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Sustainable Grain Protectants: Recruiting Entomopathogenic Nematodes against Stored-Product Coleopterans

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Abstract: Stored-product commodities are attacked by numerous insect species. The adulticidal effects of entomopathogenic nematodes (EPNs) on grains remain uninvestigated. Thus, in the current study, seven doses of the EPNs *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae), and *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) were inoculated on wheat kernels against adults of *Trogoderma granarium* Everts (Coleoptera: Dermestidae), *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), and *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). Complete mortality (100.0%) of *T. granarium* was recorded after exposure for eight days to the highest dose of 50,000 Infective Juveniles/mL (IJs/mL) of all tested EPN species. At the same exposure interval, 62.2%, 85.6%, and 76.7% of *T. molitor* were killed by 50,000 IJs/mL of *H. bacteriophora*, *S. carpocapsae,* and *S. feltiae*, respectively. The highest mortality of *A. diaperinus* (11.1%) was documented eight days post-exposure to 50,000 IJs/mL of *H. bacteriophora*. In general, *T. granarium* was highly susceptible, followed by *T. molitor* and *A. diaperinus*. Concerning EPN species, *S. carpocapsae* exhibited the highest insecticidal capacity, followed by *S. feltiae* and *H. bacteriophora*. *Trogoderma granarium* and *T. molitor* can be sufficiently managed by the highest dose of 50,000 IJs/mL of all three EPNs and by *S. carpocapsae*, respectively. However, *A. diaperinus* was not affected by any EPNs.

Keywords: khapra beetle; yellow mealworm; lesser mealworm; entomopathogenic nematodes; stored wheat

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1. Introduction

Entomopathogenic nematodes (EPNs) are obligate or on occasion facultative roundworm insect parasites that can be employed within a sustainable control regime of stored-product pests since they are not harmful for humans, non-target organisms, and environment [1–13]. They penetrate the host body through natural entrances i.e., spiracles, anus, oral cavity, or sometimes the cuticular intersegmental membrane, when they reach the third, infective stage of juveniles (IJ) [14,15]. These organisms are carriers of intestinal entomopathogenic bacteria that belong to the genera *Photorhabdus* (Enterobacterales: Morganellaceae) and *Xenorhabdus* (Enterobacterales: Morganellaceae) [15–19]. These microorganisms exit EPN bodies through the anus, oral cavity or by defecation. Then, they proliferate into the nutrient-rich haemocoel of insects [14,17,18,20,21]. The microorganisms secrete proteins and toxins that cause septicemia and eventually kill the insects [22–25]. Additionally, *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) was recently reported to be able to kill insects by releasing venom proteins [17].

Entomopathogenic nematodes are members of two genera: *Steinernema* (Steinermatidae) and *Heterorhabditis* (Heterorhabditidae). Their life cycle comprises six stages: egg, the first, second, third, and fourth juvenile stages (indicated as J1-4), and adult. Stage J3 is also

referred to as IJ (infective juvenile), since it is the stage at which the EPN enters the host body. Infective juveniles are soil-inhabiting, free-living, and non-feeding, and they are the only stage occurring outside the body of the host insect [9]. When they enter the host body, IJs release mutualistic bacteria that produce toxins and suppress the host's immune system, causing its septicemic or toxemic death, within 24–48 h. Insects that die on account of the *Steinernema–Xenorhabdus* duo become pale or greyish depending on the species of the EPN, whereas those killed by the *Heterorhabditis–Photorhabdus* duo acquire a dark-red color due to the production of luminescent anthraquinone pigments by the bacteria [26,27].

To date, the most effective EPN species are *S. carpocapsae*, *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae), and *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) [5,11]. In the past, these three EPNs have been evaluated against several stored-product insects. Ramos-Rodríguez et al. [4] tested *S. carpocapsae*, *S. feltiae* and *Steinernema riobrave* Cabanillas, Poinar & Raulston (Rhabditida: Steinernematidae) on filter paper against adults, pupae and larvae of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), *Trogoderma variabile* Ballion (Coleoptera: Dermestidae), *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), as well as *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) adults. The least susceptible species was *S. oryzae*, while the most susceptible was *E. kuehniella*. Similarly, Yuksel et al. [28] studied two strains of *S. feltiae* and two strains of *H. bacteriophora*, inoculated on filter paper, against adults of *R. dominica* and *S. oryzae* and larvae of *E. kuehniella*. Eight days post-exposure, *E. kuehniella* larvae were the most susceptible species followed by *S. oryzae* and *R. dominica* adults. Furthermore, three isolates of *S. feltiae* applied on filter paper caused variable mortality rates to *S. oryzae* adults at variable temperatures [29].

Trogoderma granarium Everts (Coleoptera: Dermestidae), *T. molitor,* and *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) are species that cause high qualitative damage to stored commodities, as well as allergies that can potentially harm public health [30–37]. More specifically, *T. granarium, T. molitor,* and *A. diaperinus* are documented to infest 94, 46 and 77 commodities, respectively, e.g., grains, cereals, seeds, nuts, spices, tobacco and products of animal origin [33]. *Trogoderma granarium* is a primary stored-product pest, while the other two species are secondary pests [32,38]. All of them are cosmopolitan and can usually be found in storage facilities, grains, pet shops, and mills [32,33,38]. Research efforts tend to natural insecticides and/or biological control for their efficient management to avoid environmental and human health risks [39–43]. Therefore, even though essential oils (EOs), nanoemulsions (NEs), microemulsions (MEs), diatomaceus earths (DEs), and entomopathogenic fungi have been used as grain protectants [42–49], there is little knowledge about the efficacy of EPNs as grain protectants [5,11].

Previously, Athanassiou et al. [5] inoculated *H. bacteriophora, S. carpocapsae* and *S. feltiae* on wheat against *E. kuehniella* larvae, *R. dominica* adults, *S. oryzae* adults, and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) adults or larvae at 20 °C and 30 °C. Entomopathogenic nematodes provided low-to-moderate mortalities at both temperatures. Similarly, Karanastasi et al. [11] studied the aforementioned EPN applied on wheat kernels to manage the tolerant *T. granarium* larvae [43]. Results were promising for both sizes of larvae, since mortality reached 98.9% and 87.8% for small and large larvae, respectively, after an 8-day exposure to 50,000 IJs/mL *S. feltiae*. Yet, there are no data about the adulticidal effects of EPNs *H. bacteriophora, S. carpocapsae,* and *S. feltiae* against *T. granarium, T. molitor,* and *A. diaperinus* adults. Thus, the current study examines the efficacy of these three EPNs inoculated on stored wheat against adults of the aforementioned coleopterans.

