Primo Star (Carl Zeiss Microscopy GmbH, Jena, Germany) at 400× magnification. The median sporulation time was determined for fungal biopesticides.

2.5. Statistical Analysis

The corrected percent mortality was calculated while using Abbott's formula [23] and, prior to analysis, these 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 ver. 23 (IBM Corp. 2015, Armonk, NY, USA) [24]. In the case of significant *F* values, means were compared while using the Bonferroni test. Median lethal time of *T. confusum* adults and LC50 were calculated by probit analysis with 95% confidence interval (CI). The Cox Regression method [25] was selected to determine the hazard effect of the factors over *T. confusum* adults. It is a survival analysis regression model that describes the relation between the event incidence and a set of covariates. Comparison of survival distributions was obtained while using the Breslow test (Generalized Wilcoxon) (SPSS ver. 23). The percentages of sporulating cadavers and the median sporulation time were compared between isolates using the *T*- Test of the SPSS. A comparison of median lethal time was performed using one-way ANOVA (Biopesticide as a factor).

3. Results

3.1. Mortality Effect of Commercial Biopesticides against Adults of T. confusum

The mortality percentage depended on the treatment, dose of the commercial biopesticides, and the product. The final mortality percentages of *T. confusum* adults, on day 28 after exposure, were 17 to 30% in the treatments with zeolite, 27 to 50% in the treatments with Metab, and 23 to 77% in the treatments with Lecan, on hull *L. usitatissimum* seeds; 97 to 100% in the treatments with zeolite, 13 to 47% in the treatments with Metab, and 17 to 50% in the treatments with Lecan, on no hull *L. usitatissimum* seeds (Tables 1 and 2). The final mortality percentages of *T. confusum* adults were 30 to 53% in the treatments with zeolite, 13 to 40% in the treatments with Metab, and 13 to 50% in the treatments with Lecan, on hull *A. sativa* seeds; 70 to 100% in the treatments with zeolite, 33 to 50% in the treatments with Metab, and 37 to 67% in the treatments with Lecan, on no hull *A. sativa* seeds (Tables 3 and 4). For control adults who had only been treated with ddH_2O, mortality was 3% on no hull *A. sativa* seeds, 2% on hull *A. sativa* seeds, 4% on hull *L. usitatissimum* seeds, and 2% on no hull *L. usitatissimum* seeds, at the end of the experiment (Tables 1–4).

Table 1. Mean mortality (% ± SD) and median lethal concentration (LC_{50} with Slope (Sl) and Intercept (Int) values) of *T. confusum* adults, exposed for 28 days to no hull *L. usitatissimum* seeds that had been treated with Metab, Lecan and Zeolite, at three dose rates. Mean ± SD values with the same letter within a column are not significantly different ($p < 0.05$) ($F = 1.068$, df = 6.96, $p < 0.001$).

Product (Seeds)	Biopesticide	Dose (ppm)	Mortality (% ± SD)				LC_{50}
			Days				
			7	14	21	28	
no hull *L. usitatissimum*	Metab	250	7 ± 6a	13 ± 6a	13 ± 6a	13 ± 6a	3500 ppm (Sl: 1.89) (Int: −6.72)
		500	7 ± 6a	13 ± 6a	17 ± 11a	17 ± 11a	
		1000	7 ± 11a	20 ± 10a	37 ± 6b	47 ± 15a	
	Lecan	250	7 ± 6a	17 ± 6a	17 ± 6a	17 ± 6a	1716 ppm (Sl: 2.22) (Int: −7.18)
		500	7 ± 11a	20 ± 10a	23 ± 6a	27 ± 6a	
		1000	13 ± 6a	20 ± 10a	37 ± 6b	50 ± 10a	
	Zeolite	250	20 ± 0a	57 ± 6b	73 ± 11c	97 ± 0b	793 ppm (Sl: 0.15) (Int: −0.04)
		500	47 ± 6a	80 ± 10c	93 ± 6c	100 ± 0b	
		1000	83 ± 12b	93 ± 6c	100 ± 0d	100 ± 0b	
	Control	dd H_2O	0 ± 0c	0 ± 0d	2 ± 0f	2 ± 0c	

Table 2. Mean mortality (% ± SD) and median lethal concentration (LC_{50} with Slope (Sl) and Intercept (Int) values) of *T. confusum* adults, exposed for 28 days to hull *L. usitatissimum* seeds that had been treated with Metab, Lecan and Zeolite, at three dose rates. Mean ± SD values with the same letter within a column are not significantly different ($p < 0.05$) ($F = 1.699$, df = 6.96, $p = 0.035$).

