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Insecticidal Effect of an Enhanced Attapulgite for the Control of Four Stored-Product Beetle Species

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Abstract: We examined the insecticidal effect of three different dust formulations, which were attapulgite (W), attapulgite mix with oregano essential oil with 52% carvacrol (D1), and attapulgite mix with oregano essential oil with 75% carvacrol (D2), in four major stored-product beetle species, *Sitophilus oryzae* (L.), *Tribolium confusum* Jacquelin du Val, *Rhyzopertha dominica* (F.), and *Trogoderma granarium* Everts. *Sitophilus oryzae* was the most susceptible at all three formulations, followed by *T. confusum* and *R. dominica*. In contrast, *T. granarium* larvae showed the lowest mortality rates even on the 14th day of observation and at the highest concentration (2000 ppm). Progeny production was particularly reduced for all species relative to the controls. Nevertheless, complete suppression of the offspring was observed only in the case of *T. confusum* and *S. oryzae*, while *R. dominica* was less susceptible to all three dust formulations, giving an average of up to 20 individuals per vial at 2000 ppm. To our knowledge, this study is the first that has examined the insecticidal activity of oregano compounds in combination with attapulgite for the control of stored-grain insect species. Additional experimentation is required to indicate the rationale of using these natural resource-based materials under a non-chemical control strategy at the post-harvest stages of agricultural commodities.

Keywords: essential oils; botanical formulations; attapulgite; stored product insects

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1. Introduction

Essential oils are secondary metabolites derived from aromatic plants and are characterized by a strong odor and volatility [1,2]. Many botanicals have been thoroughly investigated by the scientific community because of their antibacterial, antifungal, and insecticidal activities [1,2]. So far, more than 3000 essential oils have been found of which 300 are commercially available as they are used in the pharmaceutical, food, sanitary, and agronomic industries [1,2]. The application of essential oils as insecticides has gained many supporters as they provide a set of incontestable advantages over the use of conventional compounds. In addition, in most of the cases they do not pose considerable risks for the human health and non-target organisms, and are also environmentally compatible, as they do not leave toxic residues in the soil and the atmosphere [2–10].

The insecticidal value of the essential oils of species of the Lamiaceae family has been well studied in many application scenarios [2,11–16]. For instance, in the research of Obeng-Ofori et al. [12] the essential oil of *Ocimum kenyense* Ayob. ex A. J. Paton (Lamiales: Lamiaceae) was effective against the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), the maize weevil, *S. zeamais* Motschulsky (Coleoptera: Curculionidae), the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae). Similar results have also been reported in the case of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) adults when they were exposed to essential oils of *Lavandula officinalis* (L.)

(Lamiales: Lamiaceae) with a LD_{50} value of 0.07 mg/cm^2 [17]. Moreover, Ayvaz et al. [18] noted that the essential oils of savory, *Satureja thymbra* L. (Lamiales: Lamiaceae) were highly effective against the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) and the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), with 100% mortality obtained after 24 h at 9 and 25 µL/l air, respectively.

Origanum vulgare (L.) is an annual, perennial, and herbaceous plant of the Lamiaceae family. This plant is native to Mediterranean, Euro-Siberian, and Irano-Siberian regions, but has now naturalized in other tempered regions of the northern hemisphere [19–21]. It is known for its plethora of therapeutic, medicinal, and antimicrobial properties, which had been widely investigated [22–24]. There are several papers that demonstrate the antimicrobial, antioxidant, antifungal, and acaricidal activities of the essential oils and extracts obtained from the species of the genus *Origanum* [25–32]. Indicatively, Calmasur et al. [27] reported a toxic effect of *O. vulgare* essential oil on the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Bouchra et al. [26] found that essential oil of *Origanum compactum* Benth (Lamiales: Lamiaceae) inhibits 100% the growth of *Botrytis cinerea* Pers Fr (Helotiales: Sclerotiniaceae) at 100 ppm. Additionally, *O. vulgare* essential oil has exhibited antioxidant activity as shown by the consistent values of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging inhibition (59.09%) at 1000 ppm oil concentration [32]. In the same work, the authors indicated the increased antimicrobial properties of *O. vulgare* essential oil against *Bacillus cereus* Frankland (Bacillales: Bacillaceae) [32]. *Origanum* plant species have essential oils which are rich in terpenoids, with carvacrol, thymol, γ-terpinene, and p-cymene as major components [24,29,33–35]. In addition to their antimicrobial, antifungal, and acaricidal properties, *Origanum* essential oils have strong insecticidal value against a wide range of stored product insects and moths [15,18,29,35–39]. For instance, the essential oil extracted form *Origanum onites* (L.) (Lamiales: Lamiaceae) was effective against larvae of *P. interpunctella*, when it was used as a fumigant [18], while the essential oil of *O. majorana* was the most effective among the seven essential oils of different plants tested against larvae of *E. kuehniella* [37]. Encouraging results have been also reported in the research of Kordali et al. [29], in which the essential oils of *Origanum acutidens* (Hand.-Mazz.) (Lamiales: Lamiaceae) showed some insecticidal activity against the *S. granarius* and *T. castaneum*. Moreover, *O. vulgare* essential oil exhibited strong contact toxicity against *T. castaneum* [35], the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) [39], and *S. oryzae* [38]. Although the available literature on the efficacy of *O. vulgare* essential oils when applied alone for the control of stored product insects is abundant [35,38,39], the corresponding literature on the combined use of the particular essential oil with inert dusts is limited, despite the fact that there are data that underline the positive effects of the combined effects of plant extracts with inert materials in stored product protection [10,40–42].