2. Materials and Methods

2.1. Insects and Commodity

All three insect species were kept at the Laboratory of Agricultural Zoology and Entomology (Agricultural University of Athens) at 30 °C, 65% relative humidity (RH) and

an absence of light [44,45,50,51]. To culture *T. granarium,* whole wheat was used as the rearing medium [45]. To culture *T. molitor,* oat bran plus potato cuts were used as the rearing medium [44]. Finally, to culture *A. diaperinus*, yeast (1/4) plus wheat bran (3/4) plus apple cuts were used as the rearing medium [51]. Potatoes or apples are used to enhance the moisture content of the culture [44,45,51]. The unsexed selected adult individuals of *T. granarium, A. diaperinus* and *T. molitor* were <1, 7 or 14 days old, respectively [44,45,51]. The commodity that was used for the experimentations was *Triticum durum* Desf. (Poales: Poaceae) (var. Claudio). The grains were clean from pests, pesticides and impurities. Prior to the trials, the wheat moisture content was estimated at 11.8% with a calibrated mini GAC plus moisture meter (Dickey-John Europe S.A.S., Colombes, France).

2.2. Entomopathogenic Nematodes

All tested EPNs that were utilized in the current study were provided by Bio-insecta (Nea Silata, Greece).

2.3. Bioassays

After conducting preliminary trials by using all examined coleopterans, the EPN doses that were used in the current study were 100 IJs/mL, 500 IJs/mL, 1000 IJs/mL, 5000 IJs/mL, 10,000 IJs/mL, and 50,000 IJs/mL. The experiments were conducted in glass vials (12.5 cm height; 7.5 cm diameter). Lids of the vials had one circular opening (1.5 cm diameter) for the ample aeration of their inside space. The upper internal side of these vials was covered with polytetrafluoroethylene (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) to prevent insects from escaping. Three 10 g wheat samples were placed inside each vial after being weighed by a Precisa XB3200D electronic compact balance (Alpha Analytical Instruments, Gerakas, Attica, Greece), conducted on a filter paper. Afterwards, the wheat was inoculated with 1 mL of tap water that contained the aforementioned doses of EPNs, and subsequently the grains were hand-shaken for one minute to ensure the even distribution of the EPNs to the whole mass of the commodity [11]. The tested doses were equivalent to 10 IJs/adult, 50 IJs/adult, 100 IJs/adult, 500 IJs/adult, 1000 IJs/adult, and 5000 IJs/adult [5,11]. The moisture of kernels was then recalculated, reaching 13.7%. Vials that were inoculated only with tap water were used as control. Thereupon, ten adults of each species were separately inserted into each vial. The prepared containers were conveyed into incubators at 30 °C and 65% RH until the end of the experimental period. Mortality data were acquired after 4 days and 8 days under an Olympus SZX9 stereomicroscope (Bacacos S.A., Athens, Attica, Greece). An individual was declared dead after no motion was detected when the adults were poked with a brush. The dead insects were withdrawn from the vials and subsequently dissected (Figure 1) to confirm the presence of EPNs in the dead insect bodies. The entire bioassay was repeated two more times using fresh EPNs, insects, vials and grains. In total, 5670 individuals were used in the experiments (3 replications × 3 subreplications (i.e., three dishes with wheat inoculated with a given dose of EPN) × 10 individuals × 7 doses/control × 3 EPN species × 3 insect species).

2.4. Data Analysis

Control mortality was <5%; hence, no correction of data was required. To acquire a normal variance, prior to the analysis, data were converted to log (x + 1) [52,53]. The repeated-measures model (MANOVA) was utilized for each insect separately [54]. The repeated factor, response variable and main effects were exposure, mortality and nematode dose/nematode species, respectively. Means were discriminated with the Tukey–Kramer test HSD (compare all pairs) at the 5% significance level [55]. Analysis was conducted on JMP 16.2 software [56].