Product (Seeds)	Biopesticide	Dose (ppm)	Mortality (% ± SD)				LC_{50}
			Days				
			7	14	21	28	
hull *L. usitatissimum*	Metab	250	13 ± 15a	27 ± 12a	27 ± 12a	27 ± 12a	4312 ppm (Sl: 1.04) (Int: −3.79)
		500	23 ± 15a	23 ± 21a	33 ± 21a	40 ± 16a	
		1000	23 ± 15a	40 ± 10ab	50 ± 17a	50 ± 17a	
	Lecan	250	13 ± 6	17 ± 6a	20 ± 0a	23 ± 6a	1216 ppm (Sl: 2.04) (Int: −6.3)
		500	20 ± 17a	37 ± 21ab	50 ± 10a	50 ± 10a	
		1000	20 ± 10a	57 ± 6ab	77 ± 6b	77 ± 6b	
	Zeolite	250	3 ± 6a	10 ± 0a	17 ± 6a	17 ± 6a	16545 ppm (Sl: 0.97) (Int: −4.11)
		500	7 ± 6a	20 ± 10a	23 ± 6a	23 ± 6a	
		1000	23 ± 20a	27 ± 21a	30 ± 17a	30 ± 17a	
	Control	dd H_2O	0 ± 0b	0 ± 0c	0 ± 0c	4 ± 0c	

Table 3. Mean mortality (% ± SD) and median lethal concentration (LC_{50} with (Sl) and Intercept (Int) values) of *T. confusum* adults exposed for 28 days to no hull *A. sativa* seeds that had been treated with Metab, Lecan and Zeolite, at three dose rates. Mean ± SD values with the letter within a column are not significantly different ($p < 0.05$) ($F = 1.586$, df = 6.96, $p = 0.025$).

Product (Seeds)	Biopesticide	Dose (ppm)	Mortality (% ± SD)				LC_{50}
			Days				
			7	14	21	28	
no hull *A. sativa*	Metab	250	10 ± 0a	20 ± 10a	30 ± 10a	33 ± 15a	7057 ppm (Sl: 0.31) (Int: −1.86)
		500	17 ± 6b	27 ± 15a	33 ± 6a	37 ± 6a	
		1000	27 ± 15b	40 ± 0a	40 ± 0a	40 ± 0a	
	Lecan	250	27 ± 6b	37 ± 6a	37 ± 6a	37 ± 6a	2855 ppm (Sl: 0.98) (Int: −3.45)
		500	27 ± 6b	40 ± 0a	43 ± 6a	43 ± 6a	
		1000	30 ± 10b	47 ± 12a	53 ± 12a	67 ± 10b	
	Zeolite	250	3 ± 6a	57 ± 21a	70 ± 17ab	70 ± 17b	887 ppm (Sl: 0.59) (Int: −1.74)
		500	27 ± 12b	63 ± 6a	83 ± 12ab	93 ± 6b	
		1000	47 ± 6b	100 ± 0b	100 ± 0b	100 ± 0b	
	Control	dd H_2O	0 ± 0c	1 ± 0c	3 ± 0c	3 ± 0c	

Table 4. Mean mortality (% ± SD) and median lethal concentration (LC_{50} with (Sl) and Intercept (Int) values) of *T. confusum* adults exposed for 28 days to hull *A. sativa* seeds that had been treated with Metab, Lecan, and Zeolite, at three dose rates. Mean ± SD values with the same letter within a column are not significantly different ($p < 0.05$) ($F = .939$, df = 6.96, $p = 0.047$).