Attapulgite is a rare magnesium aluminosilicate mineral, whose structure contributes to the absorption of pathogenic bacteria and toxins [43]. Because of its high adsorption and lack of toxicity, attapulgite finds application in pharmaceuticals, particularly intestinal preparations, where it is far superior to other clays in the adsorption of diphtheria toxin, bacteria, and alkaloids [44]. Particularly, there is a lot of research related to the property of attapulgite as a carrier, having a certain contribution against bacteria [45–50]. The recommended dose of attapulgite for adults is about 1.2–1.5 g per dose, up to 8.4 g per day. However, in cases of overdosing the main side effect of attapulgite is constipation, but it can also cause bloating, flatulence, stomach upset, and nausea. In addition to its medicinal use, it can serve excellently as a natural and eco-friendly soil conditioner providing reduction in water needs, improvement in nutrient absorption, soil aeration and fertility, and increase in strength and growth of plants [51]. Attapulgite's effect on stored product insects has only been studied by Mahanthi [52], who found that attapulgite clay was highly effective against *S. oryzae*, reducing its progeny production capacity, and completely controlled the rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae). Despite the fact that the utilization of inert materials, such as diatomaceous earths (DEs)

and zeolites, has been extensively studied for the control of stored product insects [53–56], there is still inadequate information on the utilization of attapulgite. Different silicaceous materials, such as artificial silicon dioxide and DE, have been utilized with success in combination with a wide range of plant extracts, for the control of major stored product insect species [10,40–42]. For instance, Athanassiou et al. [41] found that the combination of silicon dioxide with essential oils from *Juniperus oxycedrus* ssp. *oxycedrus* (Pinales: Cupressaceae) exhibited considerable insecticidal effects against adults of *S. oryzae* and *T. confusum*. Theoretically, such a combination is likely to provide an extension of the residual effect of the essential oils, as the inert material will control its release; nevertheless, this remains to be proved on the basis of key application scenarios.

Considering the limited data regarding the insecticidal effect of attapulgite, applied either alone, or in combination with essential oils, we carried out laboratory bioassays to examine the effectiveness of this application. In this context, we evaluated three different inert dust formulations, one of which was the attapulgite clay formulation alone and the other two were a mixture of attapulgite with *O. vulgare* essential oils of different carvacrol content, for the control of four major stored-product beetle species. Apart from parental mortality, suppression on progeny production capacity was also tested.

2. Materials and methods

2.1. Plant Material, Isolation of the Volatiles and Analysis

Aerial parts of *O. vulgare* were collected during June–July 2021 from different areas of Western Macedonia, Greece. All essential oils were obtained by steam-distillation using a modified Clevenger apparatus for 3 h, according to previously described procedure by Evergetis et al. [57]. The chemical composition of the essential oils was determined on an Agilent Technologies 7890A gas chromatographer (GC) coupled to an Agilent 5957C, VL MS Detector with a Triple-Axis Detector system, mass spectrometer (MS), and Flame Ionization Detector (FID) as described in Evergetis et al. [57]. Mass spectra were compared with NIST 11 and Willey 275 databases and authentic samples where available. The essential oils were stored in airtight containers in a refrigerator at 4 °C.

2.2. Preparation of the Formulations

The encapsulation process reported by Athanassiou et al. [41] was applied with some modifications. In brief, a quantity of ALGEV attapulgite powder (Geohellas, Grevena, Greece) with a granule diameter of 0–0.05 mm was placed in a closed, stainless, and rotating tank of 150 L until 60% of its capacity was filled. At the same time, 5 cm diameter stainless mesh beads containing impregnated fabric with oregano essential oil with 51.5% carvacrol (D1), equal to 5% of attapulgite weight, were added to the same tank. The tank was then placed at 50–60 °C and rotated at 15–20 rpm. During the rotation, the essential oil was extracted and encapsulated in the attapulgite powder in a period of 30–35 min. The same procedure was followed for the encapsulation of attapulgite powder in oregano essential oil with a carvacrol content of 74.7% (D2).