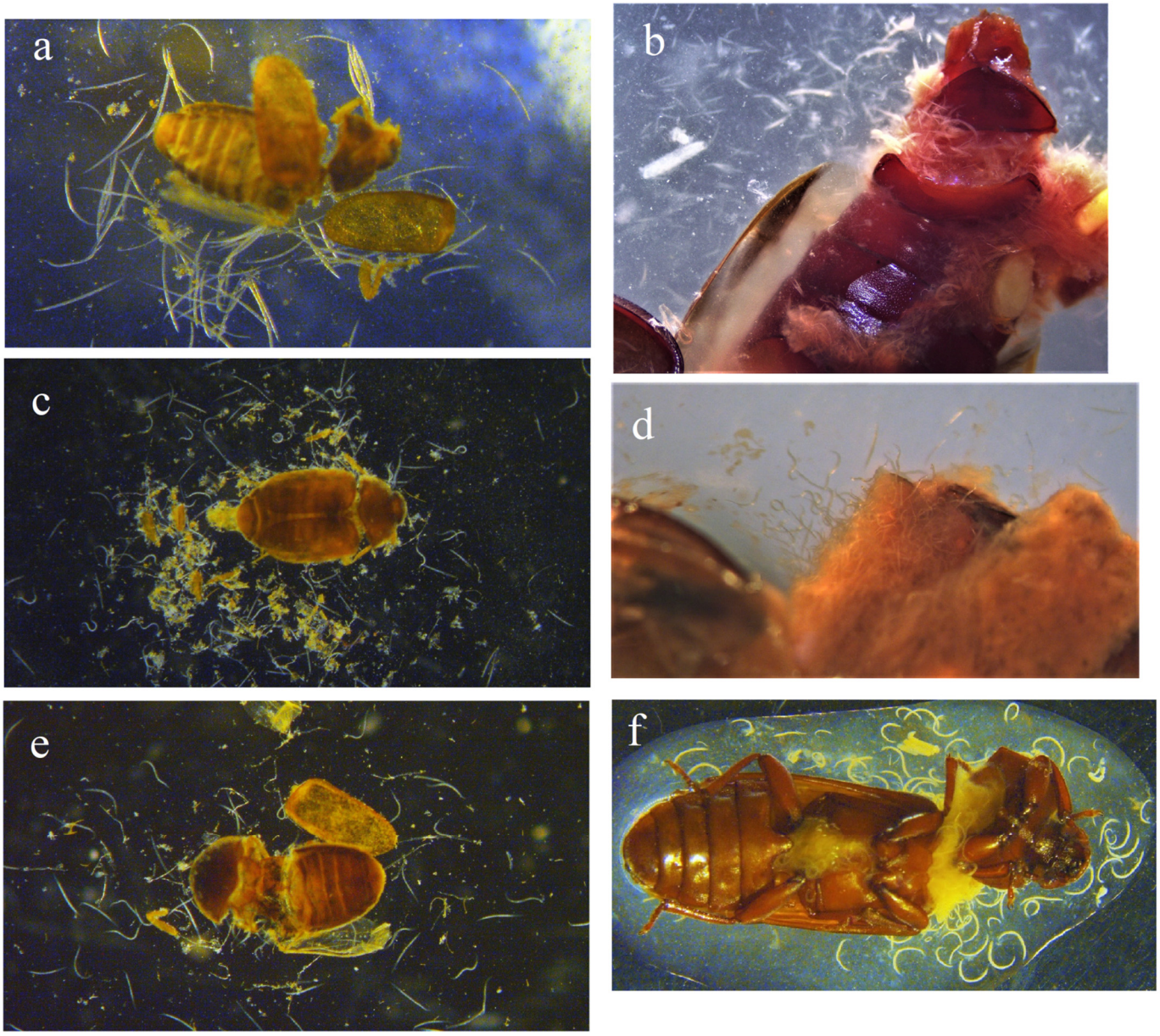

Figure 1. *Trogoderma granarium* and *Tenebrio molitor* adults infected with the entomopathogenic nematodes *Steinernema feltiae* ((**a**,**b**), respectively), *Heterorhabditis bacteriophora* ((**c**,**d**), respectively), and *Steinernema carpocapsae* ((**e**,**f**), respectively). The images were acquired at the end of the experiments (8 days).

3. Results

3.1. Mortality of Trogoderma granarium Adults

All main effects and interactions of *T. granarium* adults between and within exposure intervals were significant except for the exposure × nematode species × nematode dose (Table 1). For the first 4 days of the experiment, all doses of *H. bacteriophora*, *S. carpocapsae* and *S. feltiae* provided low-to-moderate mortalities, i.e., 21.1–36.7% at 100 IJs/mL and 46.7–72.2% at 50,000 IJs/mL (Table 2). About half of the individuals (51.1%) were dead after exposure for 4 days to 1000 IJs/mL *S. carpocapsae*. Eight days post-exposure, all EPNs provided complete mortality when the dose was 50,000 IJs/mL. *Steinernema carpocapsae* killed 96.7% and 97.8% of *T. granarium* adults at 5000 IJs/mL and 10,000 IJs/mL. The lowest dose of the three EPN species (100 IJs/mL) caused 55.6–76.7% mortality at the end

of the experiments. Control mortality (0 IJs/mL) provided 1.1% mortality at the end of the experiments.

Table 1. MANOVA parameters for main effects and associated interactions for mortality of *Trogoderma granarium, Tenebrio molitor,* and *Alphitobius diaperinus* adults between and within exposure intervals (error DF = 144).

Effect		*Trogoderma granarium*		*Tenebrio molitor*		*Alphitobius diaperinus*	
Between exposure intervals							
Source	DF	*F*	*p*	*F*	*p*	*F*	*p*
Intercept	1	39,131.4	<0.01	2600.6	<0.01	29.9	<0.01
Nematode species	2	26.3	<0.01	4.3	0.02	4.4	0.02
Nematode dose	5	27.0	<0.01	33.4	<0.01	8.3	<0.01
Nematode species × nematode dose	10	2.0	0.04	1.4	0.19	1.0	0.41
Within exposure intervals							
Exposure	1	571.0	<0.01	371.5	<0.01	25.3	<0.01
Exposure × nematode species	2	10.6	<0.01	18.2	<0.01	9.2	<0.01
Exposure × nematode dose	5	5.5	<0.01	1.4	0.21	7.5	<0.01
Exposure × nematode species × nematode dose	10	1.1	0.36	9.1	<0.01	1.9	0.05

Table 2. Mean mortality of (% ± SE) *Trogoderma granarium* adults after 4 and 8 days on wheat treated with *Heterorhabditis bacteriophora, Steinernema carpocapsae,* and *Steinernema feltiae* applied at six doses. Within each row, means followed by the same uppercase letter are not significantly different in all cases. DF = 5, 53; Tukey–Kramer test at $p = 0.05$. Within each column, means followed by the same lowercase letter are not significantly different, in all cases DF = 5, 53; Tukey–Kramer test at $p = 0.05$.

		100 IJs/mL	500 IJs/mL	1000 IJs/mL	5000 IJs/mL	10,000 IJs/mL	50,000 IJs/mL	*F*	*p*
4 Days	*H. bacteriophora*	21.1 ± 3.5^{Cc}	23.3 ± 3.7^{BCc}	28.9 ± 3.1^{ABCe}	51.1 ± 7.2^{ABb}	53.3 ± 4.4^{Acd}	55.6 ± 3.4^{Ac}	7.3	<0.01
	S. carpocapsae	36.7 ± 5.0^{Bbc}	48.9 ± 5.9^{ABb}	51.1 ± 5.6^{ABcd}	52.2 ± 6.2^{ABb}	68.9 ± 4.2^{Abc}	72.2 ± 3.6^{Ab}	7.0	<0.01
	S. feltiae	23.3 ± 4.1^{Cc}	24.4 ± 1.8^{BCc}	37.8 ± 2.2^{ABde}	38.9 ± 4.2^{ABb}	42.2 ± 7.0^{ABd}	46.7 ± 5.0^{Ac}	5.7	<0.01
8 Days	*H. bacteriophora*	55.6 ± 5.0^{Bab}	57.8 ± 3.6^{Bab}	58.9 ± 4.6^{Bbc}	83.3 ± 4.7^{Aa}	84.4 ± 2.4^{Aab}	100.0 ± 0.0^{Aa}	17.6	<0.01
	S. carpocapsae	76.7 ± 4.4^{Ba}	78.9 ± 4.6^{Ba}	86.7 ± 2.4^{ABa}	96.7 ± 1.7^{Aa}	97.8 ± 1.5^{Aa}	100.0 ± 0.0^{Aa}	11.4	<0.01
	S. feltiae	61.1 ± 5.6^{Cab}	73.3 ± 6.5^{BCa}	80.0 ± 3.7^{ABCab}	84.4 ± 2.9^{ABa}	85.6 ± 4.4^{ABab}	100.0 ± 0.0^{Aa}	7.0	<0.01
	F	11.1	31.1	26.2	16.1	18.9	34.6		
	p	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		