Product (Seeds)	Biopesticide	Dose (ppm)	Mortality (% ± SD)				LC_{50}
			Days				
			7	14	21	28	
hull *A. sativa*	Metab	250	3 ± 6a	10 ± 0a	13 ± 6a	13 ± 6a	2766 ppm (Sl: 1.86) (Int: −6.42)
		500	10 ± 10a	23 ± 12a	27 ± 6a	30 ± 17a	
		1000	10 ± 0a	27 ± 6a	40 ± 10a	40 ± 10a	
	Lecan	250	3 ± 6a	7 ± 6a	13 ± 6a	13 ± 6a	1748 ppm (Sl: 2.23) (Int: −7.25)
		500	7 ± 12a	27 ± 12a	30 ± 6a	40 ± 10a	
		1000	37 ± 21a	37 ± 21a	40 ± 17a	50 ± 20a	
	Zeolite	250	13 ± 12a	17 ± 15a	30 ± 10a	30 ± 10a	5475 ppm (Sl: 0.94) (Int: −3.53)
		500	17 ± 15a	20 ± 17a	27 ± 6a	33 ± 6a	
		1000	20 ± 10a	27 ± 15a	33 ± 12a	53 ± 15a	
	Control	dd H_2O	0 ± 0b	0 ± 0b	1 ± 0b	2 ± 2b	

Accordingly, in the case of no hull products, the estimated median lethal concentration (LC50) was lower for zeolite, as compared with Metab and Lecan (Tables 1–4), which indicated higher virulence of zeolite against *T. confusum*. In the case of hull products, the median lethal concentration (LC50) of Lecan was lower when compared to Metab and zeolite (Tables 1–4), indicating a higher virulence of Lecan against *T. confusum*.

Significant differences were recorded between product, biopesticide, doses, and the days of the experiment as factors, in relation to the dependent variable of mortality (Table 1). The effectiveness of the biopesticides was significant against *T. confusum* adults at different doses with different products (Table 5). The three-way factor model of product×days×dose, product×biopesticide×days biopesticide×dose×days and the four-way factor model of product×biopesticide×doses×days also showed a significant effect in terms of the mortality of *T. confusum* adults (Table 5).

Table 5. ANOVA parameters for mortality levels of *T. confusum* adults exposed for 28 days to three doses of Metab, Lecan, and Zeolite that had been applied to hull and no hull seeds of *A. sativa* and *L. usitatissimum.*

Factor	Df	*F*	Sig.
Seeds	3	6.643	.000
Biopesticide	3	85.373	.000
Dose	2	15.678	.000
Time	3	49.312	.000
Seed * Biopesticide	9	9.306	.000
Seeds * Dose	6	.366	.900
Seeds * Time	9	2.717	.004
Biopesticide * Dose	6	1.868	.085
Biopesticide * Time	9	8.733	.000
Dose * Time	6	1.651	.132
Seeds * Biopesticide * Dose	18	.450	.976
Seeds * Biopesticide * Time	27	4.759	.000
Seeds * Dose * Time	18	2.607	.000
Biopesticide * Dose * Time	18	4.097	.000
Seeds * Biopesticide * Dose * Time	54	1.956	.000
Error	384		
Total	576		
Corrected Total	575		

3.2. Fungal Growth on Cadavers of T. confusum after the Exposure to the Fugnal Biopesticides

Following treatment with Lecan on no hull *A. sativa* and *L. usitatissimum* seeds, we observed a high rate of mycosis on cadavers (t = 12.144, df = 7, $p < 0.001$) (Table 6), as well as the shortest sporulation time; four days on no hull *A. sativa* seeds and 4.1 days on no hull *L. usitatissimum* seeds (t = 16.578, df = 7, $p < 0.001$) (Table 6).

Table 6. The sporulation percentage and sporulation time on cadavers of *T. confusum*. Mean ± SD values with the same letter within a column are not significantly different ($p < 0.05$).