2.3. Test Insects

The species tested were the *T. confusum*, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), *S. oryzae*, and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). All species were reared at the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, at 25 °C, 65% relative humidity (r. h.), and continuous darkness. *Tribolium confusum* was reared on whole wheat flour, while the rest of the species were reared on whole wheat kernels. For *T. confusum*, *S. oryzae*, and *R. dominica* adult beetles, <1 month old individuals were used in the tests, while for *T. granarium* the individuals used were 5th instar larvae.

2.4. Bioassays

Lots of 500 g of wheat were placed in glass jars of 1000 mL in capacity and treated with the three-powder formulation: attapulgite (W), attapulgite mix with oregano essential oil with 51.5% carvacrol (D1), and attapulgite mix with oregano essential oil with 74.7% carvacrol (D2), in 3 concentration rates: 500, 1000, and 2000 ppm. An additional series of untreated lots was used as a control. Then, the jars were shaken for 5 min to ensure that the formulation was equally distributed throughout the wheat mass. The experimental units for the tests were plastic cylindrical vials (3 cm in diameter, 8 cm high, Rotilabo Sample tins Snap on lid, Carl Roth, Germany), with the top one quarter of the inside "neck" covered with Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, USA) to avoid insects' escape. Each vial contained 20 g of treated or untreated wheat, and then 20 adults were placed into each vial, using different vials for each insect species and concentration. All vials were maintained in incubators set at 26 °C, 55% r. h. and continuous darkness. The mortality of the exposed beetles was recorded after 1, 3, 7, and 14 days. For each species–concentration–dust formulation combination there were three vials, while the entire procedure was repeated three times, i.e., there were three replicates with three sub-replicates, with new lots of treated and untreated grains each time (3 jars X 3 vials each = 9 vials for each combination). At the end of this interval, all adults (dead and alive) for *T. confusum*, *S. oryzae,* and *R. dominica* and all larvae (dead and alive) for *T. granarium* were removed from the vials and the vials remained in the same conditions for an additional period of 65 days. Then, the vials were opened for the last time and the number of progenies was recorded.

2.5. Statistical Analysis

Initially, the mortality data were analyzed by using a Multivariate Analysis of Variance (MANOVA-Fit with Wilk's Lambda) with concentration, dust formulation, and exposure time as the main effects, by using the JPM 8 software (SAS Institute Inc., Cary, NC, USA). Then, in order to identify the differences between the three dust formulations, a one-way Analysis of Variance (ANOVA) was performed. Regarding progeny production, the data were analyzed by using a two-way ANOVA, with concentration rate and dust formulations as main effects. In both cases, means were separated by using the Tukey–Kramer Honestly Significant Difference (HSD) test, at a level of 0.05 [58].

3. Results

3.1. Chemical Components of Origanum vulgare Essential Oils

A total of 22 components were identified in O. vulgare essential oils, accounting for 99.1% and 99.2% of the total composition for D1 and D2, respectively (Table 1). Two isomeric monoterpene phenols, carvacrol (51.5 and 74.7% for D1 and D2, respectively) and thymol (3.29 and 3.78% for D1 and D2, respectively), along with one monoterpene hydrocarbon, p-cymene (19.9 and 8.42% for D1 and D2, respectively), were the main components of the essential oil formulations (Table 1). The other components were thujene, α-pinene, camphene, sabinene, β-pinene, β-myrcene, α-terpinene, d-limonene, γ-terpinene, linalool, borneol, 4-terpineol, α-terpineol, carvacrol methyl ether, β-caryophyllene, b-farnesene, α-caryophyllene, β-bisabolene, and caryophyllene oxide (Table 1).

Table 1. Constituents identified from two different *Origanum vulgare* essential oil formulations (D1 and D2) by gas chromatography (GC).