3.2. Mortality of Tenebrio molitor Adults

The main effects were significant between the exposure intervals for *T. molitor* adults. Within the exposure intervals, exposure × nematode species and exposure × nematode species × nematode dose were significant (Table 1). Mortalities caused by the three EPNs were low at all tested doses at 4 days post-exposure, not exceeding 7.8% for the lowest dose of 100 IJs/mL and 27.8% for the highest dose of 50,000 IJs/mL (Table 3). Mortality values 8 days post-exposure were higher but still moderate for the doses 100 IJs/mL, 500 IJs/mL, 1000 IJs/mL, and 5000 IJs/mL for all EPNs. The doses 10,000 IJs/mL and 50,000 IJs/mL of *S. carpocapsae* killed 70.0% and 85.6%, respectively, while 50,000 IJs/mL of *H. bacteriophora* and *S. feltiae* did not exceed 62.2% and 76.7% mortality, respectively, at the same exposure interval. Control mortality was 0% after 8 days of exposure.

3.3. Mortality of Alphitobius diaperinus Adults

Between and within exposures, the main effects and interactions were significant, except those of nematode species × nematode dose interaction for *A. diaperinus* adults (Table 1). Mortality values were 0.0% after 4 days and 8 days of exposure to 100–10,000 IJs/mL of *S. feltiae* and 100–1000 IJs/mL of *S. carpocapsae*, while no mortality (0.0%) was recorded 4 days or 8 days post-exposure to 100–5000 IJs/mL or 100–1000 IJs/mL of *H. bacteriophora*, respectively (Table 4). The highest doses of 50,000 IJs/mL killed 1.1–2.2% of *A. diaperinus* adults after the first four days of experimentation and 3.3–11.1% at the termination of the experimental period. Control mortality was 0% at 8 days post-exposure.

Table 3. Mean mortality of (% ± SE) *Tenebrio molitor* adults after 4 and 8 days on wheat treated with *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *Steinernema feltiae* applied at six doses. Within each row, means followed by the same uppercase letter are not significantly different in all cases. DF = 5, 53; Tukey–Kramer test at $p = 0.05$. Within each column, means followed by the same lowercase letter are not significantly different in all cases. DF = 5, 53; Tukey–Kramer test at $p = 0.05$.

		100 IJs/mL	500 IJs/mL	1000 IJs/mL	5000 IJs/mL	10,000 IJs/mL	50,000 IJs/mL	*F*	*p*
4 Days	*H. bacteriophora*	7.8 ± 2.2^{Bab}	12.2 ± 4.7^{ABab}	14.4 ± 1.8^{ABa}	16.7 ± 1.7^{ABc}	17.8 ± 2.8^{ABd}	20.0 ± 4.1^{Ab}	3.6	0.01
	S. carpocapsae	7.8 ± 2.8^{Bab}	13.3 ± 2.4^{ABa}	16.7 ± 1.7^{Aa}	21.1 ± 3.5^{Abc}	24.4 ± 2.4^{Ad}	26.7 ± 4.1^{Ab}	6.4	<0.01
	S. feltiae	2.2 ± 2.2^{Bb}	3.3 ± 1.7^{Bb}	3.3 ± 1.7^{Bb}	23.3 ± 4.1^{Abc}	26.7 ± 4.1^{Acd}	27.8 ± 2.8^{Ab}	24.0	<0.01
8 Days	*H. bacteriophora*	16.7 ± 4.4^{Ca}	20.0 ± 4.4^{BCa}	25.6 ± 3.4^{ABCa}	43.3 ± 5.5^{ABa}	46.7 ± 2.4^{Aab}	62.2 ± 8.5^{Aa}	6.9	<0.01
	S. carpocapsae	12.2 ± 4.0^{Dab}	18.9 ± 3.1^{CDa}	27.8 ± 4.0^{BCa}	51.1 ± 6.8^{ABa}	70.0 ± 4.1^{ABa}	85.6 ± 3.4^{Aa}	13.4	<0.01
	S. feltiae	15.6 ± 3.4^{Ca}	20.0 ± 2.9^{BCa}	24.4 ± 3.8^{BCa}	40.0 ± 5.8^{ABab}	42.2 ± 4.9^{ABbc}	76.7 ± 6.7^{Aa}	10.6	<0.01
	F	3.3	4.8	20.0	8.1	19.8	24.7		
	p	0.01	<0.01	<0.01	<0.01	<0.01	<0.01		

Table 4. Mean mortality of (% ± SE) *Alphitobius diaperinus* adults after 4 days and 8 days on wheat treated with *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *Steinernema feltiae* applied at six doses. Within each row, means followed by the same uppercase letter are not significantly different in all cases. DF = 5, 53; Tukey–Kramer test at $p = 0.05$. Within each column, means followed by the same lowercase letter are not significantly different in all cases. DF = 5, 53; Tukey–Kramer test at $p = 0.05$.