Product (Seeds)	Biopesticide (Fungal)	Sporulation on Cadavers (% + SD)	Sporulation Time on Cadavers (Days + SD)
no hull *A. sativa*	Metab	44 ± 11^{a}	4.9 ± 0.2^{a}
	Lecan	58 ± 8^{a}	4.0 ± 0.5^{b}
hull A. sativa	Metab	50 ± 17^{a}	5.6 ± 1.1^{ab}
	Lecan	44 ± 11^{a}	5.9 ± 0.8^{a}
no hull *L. usitatissimum*	Metab	54 ± 11^{a}	5.3 ± 0.6^{a}
	Lecan	62 ± 13^{a}	4.1 ± 0.5^{b}
hull *L. usitatissimum*	Metab	57 ± 10^{a}	5.7 ± 0.5^{a}
	Lecan	47 ± 9^{a}	5.1 ± 1^{ab}

3.3. Median Lethal Time of T. confusum Adults after Exposure to the Biopesticides

The median lethal time of *T. confusum* adults that were treated with Metab, Lecan, and zeolite were statistically significant in relation to the median lethal time of control adults (F = 3.730, df = 3, $p < 0.001$). The median lethal time of control adults was very low as compared with the median lethal time of adults that had been sprayed with the biopesticides. More specifically, after the treatment with zeolite, the median lethal time of *T. confusum* adults was 32% lower than the median lethal time of control individuals ((18.78 days (CI: 17.85–19.70 days)); after the treatment with Lecan, the median lethal time was 15% lower ((22.82 days (CI: 22.00–23.65 days)), and after the treatment with Metab, it was 17% lower ((23.45 days (CI: 22.66–24.23 days)). The medial lethal time of control individuals was 27.65 days (CI: 27.42–27.87 days).

3.4. Factor Effect on Mortality of T. confusum Adults

Treatment and Dose are statistically significant with p values of < 0.001 (Table 7). Treatments and Doses had a major effect on the lethal time of *T. confusum* adults. The Exp(B) for Upper limit 95.0% CI will be associated with increased hazard as recorded for *T. confusum* adults on no hull *A. sativa* and no hull *L. usitatissimum* seeds. Exp(B) for Product was 0.962, meaning that product as a factor will be associated with lower hazard, longer survival, and less of an effect on the lethal time of the Coleopteran. The B coefficient for Dose was positive with Exp(B) > 1. Higher dose values are associated with greater hazard and therefore shorter survival of *T. confusum* adults. Treatment also displayed positive B coefficient with Exp(B) > 1. This showed greater hazard and therefore shorter survival of adults in comparison to the control treatment (Control Exp(B) = 1). Product, on the other hand, had a negative coefficient and Exp(B) < 1. This means that some products will be associated with lower hazard and longer survival.

Table 7. Variables in the Equation from Cox regression (*Chi–square*: 301.764, df = 3, $p < 0.001$).

	B	Sd	df	Sig.	Exp(B)	95.0% CI for Exp(B)	
						Lower	Upper
Treatment	.748	.049	1	.000	1.473	1.430	1.521
Dose	.308	.057	1	.000	1.360	1.216	1.521
Product (Seeds)	−.039	.040	1	.333	.962	.889	1.041

4. Discussion

The insecticidal efficacy of biopesticides is interlinked with several factors, including insect's behavior and other genetic and physiological information, the extent to which the physiology and morphology of the host render it sensitive to biological control agents, such as biopesticides [26–29], as well as the product. Our results showed that the different efficacy of the tested biopesticides depended on the product, on the dose, on the isolates, and their interaction. Our results indicated that stored product pests can be controlled with biopesticides, especially zeolite (lower LC50 on no hull seeds) and Lecan (*L. lecanii*) (lower LC_{50} on hull seeds), which produced good results. Several published studies are available on the efficacy of the biopesticides against stored grain insect pests [16], [30–32], but there are no references regarding the susceptibility of *T. confusum* to these biopesticides, to be compared with the results that were obtained in this study.

Literature presents several hypotheses regarding which factors may influence the efficacy of biopesticide residues in stored products and many of these hypotheses have not yet been fully tested due to the numerous variables involved. Some of the main factors that can influence the efficacy of biopesticides are bio-pathogens and dose [28,30,33], abiotic factors [34–37], as well as the product [10]. In our experiment, the lethal time parameter indicates that the biopesticides are as effective as the other isolates mentioned in the literature, especially the raw zeolite. The pathogenicity of the zeolite was the highest recorded among the three biopesticides, on no hull seeds. More specifically, *T. confusum* adults on no hull seeds had the lowest medial lethal time after exposure to zeolite, in comparison to the other tested biopesticides.