Component	Relative Composition Ratio (%)	
	D1	D2
Thujene	<0.05	<0.05
α-Pinene	1.20	0.32
Camphene	0.39	0.10
Sabinene	1.24	0.72

Table 1. *Cont.*

Component	Relative Composition Ratio (%)	
	D1	D2
β-Pinene	0.35	0.14
β-Myrcene	2.74	0.86
α-Terpinene	1.50	0.48
p-Cymene	19.9	8.42
d-limonene	0.97	0.35
γ-Terpinene	8.0	2.36
linalool	0.28	0.22
borneol	0.84	0.90
4-Terpineol	1.31	1.70
α-Terpineol	0.58	0.71
Carvacrol methyl ether	0.65	0.52
Thymol	3.29	3.78
Carvacrol	51.5	74.7
β-Caryophyllene	2.45	1.38
b-farnesene	<0.05	<0.05
α-Caryophyllene	0.29	0.37
β-bisabolene	1.20	0.88
caryophyllene oxide	0.36	0.24
Total	99.1	99.2

3.2. Adult Mortality

The concentration was found to be significant for all insect species examined (Table 2). Furthermore, the exposure time of adults to the different formulations, as well as its interaction with the concentration, were found to be significant for *T. confusum*, *S. oryzae,* and *R. dominica* (Table 2). Nevertheless, only in the case of *S. oryzae* did the interaction of exposure time with concentration and formulation significantly affect adult mortality (Table 2). On day 1 of exposure to the three dust formulations, mortality was low at all concentrations tested for all insect species (Table 3). Significant differences were noted only in the case of D2 as compared to the other two formulations examined for *R. dominica* at 1000 ppm, but in all treatments, mortality was low (Table 3). Over time, an increase in mortality was observed for all species (Table 4). Higher mortality rates on day 3 of exposure were noted at 2000 ppm for *S. oryzae*, but there were no significant differences (Table 4). At the 7-d exposure interval, D1 was found to be highly effective for *T. confusum* and *R. dominica* at 500 and 1000 ppm, respectively (Table 5). Finally, at the 14-d exposure interval, efficacy of D2 was significantly lower than that of the other two formulations, i.e., D1 and W for *T. confusum* and W for *S. oryzae* (Table 6). In contrast, all three dust formulations were not effective for the control of *T. granarium*, regardless of the combinations tested (Table 6).

Table 2. Repeated measures MANOVA parameters for mortality of the tested species exposed to three different dust formulations (D1, D2, and W) and four concentration rates (500, 1000, 2000 ppm, and control) [total *degrees of freedom (df) = 96*]. * Wilks' Lamda approximate F value.

	df	*Tribolium confusum*		*Trogoderma granarium*		*Sitophilus oryzae*		*Rhyzopertha dominica*	
		F	***P***	**F**	***P***	**F**	***P***	**F**	***P***
All between	11	49.50	**<0.01**	1.63	0.09	234,58	**<0.01**	32.09	**<0.01**
Intercept	1	948.23	**<0.01**	5.80	**0.01**	9188.23	**<0.01**	550.98	**<0.01**
Formulation	2	2.55	0.08	1.00	0.36	0.80	0.44	3.31	**0.04**
Dose	3	175.80	**<0.01**	4.01	**<0.01**	857.52	**<0.01**	114.06	**<0.01**
Formulation * Dose	6	1.99	0.07	0.66	0.68	1.05	0.39	0.70	0.65
All within interactions	33	14.38 *	**<0.01**	1.62 *	**0.02**	28.09 *	**<0.01**	7.15 *	**<0.01**
Time	3	371.10	**<0.01**	2.02	0.11	2785.47	**<0.01**	193.06	**<0.01**
Time * Formulation	6	1.52 *	0.17	2.01 *	0.06	0.73 *	0.62	1.50 *	0.17
Time * Dose	9	54.57 *	**<0.01**	1.49 *	0.15	111.43 *	**<0.01**	24.35 *	**<0.01**
Time * Formulation * Dose	18	1.52 *	0.08	1.51 *	0.08	2.08 *	**<0.01**	1.15 *	0.29

Table 3. Mean mortality (% ± SE) of *Tribolium confusum*, *Sitophilus oryzae*, *Rhyzopertha dominica* adults and *Trogoderma granarium* larvae after exposure to three different dust formulations (D1, D2, and W) applied at four concentration rates (500, 1000, 2000 ppm, and control) for 1 day (in all cases *d.f.* = 2.26).