		100 IJs/mL	500 IJs/mL	1000 IJs/mL	5000 IJs/mL	10,000 IJs/mL	50,000 IJs/mL	*F*	*p*
4 Days	*H. bacteriophora*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 1.1^{ab}	1.1 ± 1.1^{b}	0.8	0.56
	S. carpocapsae	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 1.1	2.2 ± 2.2^{ab}	2.2 ± 2.2^{b}	0.6	0.70
	S. feltiae	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0^{b}	1.1 ± 1.1^{b}	1.0	0.43
8 Days	*H. bacteriophora*	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}	6.7 ± 3.3^{AB}	7.8 ± 3.2^{ABa}	11.1 ± 2.6^{Aa}	6.7	<0.01
	S. carpocapsae	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 1.5	2.2 ± 2.2^{ab}	5.6 ± 2.9^{ab}	1.9	0.11
	S. feltiae	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}	0.0 ± 0.0^{Bb}	3.3 ± 1.7^{Aab}	4.0	<0.01
	F	-	-	-	2.2	2.4	3.6		
	p	-	-	-	0.07	0.05	0.01		

4. Discussion

The current study sheds light for the first time on the adulticidal efficacy of *H. bacteriophora*, *S. carpocapsae* and *S. feltiae* against *T. granarium*, *T. molitor* and *A. diaperinus* as grain protectants. Regarding *T. granarium* adults, the results revealed the remarkable efficacy of all EPN species at the highest dose. The effective management of this stage is crucial for this species, since its offspring at the egg or larva stages are tolerant to numerous synthetic or natural insecticides [43,46,57–59]. Therefore, adults determine the growth of the population of this species and the establishment of larvae in storage facilities, leading to dangerous population outbreaks [45,49]. Recently, Karanastasi et al. [11] reported almost total mortality to small *T. granarium* larvae and ~88% to large *T. granarium* larvae. Taking these results into account along with those of the current study, we can conclude that the use of *H. bacteriophora*, *S. carpocapsae* and *S. feltiae* on wheat can efficiently control adults and larvae (large and small) of this noxious insect. Furthermore, we propose the dose of 50,000 IJs/mL for all EPN species to achieve complete or almost complete adult or larval mortalities.

Regarding *T. molitor* adults, *S. carpocapsae* provided higher mortality (85.6%) than *H. bacteriophora* (62.2%) or *S. feltiae* (76.7%). Previously, 10 IJs/adult of *S. carpocapsae*, *S. feltiae*, and *S. riobrave* have been tested against *T. molitor* adults on filter paper [4]. *Steinernema carpocapsae* and *S. riobrave* could kill 90% and 95% of the exposed individuals, while *S. feltiae* could kill only 20% after 4 days of exposure. Virulence differences within the same EPN species can be attributed to the different strains of EPN that are used to manage *T. molitor* adults, a fact that could partially explain the high mortality related to the utilization of *S. feltiae* in our results vs. the low levels of mortality reported by Ramos-Rodríguez et al. [4]. For instance, four strains of *H. bacteriophora* caused the death of 30–90% of the exposed *T. molitor* larvae [7]. Similarly, Mbata and Shapiro-Ilan [3] noticed significant differences in the mortality values exhibited by *H. bacteriophora* strains against *P. interpunctella* adults.

In a recent study, 2000 IJs/mL *S. riobrave* or 3000 IJs/mL *H. bacteriophora* applied on filter paper could kill 10.0% or 20.0% of the exposed *T. granarium* individuals, three days post-exposure [13]; these are very low percentages compared to our findings (100.0%).

In the case of *A. diaperinus*, former studies have confirmed that mortality levels depend on the strain of the EPN. Ten strains of *Heterorhabditis* sp. applied on filter paper killed between 1.0 and 40.0% of the exposed *A. diaperinus* adults [2]. The results of this study revealed that none of the three EPNs could kill more than 11.1% of the individuals 8 days post-exposure. In general, the existing studies of EPN efficacy against *A. diaperinus* adults show tolerance of this species to EPN. For instance, 60 or 120 IJs/adult *Steinernema glaseri* (Steiner) (Rhabditida: Steinernematidae) killed 0.0% or 3.3% of the *A. diaperinus* individuals, respectively, after 7 days of exposure on filter paper. On the other hand, 60 IJs/adult *S. carpocapsae* led to 26.7% mortality of the *A. diaperinus* individuals after 7 days of exposure on filter paper [1]. Furthermore, different strains of insects may have variable susceptibility to EPNs. For example, Koc et al. [60] studied four geographical strains of *A. diaperinus*. After 5 days of exposure to 200 IJs/mL *S. carpocapsae*, adult mortalities ranged between 40.0% and 66.6%, while larval mortalities ranged from 73.3% to 90.0%. It is worth noting that mortalities caused by EPNs can vary when they are applied to different types of materials. For example, Del Valle et al. [8] revealed that the strains CUL of *Steinernema rarum* (Doucet) (Rhabditida: Steinernematidae) and SMC of *H. bacteriophora* killed 32.7% and 60.0% of *A. diaperinus* adults when they were applied to filter paper while they killed only 2.7% and 16.7% when they were applied to dry rice hull, 4 days post-exposure, respectively. In the same study, when the rice hull was wet, mortalities were higher, reaching 9.3% and 33.3% for the *S. rarum* CUL and *H. bacteriophora* SMC, respectively.

Another factor affecting the virulence of the EPN is temperature. For instance, the B49 and B30 strains of *S. feltiae* caused the death of more *S. oryzae* adults at 20 °C than at 15 °C, 25 °C, or 30 °C, vs. the 3162 strain, which caused higher mortality at 25 °C than at all other temperature levels [61]. Similarly, *S. carpocapsae* and *S. feltiae* killed more *E. kuehniella* larvae at 20 °C than at 30 °C, but the same temperature increase yielded opposite results regarding *H. bacteriophora* [5]. Interestingly, the same temperature change (from 20 °C to 30 °C) increased the efficacy of *S. feltiae* against *R. dominica* adults and decreased the virulence of *H. bacteriophora* against *T. confusum* larvae and adults. However, mortalities caused by *S. carpocapsae*, *H. bacteriophora* and *S. feltiae* to *S. oryzae* adults were approximately the same when temperature increased [5]. At 30 °C, Karanastasi et al. [11] provided >91% and >98% mortality of *T. granarium* small larvae after 8 days of exposure to 50,000 IJs/mL of *S. carpocapsae* and *S. feltiae*, respectively, an issue that was confirmed in the case of adults in the current study. Whether the complete mortality (100.0%) of *T. granarium* adults found in the current study remains the same under lower or higher temperatures merits further investigation. Moreover, Kung et al. [62] revealed that as RH levels decreased from 100% to 25%, the numbers of *S. carpocapsae* and *S. glaseri* IJ gradually decreased. Interestingly, the same RH decrease led to gradually lower pathogenicity of the aforementioned EPNs. Therefore, abiotic factors such as temperature and RH play a significant role in the survival and the efficacy of the EPN. Due to variable findings, apparently there is no general trend between temperature and the virulence of EPN.