Although no data is available regarding the susceptibility of *T. confusum* to zeolite, *Tribolium* spp. individuals are the most tolerant among stored product insects [38–41]. Nevertheless, Vayias and Athanassiou [42] showed that *T. confusum* larvae were affected by diatomaceous earth dust and, therefore, even though adults are resilient, control can be achieved by exposing young larvae. Our study showed a significant high mortality percentage of *T. confusum* adults treated with the zeolite aqueous solution, in all tested doses.

Different strains of entomopathogenic fungi are known to differ in their pathogenicity-related characteristics [43–45], as confirmed by our results. *B. bassiana* and *M. anisopliae* both have a wide

range of hosts [46]. Batta [14] reported that high mortality of *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) was obtained seven days after treatment with *M. anisopliae*. Wakefield et al. [47] reported 100% mortality of *Oryzaephilus surinamenis* L. (Coleoptera: Silvanidae) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) after 10 days of treatment with 10^8 conidia/mL of *B. bassiana*. Hluchy and Samsinakova [48] noted that Boverosil® caused 90% mortality of *O. surinamensis* at 10^8 conidia/mL.

Moino et al. [28] and Dal Bello et al. [49] reported that inoculation with *Beauveria* isolates produced a greater mortality of stored product pests than inoculation with *Metarhizium* isolates. Būda and Pečiulytė [50] found that all four fungal isolates of *B. bassiana*, *L. lecanii*, *M. anisopliae* var. *anisopliae*, and *Isaria farinose* were pathogenic to adults of the Indian meal moth [*Plodia interpunctella* Hübner (Lepidoptera: Pyralidae)]. Moreover, the treatment of stored wheat grains with formulated *B. bassiana* in milled rice significantly restricted the total grain weight loss that was caused by *S. oryzae* infestation and generated high rates of *S. oryzae* mortality [10]. On the contrary, Dal Bello et al. [51] reported that the treatment of *S. oryzae* with *M. anisopliae* was ineffective. Akbar et al. [13] concluded that *B. bassiana*, at a concentration of 10^9 conidia/mL, had very little virulence against adults of *T. castaneum*.

In our study, all the fungal biopesticides were found to cause mortality to *T. confusum*. Lecan proved to be the most pathogenic to *T. confusum* on hull seeds, with higher sporulation percentage in cadavers and, in some cases, the shortest sporulation time. In fact, Lecan was the fastest in causing mortality and it also produced the highest number of cadavers that showed signs of infection after death. Moreover, the lowest median lethal time was recorded in the treatment with Lecan.

In all cases, the mortality of *T. confusum* adults was satisfactory. On the no hull seeds, the mortality that is caused by the zeolite was the highest among the three tested treatments. The fungal biopesticides also proved more pathogenic on the no hull than on the hull seeds. The type of seed can play role in the pathogenicity or effectiveness of the biopesticides. Generally, studies have shown that the presence of hull in the stored product confers some level of protection against infestation and facilitates post-harvest management [52,53]. Conversely, varieties with more cracks and splits in the hull provide a pathway for the entry of neonates [54,55]. This also supports our results that the level of mortality of stored product pests that is caused by biopesticides might also vary according to the type of seed.

5. Conclusions

For the protection of stored products, biopesticides, fungal or not, can be interchangeably used or together with other insecticides, to restrict the quantities of chemicals and to possibly lessen or delay the development of pest resistance. One advantage of pathogen-based control systems is the disease cycling. Upon death, the cadaver releases many infective agents, thus renewing the inoculum at the place where the insects had died. In this way, insect pests are exposed to lethal doses of the entomopathogen from the sporulating cadavers. Disease cycling can increase insecticidal effects in the long run, while zeolite formulations are effective in the short run. The use of these biopesticides could more effectively benefit the environment and protect stored grains.

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