Insect Species	Dusts	500	1000	2000	Control
T. confusum	D1	0.0 ± 0.0	0.0 ± 0.0	2.22 ± 1.21	0.0 ± 0.0
	D2	0.0 ± 0.0	0.0 ± 0.0	1.11 ± 0.73	0.0 ± 0.0
	W	0.0 ± 0.0	0.55 ± 0.55	1.11 ± 0.73	0.0 ± 0.0
	F	-	1.00	0.48	-
	P	-	0.38	0.62	-
T. granarium	D1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	D2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	W	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	F	-	-	-	-
	P	-	-	-	-
S. oryzae	D1	0.0 ± 0.0	0.55 ± 0.55	1.66 ± 0.83	0.0 ± 0.0
	D2	1.66 ± 0.83	0.0 ± 0.0	1.66 ± 1.17	0.0 ± 0.0
	W	0.55 ± 0.55	0.0 ± 0.0	1.66 ± 1.17	0.0 ± 0.0
	F	2.15	1.00	0.00	-
	P	0.13	0.38	1.00	-
R. dominica	D1	2.22 ± 0.87	3.33 ± 1.44b	18.88 ± 4.69	0.0 ± 0.0
	D2	0.55 ± 0.55	5.00 ± 0.83a	12.22 ± 2.77	0.0 ± 0.0
	W	0.55 ± 0.55	0.00 ± 0.00b	13.88 ± 3.41	0.0 ± 0.0
	F	2.00	7.00	0.87	-
	P	0.15	0.04	0.43	-

Within each column and species, means followed by the same letter are not significantly different (Tukey–Kramer HSD test at 0.05; where no letters exist, no significant differences were noted).

Table 4. Mean mortality (% ± SE) of *Tribolium confusum*, *Sitophilus oryzae*, *Rhyzopertha dominica* adults and *Trogoderma granarium* larvae after exposure to three different dust formulations (D1, D2, and W) applied at four concentration rates (500, 1000, 2000 ppm, and control) for Day 3 (in all cases *df* = 2.26).

Insect Species	Dusts	500	1000	2000	Control
T. confusum	D1	2.77 ± 1.46	2.77 ± 1.21	41.66 ± 10.34	1.11 ± 1.11
	D2	2.22 ± 1.21	2.77 ± 1.68	32.22 ± 5.39	0.00 ± 0.00
	W	0.55 ± 0.55	5.55 ± 2.11	56.66 ± 4.48	0.55 ± 0.55
	F	1.02	0.87	2.81	0.60
	P	0.37	0.42	0.08	0.55
T. granarium	D1	0.0 ± 0.0	0.0 ± 0.0	1.11 ± 0.73	0.0 ± 0.0
	D2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	W	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	F	-	-	2.28	-
	P	-	-	0.12	-
S. oryzae	D1	64.44 ± 7.92	98.88 ± 0.73	99.44 ± 0.55	0.55 ± 0.55
	D2	53.88 ± 5.45	98.88 ± 0.73	100.00 ± 0.00	0.55 ± 0.55
	W	73.33 ± 4.78	98.83 ± 0.83	97.77 ± 2.22	0.55 ± 0.55
	F	2.46	0.17	0.76	0.00
	P	0.10	0.84	0.47	1.00
R. dominica	D1	11.11 ± 1.82	35.55 ± 6.14	57.77 ± 5.71	0.0 ± 0.0
	D2	8.33 ± 2.35	21.11 ± 3.88	56.11 ± 4.84	0.0 ± 0.0
	W	11.11 ± 4.62	26.11 ± 2.97	52.22 ± 6.29	0.0 ± 0.0
	F	0.25	2.61	0.25	-
	P	0.77	0.09	0.77	-

Table 5. Mean mortality (% ± SE) of *Tribolium confusum*, *Sitophilus oryzae*, *Rhyzopertha dominica* adults and *Trogoderma granarium* larvae after exposure to three different dust formulations (D1, D2, and W) applied at four concentration rates (500, 1000, 2000 ppm, and control) for Day 7 (in all cases *df* = 2.26).

Insect Species	Dusts	500	1000	2000	Control
T. confusum	D1	11.11 ± 3.51a	27.77 ± 3.23	83.33 ± 6.23	18.88 ± 12.04
	D2	4.44 ± 1.75ab	25.00 ± 7.31	79.44 ± 6.14	7.77 ± 7.77
	W	2.22 ± 0.87b	36.11 ± 4.06	96.11 ± 1.82	0.55 ± 0.55
	F	3.96	1.24	2.85	1.24
	P	0.03	0.30	0.07	0.30
T. granarium	D1	0.0 ± 0.0	0.0 ± 0.0	1.11 ± 0.73	0.0 ± 0.0
	D2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	W	0.0 ± 0.0	0.55 ± 0.55	1.66 ± 1.17	0.0 ± 0.0
	F	-	1.00	1.12	-
	P	-	0.38	0.34	-
S. oryzae	D1	96.66 ± 1.17	100.00 ± 0.00	100.00 ± 0.00	4.44 ± 2.93
	D2	90.00 ± 3.72	100.00 ± 0.00	100.00 ± 0.00	6.66 ± 6.06
	W	92.77 ± 3.64	100.00 ± 0.00	98.33± 1.66	13.33 ± 6.82
	F	1.17	-	1.00	0.69
	P	0.32	-	0.38	0.50
R. dominica	D1	23.33 ± 3.11	50.00 ± 8.20a	72.22 ± 6.40	3.33 ± 2.35
	D2	18.33 ± 3.99	29.44 ± 4.59b	64.44 ± 6.14	2.77 ± 2.22
	W	16.66 ± 5.00	33.88 ± 2.32ab	65.55 ± 6.31	2.22 ± 2.22
	F	0.71	3.73	0.44	0.06
	P	0.50	0.03	0.64	0.94

Within each column and species, means followed by the same letter are not significantly different (Tukey–Kramer HSD test at 0.05; where no letters exist, no significant differences were noted).