5. Conclusions

In conclusion, the highest dose (50,000 IJs/mL) of the EPNs *H. bacteriophora*, *S. carpocapsae* and *S. feltiae* inoculated on wheat are highly efficient for the management of adults of *T. granarium*, an important quarantine species in several countries of the world. The same dose of *S. carpocapsae* can provide adequate protection of wheat from *T. molitor* adults, while all EPNs were ineffective against *A. diaperinus* adults. The susceptibility rank (from most to least susceptible) of the tested insect species was *T. granarium* > *T. molitor* > *A. diaperinus*. The EPN efficiency rank (from most to least efficient) was *S. carpocapsae* > *S. feltiae* > *H. bacteriophora*. Entomopathogenic nematodes have already been suggested for insect management in soil-less cryptic environments, as they are an excellent substitute for chemi-

cal control. The current study provides additional information on their adulticidal ability; nonetheless, to assess their entire potential as grain protectants, additional experimentation is required, including experimentation on more EPN species/strains and the use of a wide variety of grain commodities against stored-product insects and their developmental stages under a series of variable abiotic conditions.

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References

1. Alves, L.F.A.; Rohde, C.; Alves, V.S. Patogenicidade de *Steinernema glaseri* e *S. carpocapsae* (Nematoda: Rhabdita) contra o cascudinho, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). *Neotrop. Entomol.* **2005**, *34*, 139–141. [CrossRef]
2. Alves, V.S.; Neves, P.M.J.D.O.; Alves, L.F.A.; Moino, A., Jr.; Holz, N. Entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) screening for lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) control. *Rev. Colomb. Entomol.* **2012**, *38*, 76–80. [CrossRef]
3. Mbata, G.N.; Shapiro-Ilan, D.I. Laboratory evaluation of virulence of Heterorhabditid nematodes to *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). *Environ. Entomol.* **2005**, *34*, 676–682. [CrossRef]
4. Ramos-Rodríguez, O.; Campbell, J.F.; Ramaswamy, S.B. Pathogenicity of three species of entomopathogenic nematodes to some major stored-product insect pests. *J. Stored Prod. Res.* **2006**, *42*, 241–252. [CrossRef]
5. Athanassiou, C.G.; Kavallieratos, N.G.; Menti, H.; Karanastasi, E. Mortality of four stored product pests in stored wheat when exposed to doses of three entomopathogenic nematodes. *J. Econ. Entomol.* **2010**, *103*, 977–984. [CrossRef] [PubMed]
6. Boemare, N.; Laumond, C.; Mauleon, H. The entomopathogenic nematode-bacterium complex: Biology, life cycle and vertebrate safety. *Biocontrol Sci. Technol.* **2010**, *6*, 333–346. [CrossRef]
7. de Carvalho Barbosa Negrisoli, C.R.; Negrisoli, A.S.J.; Berbardi, D.; Garcia, M.S. Activity of eight strains of entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) against five stored product pests. *Exp. Parasitol.* **2013**, *134*, 384–388. [CrossRef] [PubMed]
8. Del Valle, E.E.; Frizzo, L.S.; Malmierca, M.; Zbrun, M.V.; Lax, P.; Doucet, M.E. Biological control of *Alphitobius diaperinus* with *Steinernema rarum* CUL and *Heterorhabditis bacteriophora* SMC and feasibility of application in rice hull. *J. Pest Sci.* **2016**, *89*, 161–170. [CrossRef]
9. Gulcu, B.; Cimen, H.; Raja, R.K.; Hazir, S. Entomopathogenic nematodes and their mutualistic bacteria: Their ecology and application as microbial control agents. *Biopestic. Int.* **2017**, *3*, 79–112.
10. Javed, S.; Khanum, T.A.; Khan, S. Biocontrol potential of entomopathogenic nematode species against *Tribolium confusum* (Jac.) (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae) under laboratory conditions. *Egypt. J. Biol. Pest Control* **2020**, *30*, 5. [CrossRef]
11. Karanastasi, E.; Kavallieratos, N.G.; Boukouvala, M.C.; Christodoulopoulou, A.D.; Papadopoulou, A.A. Effect of three entomopathogenic nematode species to *Trogoderma granarium* Everts (Coleoptera: Dermestidae) larvae on stored-wheat. *J. Stored Prod. Res.* **2020**, *88*, 101641. [CrossRef]
12. Erdoğuş, F.D. On the efficiency of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) on rust red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). *Egypt. J. Biol. Pest Control* **2021**, *31*, 116. [CrossRef]
13. Qader, F.; Mohammed, B.; Ameen, H. Efficacy of entomopathogenic nematodes against three species of stored product insects. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *910*, 012047. [CrossRef]
14. Kaya, H.K.; Gaugler, R. Entomopathogenic nematodes. *Annu. Rev. Entomol.* **1993**, *38*, 181–206. [CrossRef]
15. Singh, A.K.; Kumar, M.; Ahuja, A.; Vinay, B.K.; Kommu, K.K.; Thakur, S.; Paschapur, A.U.; Jeeven, B.; Mishra, K.K.; Meena, R.P.; et al. Entomopathogenic nematodes: A sustainable option for insect pest management. In *Biopesticides*; Rakshit, A., Meena, V.S., Abhilash, P.C., Sarma, B.K., Singh, H.B., Fraceto, L., Parihar, M., Singh, A.K., Eds.; Elsevier Academic Press Inc.: Duxford, UK, 2022; pp. 73–92.

16. Boemare, N. Interactions between the partners of the entomopathogenic bacterium nematode complexes, *Steinernema-Xenorhabdus* and *Heterorhabditis-Photorhabdus*. *Nematology* **2002**, *4*, 601–603. [CrossRef]
17. Ablel Razek, A.S. Pathogenic effects of *Xenorhabdus nematophilus* and *Photorhabdus luminescens* (Enterobacteriaceae) against pupae of the diamond backmoth, *Plutella xylostella* (L.). *J. Pest Sci.* **2003**, *76*, 108–111. [CrossRef]
18. Ciche, T.A.; Ensign, J.C. For the insect pathogen *Photorhabdus luminescens*, which end of a nematode is out? *Appl. Environ. Microbiol.* **2003**, *69*, 1890–1897. [CrossRef] [PubMed]
19. Lu, D.; Macchietto, M.; Chang, D.; Barros, M.M.; Baldwin, J.; Mortazavi, A.; Dillman, A.R. Activated entomopathogenic nematode infective juveniles release lethal venom proteins. *PLoS Pathog.* **2017**, *13*, e1006302. [CrossRef]
20. Martens, E.C.; Heungens, K.; Goodrich-Blair, H. Early colonization events in the mutualistic association between *Steinernema carpocapsae* nematodes and *Xenorhabdus nematophila* bacteria. *J. Bacteriol.* **2003**, *185*, 3147–3154. [CrossRef]
21. Ciche, T.A.; Darby, C.; Ehlers, R.U.; Forst, S.; Goodrich-Blair, H. Dangerous liaisons: The symbiosis of entomopathogenic nematodes and bacteria. *Biol. Control* **2006**, *38*, 22–46. [CrossRef]
22. Bowen, D.J.; Ensign, J.C. Purification and characterization of a high-molecular-weight insecticidal protein complex produced by the entomopathogenic bacterium *Photorhabdus luminescens*. *Appl. Environ. Microbiol.* **1998**, *64*, 3029–3035. [CrossRef] [PubMed]
23. Bowen, D.; Rocheleau, T.A.; Blackburn, M.; Andreev, O.; Golubeva, E.; Bhartia, R.; French-Constant, R.H. Insecticidal toxins from the bacterium *Photorhabdus luminescens*. *Science* **1998**, *280*, 2129–2132. [CrossRef] [PubMed]
24. Guo, L.; Fatig, R.O., III; Orr, G.L.; Schafer, B.W.; Strickland, J.A.; Sukhapinda, K.; Woodsworth, A.T.; Petell, J.K. *Photorhabdus luminescens* W-14 insecticidal activity consists of at least two similar but distinct proteins: Purification and characterization of toxin A and toxin B. *J. Biol. Chem.* **1999**, *274*, 9836–9842. [CrossRef] [PubMed]
25. Park, Y.; Kim, Y. Eicosanoids rescue *Spodoptera exigua* infected with *Xenorhabdus nematophilus*, the symbiotic bacteria to the entomopathogenic nematode *Steinernema carpocapsae*. *J. Insect Physiol.* **2000**, *46*, 1469–1476. [CrossRef] [PubMed]
26. Gupta, R.K.; Patterson, S.S.; Ripp, S.; Simpson, M.L.; Sayler, G.S. Expression of the *Photorhabdus luminescens* lux genes (luxA, B, C, D, and E) in *Saccharomyces cerevisiae*. *FEMS Yeast Res.* **2003**, *4*, 305–313. [CrossRef]
27. Boemare, N.; Akhurst, R. The genera *Photorhabdus* and *Xenorhabdus*. *Prokaryotes* **2006**, *6*, 451–494.
28. Yuksel, E.; Canhilal, R.; Imren, M. Potential of four Turkish isolates of entomopathogenic nematodes against three major stored products insect pests. *J. Stored Prod. Res.* **2019**, *83*, 317–321. [CrossRef]
29. Laznik, Ž.; Tóth, T.; Lakatos, T.; Vidrih, M.; Trdan, S. The activity of three new strains of *Steinernema feltiae* against adults of *Sitophilus oryzae* under laboratory conditions. *J. Food Agric. Environ.* **2010**, *8*, 150–154.
30. Okumura, G.T. A report of canthariasis and allergy caused by *Trogoderma* (Coleoptera: Dermestidae). *Calif. Vector Views* **1967**, *14*, 19–22.
31. Jood, S.; Kapoor, A.C.; Singh, R. Effect of insect infestation and storage on lipids of cereal grains. *J. Agric. Food Chem.* **1996**, *44*, 1502–1506. [CrossRef]
32. Rees, D. *Insects of Stored Products*; Manson Publishing: London, UK, 2004.
33. Hagstrum, D.W.; Subramanyam, B. *Stored-Product Insect Resource*; AACC International: St. Paul, MN, USA, 2009.
34. Agabou, A.; Alloui, N. Importance of *Alphitobius diaperinus* (Panzer) as a reservoir for pathogenic bacteria in Algerian broiler houses. *Vet. World* **2010**, *3*, 71–73.
35. Van Broekhoven, S.; Bastiaan-Net, S.; de Jong, N.W.; Witchers, H.J. Influence of processing and in vitro digestion on the allergic cross-reactivity of three mealworm species. *Food Chem.* **2016**, *196*, 1075–1083. [CrossRef] [PubMed]
36. Krinsky, W.L. Beetles (Coleoptera). In *Medical and Veterinary Entomology*; Mullen, G.R., Durden, L.A., Eds.; Academic Press: London, UK, 2019; pp. 129–143.
37. Garino, C.; Mielke, H.; Knüppel, S.; Selhorst, T.; Broll, H.; Braeuning, A. Quantitative allergenicity risk assessment of food products containing yellow mealworm (*Tenebrio molitor*). *Food Chem. Toxicol.* **2020**, *142*, 111460. [CrossRef] [PubMed]
38. Hill, D.S. *Pests of Stored Foodstuffs and their Control*; Kluwer Academic Publishers: New York, NY, USA, 2003.
39. Desneux, N.; Decourtye, A.; Delpuech, J.M. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* **2007**, *52*, 81–106. [CrossRef] [PubMed]
40. Ansari, M.S.; Moraiet, M.A.; Ahmad, S. Insecticides: Impact on the environment and human health. In *Environmental Deterioration and Human Health*; Malik, A., Grohmann, E., Akhtar, R., Eds.; Springer: Dordrecht, The Netherlands, 2014; pp. 99–123.
41. Benelli, G.; Wilke, A.B.; Bloomquist, J.R.; Desneux, N.; Beier, J.C. Overexposing mosquitoes to insecticides under global warming: A public health concern? *Sci. Total Environ.* **2021**, *762*, 143069. [CrossRef]
42. Wakil, W.; Kavallieratos, N.G.; Ghazanfar, M.U.; Usman, M.; Habib, A.; El-Shafie, H.A.F. Efficacy of different entomopathogenic fungal isolates against four key stored-grain beetle species. *J. Stored Prod. Res.* **2021**, *93*, 101845. [CrossRef]
43. Kavallieratos, N.G.; Nika, E.P.; Skourti, A.; Perinelli, D.R.; Spinozzi, E.; Bonacucina, G.; Cappellacci, L.; Morshedloo, M.R.; Canale, A.; Benelli, G.; et al. Apiaceae essential oil nanoemulsions as effective wheat protectants against five arthropod pests. *Ind. Crops Prod.* **2022**, *186*, 115001. [CrossRef]
44. Kavallieratos, N.G.; Michail, E.J.; Boukouvala, M.C.; Nika, E.P.; Skourti, A. Efficacy of pirimiphos-methyl, deltamethrin, spinosad and silicoSec against adults and larvae of *Tenebrio molitor* L. on wheat, barley and maize. *J. Stored Prod. Res.* **2019**, *83*, 161–167. [CrossRef]