Table 6. Mean mortality (% ± SE) of *Tribolium confusum*, *Sitophilus oryzae*, *Rhyzopertha dominica* adults and *Trogoderma granarium* larvae after exposure to three different dust formulations (D1, D2, and W) applied at four concentration rates (500, 1000, 2000 ppm, and control) for Day 14 (in all cases *df* = 2.26).

Insect Species	Dusts	500	1000	2000	Control
T. confusum	D1	51.66 ± 5.89	80.00 ± 5.71	100.00 ± 0.00a	25.55 ± 13.85
	D2	46.66 ± 5.65	69.44 ± 6.26	98.33 ± 0.83b	10.00 ± 8.20
	W	46.66 ± 6.45	88.33 ± 4.08	100.00 ± 0.00a	6.66 ± 6.06
	F	0.23	3.03	4.00	1.03
	P	0.79	0.06	0.03	0.37
T. granarium	D1	0.0 ± 0.0	0.0 ± 0.0	1.11 ± 0.73	0.0 ± 0.0
	D2	0.0 ± 0.0	0.0 ± 0.0	0.55 ± 0.55	0.0 ± 0.0
	W	0.0 ± 0.0	0.55 ± 0.55	1.66 ± 1.17	0.0 ± 0.0
	F	-	1.00	0.41	-
	P	-	0.38	0.66	-
S. oryzae	D1	97.77 ± 1.21ab	100.00 ± 0.00	100.00 ± 0.00	8.88 ± 5.82
	D2	93.88 ± 2.16b	100.00 ± 0.00	100.00 ± 0.00	13.88 ± 8.06
	W	100.00 ± 0.00a	100.00 ± 0.00	99.44± 0.55	13.33 ± 6.82
	F	4.65	-	1.00	0.15
	P	0.02	-	0.38	0.85
R. dominica	D1	28.33 ± 3.81	51.11 ± 7.89	76.66 ± 5.83	4.44 ± 3.05
	D2	28.33 ± 4.48	32.77 ± 5.21	67.77 ± 6.24	2.77 ± 2.22
	W	22.22 ± 5.27	35.55 ± 2.69	67.22 ± 5.95	2.77 ± 2.22
	F	0.59	3.02	0.77	0.14
	P	0.55	0.06	0.47	0.86

Within each column and species, means followed by the same letter are not significantly different (Tukey–Kramer HSD test at 0.05, where no letters exist, no significant differences were noted).

3.3. Progeny Production

Regarding progeny production counts, concentration had a significant effect for all species, with the exception of *T. granarium*, while formulation was significant only in the case of *R. dominica* (Table 7). Zero offspring production was observed in the case of *T. castaneum* and *T. granarium* for all concentrations and dust formulations tested (Table 8). Progeny production was also low for *S. oryzae*, where the highest number of adults per vial did not exceed 0.77 (Table 8). For *R. dominica*, the increase in concentration decreased progeny production (Table 8). Nevertheless, even at the highest concentration, progeny production for this species was high and exceeded 9, 15, and 20 individuals per vial for D1, D2, and W, respectively (Table 8).

Table 7. ANOVA parameters for progeny production counts for each species exposed to three different dust formulations (D1, D2, and W) and four concentration rates (500, 1000, 2000 ppm, and control).

	df	*Tribolium confusum*		*Trogoderma granarium*		*Sitophilus oryzae*		*Rhyzopertha dominica*	
		F	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Model	11	1.88	0.05	-	-	21.40	<0.01	4.98	<0.01
Intercept	1	6.25	0.01	-	-	79.25	<0.01	194.64	<0.01
Formulation	2	0.25	0.77	-	-	0.80	0.92	3.09	**0.04**
Concentration	3	6.25	**<0.01**	-	-	78.22	**<0.01**	14.16	**<0.01**
Formulation * Concentration	6	0.25	0.95	-	-	0.09	0.99	1.02	0.41

* No progeny production was recorded.

Table 8. Mean progeny production (number of adults per vial ± SE) of *Tribolium confusum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Trogoderma granarium*, 65 days after the removal of the parental individuals at three different dust formulations (D1, D2, and W) and four concentration rates (500, 1000, 2000 ppm, and control).