45. Kavallieratos, N.G.; Boukouvala, M.C.; Ntalli, N.; Skourti, A.; Karagianni, E.S.; Nika, E.P.; Kontodimas, D.C.; Cappellacci, L.; Petrelli, R.; Cianfaglione, K.; et al. Effectiveness of eight essential oils against two key stored-product beetles, *Prostephanus truncatus* (Horn) and *Trogoderma granarium* Everts. *Food Chem. Toxicol.* **2020**, *139*, 111255. [CrossRef]
46. Kavallieratos, N.G.; Boukouvala, M.C.; Ntalaka, C.T.; Skourti, A.; Nika, E.P.; Maggi, F.; Spinozzi, E.; Mazzara, E.; Petrelli, R.; Lupidi, G.; et al. Efficacy of 12 commercial essential oils as wheat protectants against stored-product beetles, and their acetylcholinesterase inhibitory activity. *Entomol. Gen.* **2021**, *41*, 385–414. [CrossRef]
47. Kavallieratos, N.G.; Skourti, A.; Nika, E.P.; Mártonfi, P.; Spinozzi, E.; Maggi, F. *Tanacetum vulgare* essential oil as grain protectant against adults and larvae of four major stored-product insect pests. *J. Stored Prod. Res.* **2021**, *94*, 101882. [CrossRef]
48. Kavallieratos, N.G.; Nika, E.P.; Skourti, A.; Spinozzi, E.; Ferrati, M.; Petrelli, R.; Maggi, F.; Benelli, G. *Carlina acaulis* essential oil: A candidate product for agrochemical industry due to its pesticidal capacity. *Ind. Crops Prod.* **2022**, *188*, 115572. [CrossRef]
49. Papanikolaou, N.E.; Kavallieratos, N.G.; Iliopoulos, V.; Evergetis, E.; Skourti, A.; Nika, E.P.; Haroutounian, S.A. Essential oil coating: Mediterranean culinary plants as grain protectants against larvae and adults of *Tribolium castaneum* and *Trogoderma granarium*. *Insects* **2022**, *13*, 165. [CrossRef]
50. Sagheer, M.; Aman, Y.; Mansoor-ul-Hasan; Ahmed, F.; Ranjha, M.H.; Ali, Q.; Ali, K.; Sidra-tul-Muntaha. Fumigant bioactivity of extracts of *Citrulus colocynthes*, *Moringa oleifera* and *Azadirachta indica* against *Tribolium castaneum* and *Alphitobius diaperinus* under laboratory conditions. In Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2016), New Delhi, India, 7–11 November 2016; Navarro, S., Jayas, D.S., Alagusundaram, K., Eds.; CAF Permanent Committee Secretariat: Winnipeg, MB, Canada, 2016; pp. 459–464.
51. Kavallieratos, N.G.; Nika, E.P.; Skourti, A.; Filintas, C.S.; Goumenou, T.D. Short- and long-term mortalities of small and large larvae of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) on concrete surfaces treated with three insecticides: Impact of food. *Insects* **2022**, *13*, 366. [CrossRef] [PubMed]
52. Zar, J.H. *Biostatistical Analysis*; Pearson Education Limited: Essex, UK, 2014.
53. Scheff, D.S.; Arthur, F.H. Fecundity of *Tribolium castaneum* and *Tribolium confusum* adults after exposure to deltamethrin packaging. *J. Pest Sci.* **2018**, *91*, 717–725. [CrossRef]
54. Sall, J.; Lehman, A.; Creighton, L. JMP start statistics. In *A Guide to Statistics and Data Analysis Using JMP and JMP in Software*; Duxbury Press: Belmont, ON, Canada, 2001.
55. Sokal, R.R.; Rohlf, F.J. *Biometry*; Freeman & Company: New York, NY, USA, 1995.
56. SAS Institute Inc. *Using JMP 16.2*; SAS Institute Inc.: Cary, NC, USA, 2021.
57. Kousar, N.; Riaz, T.; Feroz, A.; Shakoori, A.R.; Rauf, F. Efficacy of diatomaceous earth and deltamethrin alone and in combinations on mortality and energy reserves of insecticide resistant strains of stored grain pest, *Trogoderma granarium*. *Pakistan J. Zool.* **2021**, *53*, 2183–2193. [CrossRef]
58. Ali, H.; Raza, A.B.M.; Majeed, M.Z.; Hamid, M.I. Laboratory evaluation of selected botanical and microbial formulations against khapra beetle *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *Pakistan J. Agric. Res.* **2022**, *35*, 154–164. [CrossRef]
59. Saad, M.M.G.; El Gendy, A.N.G.; Elkhateeb, A.M.; Abdelgaleil, S.A.M. Insecticidal properties and grain protective efficacy of essential oils against stored product insects. *Int. J. Trop. Insect Sci.* **2022**, *42*, 3639–3648. [CrossRef]
60. Koc, S.; Polat, B.; Cengiz, A.; Kahraman, S.; Tufan-Cetin, O.; Cetin, H. Pathogenicity of an entomopathogenic nematode, *Steinernema carpocapsae* on *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) strains from Turkey. *J. Econ. Entomol.* **2022**, *115*, 1303–1309. [CrossRef] [PubMed]
61. Laznik, Ž.; Trdan, S. Intraspecific variability of *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) as biological control agent of rice weevil (*Sitophilus oryzae* [L.], Coleoptera, Curculionidae) adults. *Acta Agric. Slov.* **2010**, *95*, 51–59.
62. Kung, S.P.; Gaugler, R.; Kaya, H.K. Effects of soil temperature, moisture and relative humidity on entomopathogenic nematode persistence. *J. Invertabr. Pathol.* **1991**, *57*, 242–249. [CrossRef]