Insect Species	Dusts	500	1000	2000	Control
T. confusum	D1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.44 ± 0.33
	D2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.44 ± 0.33
	W	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.77 ± 0.46
	F	-	-	-	0.25
	P	-	-	-	0.78
T. granarium	D1	-	-	-	-
	D2	-	-	-	-
	W	-	-	-	-
	F	-	-	-	-
	P	-	-	-	-
S. oryzae	D1	0.00 ± 0.00A	0.00 ± 0.00A	0.0 ± 0.0A	152.44 ± 33.50B
	D2	0.22 ± 0.14A	0.11 ± 0.11A	0.0 ± 0.0A	141.88 ± 25.57B
	W	0.77 ± 0.66A	0.22 ± 0.14A	0.0 ± 0.0A	135.66 ± 24.05B
	F	1.04	1.09	-	0.09
	P	0.36	0.35	-	0.91
R. dominica	D1	32.33 ± 5.72	28.66 ± 5.24	9.22 ± 4.35	45.44 ± 17.64
	D2	23.66 ± 7.22A	40.22 ± 6.58A	14.77 ± 3.29A	83.44 ± 17.00B
	W	53.88 ± 9.08AB	35.11 ± 3.57AB	20.11 ± 2.37A	67.22 ± 14.83B
	F	2.61	1.20	2.50	1.32
	P	0.09	0.31	0.10	0.28

Within each row, means followed by the same letter are not significantly different (Tukey–Kramer HSD test at 0.05; in all cases *df* = 3.35, where no letters exist, no significant differences were noted). ANOVA parameters for *T. confusum* were: D1: F = 1.73, *P* = 0.181, D2: F = 1.73, *P* = 0.181, W: F = 2.80, *P* = 0.056, for *S. oryzae* were: D1: F = 20.70, *P* < 0.01, D2: F = 30.60, *P* < 0.01, W: F = 31.63, *P* < 0.01, for *R. dominica* were: D1: F = 2.30, *P* = 0.095, D2: F = 8.52, *P* < 0.01, W: F = 5.34, *P* < 0.01.

4. Discussion

To our knowledge, this is the first work that has examined the insecticidal activity of *O. vulgare* essential oils in combination with attapulgite for the control of the four species tested here. For some of the species and concentrations examined, we found that the combination of the two compounds was effective. In principle, it is generally regarded that the combined application of botanicals with inert materials prevents the essential oils from rapid breakdown, and such a formulation can be used with success for long-term protection strategies. For instance, Athanassiou et al. [41] found that the combined use of *J. oxycedrus* ssp. *oxycedrus* with silica was very effective for the control of *S. oryzae*, at concentrations that are generally lower than those that have been used when inert materials are applied alone [59,60]. Apart from breakdown disruption, the insecticidal effect of the inert material can also provide a sufficient insecticidal effect, even after the dissipation of the essential oil. In our tests, we postulate that both substances were effective for some of the species tested, but additional work is needed to illustrate the individual contribution of each substance.

The results of our research underline that the three different dust formulations are effective in controlling the three of the four stored product insect species (*S. oryzae*, *T. confusum* and *R. dominica*), when admixed with wheat and can therefore serve as alternative environmentally safe grain protectants. The concentration rates tested here are comparable with the ones that have been suggested for DEs, which are usually registered for direct application on grains at concentrations that range between 500 and 1000 ppm. From the species examined, *S. oryzae* was the most susceptible at all three formulations, followed by *T. confusum* and *R. dominica*. In contrast, *T. granarium* larvae showed the lowest mortality rates even on the last day of observation and at the highest concentration (2000 ppm). This finding is in agreement with previous studies, which reported the tolerance of *T. granarium* larvae to conventional insecticides [61–64], DEs [65] and essential oils [66–68]. Kavallieratos et al. [68] examined the essential oils of 12 different plant species, of which another Lamiaceae plant, *Thymus vulgaris* L., caused the second lowest mortality rate in the larvae of *T. granarium* (27.8%), at 1000 ppm. Kousar et al. [65] found that the 4th instar larvae of four populations of *T. granarium* that were collected from different regions of Pakistan were highly tolerant to DEs with LC_{50} values ranging from 2065 to 3606 ppm. Indeed, the above research is in agreement with ours regarding the formulations tested. Nevertheless, the experimental conditions tested here, and especially the temperature regime, might have caused larval induction to diapause [69–72]. Therefore, diapause may have increased their tolerance to the agents tested, as diapausing larvae of this species have been found considerably more tolerant than the non-diapausing ones to a wide range of active ingredients, such as fumigants and contact insecticides [64,73,74].

In contrast with *T. granarium* larvae, adults of *S. oryzae* and *T. confusum* were found to be susceptible to the three formulations tested. According to Campolo et al. [75] essential oils derived from plants belonging to the Lamiaceae family can be considered as highly effective for the control of *S. oryzae*. Moreover, *O. vulgare, Salvia fruticose* Miller *S. officinalis* (L.), *S. pomifera* ssp *pomifera, Thymbra capitata* (L.), and *Thymus persicus* (Roniger ex Reach F.) exhibited high toxicity on *S. oryzae*, with LC_{50} values ranging between 1.5 and 9 μL/L [38,76,77]. Goswami et al. [78] reported 100% mortality of *S. oryzae* when exposed for 7 days to 2000 and 1000 ppm of hydrophobic and lipophilic SiO_2 and hydrophilic Al_2O_3, respectively. Given that attapulgite is a layered magnesium aluminum, it appears that aluminosilicate components are particularly effective as grain protectants against *S. oryzae* in both rice and wheat [78,79].

The complete suppression of progeny production in the case of *T. confusum* could be partially attributed to the increased parental mortality of this species. Moreover, given that *T. confusum* is a secondary colonizer, this suppression could be enhanced by the inability of this species to infest sound grain kernels. Conversely, our results indicate that the formulations tested here were not effective for *R. dominica* adults. The same holds for the newly hatched larvae of this species that exhibited high survival rates, since this

species oviposits at the external part of the grain kernel, and larvae initially act as external feeders, before entering the grain. Earlier studies demonstrate the tolerance of *R. dominica* to DEs [53] and plant powders [80], that are used with admixture with the grains. In contrast, suppression of progeny production of *S. oryzae* was high, which could be attributed to the increased mortality of the parental adults of this species in a relatively short period of time, which might have drastically reduced the interval that was required for oviposition inside the kernels. Furthermore, this progeny suppression may indicate that the immatures individuals of this species are more susceptible than those of *R. dominica* to the plant-based agents that penetrated the grain kernel. This is particularly important, as it has been shown that certain inert materials are not very effective for the control of *S. oryzae* [81,82].

In summary, it is demonstrated that the formulations tested here, although extremely different in their composition of compounds or compound rates, are quite potent as insecticidal agents with effects comparable to those obtained using chemical insecticides for the control of stored product pests. Moreover, the combined application of inert materials and plant-derived substances in stored product protection merits additional investigation, on the basis of the development of eco-friendly and green grain protectants, which can be used in a non-chemical control strategy at the post-harvest stages of agricultural commodities in warehouses and food processing facilities. Additionally, we found that attapulgite, applied either alone or in combination with botanicals, has considerable insecticidal properties, which are comparable with those of other inert materials. In this regard, the data reported here underline the efficacy of attapulgite as a standalone formulation in stored-product protection, and merits additional investigation. In addition, we observed notable deviations in the susceptibility levels among the different species, which might have partially been caused by the conditions of our bioassays and their interaction with certain biological traits. Finally, for the application of these dust formulations in realistic scenarios in stored-product protection, further research is needed to achieve reduction in the relatively high application dose, perhaps in combination with low mammalian toxicity insecticides, botanicals, or food-grade additives, as successfully preceded by other inert materials, such as diatomaceous earth and zeolite [83].

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References

1. Bakkali, F.; Averbecka, S.; Averbecka, D.; Idaomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [CrossRef] [PubMed]
2. Dimetry, N.Z. Different Plant Families as Bioresource for Pesticides. In *Advances in Plant Biopesticides*; Singh, D., Ed.; Springer: Berlin/Heidelberg, Germany, 2014; pp. 1–20.
3. Kim, S.I.; Park, C.; Ohh, M.H.; Cho, H.C.; Ahn, Y.J. Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: Anobiidae). *J. Stored Prod. Res.* **2003**, *39*, 11–19. [CrossRef]

4. Sim, M.J.; Choi, D.R.; Ahn, Y.J. Vapor phase toxicity of plant essential oils to *Cadra cautella* (Lepidoptera: Pyralidae). *J. Econ. Entomol.* **2006**, *99*, 593–598. [CrossRef] [PubMed]
5. Guleria, S.; Tiku, A.K. Botanicals in pest management, current status and future perspectives. In *Integrated Pest Management, Innovation-Development Process*; Springer: Berlin/Heidelberg, Germany, 2009; pp. 317–329.
6. Dubey, N.K.; Shukla, R.; Kumar, A.; Singh, P.; Prakash, B. Prospects of botanical pesticides in sustainable agriculture. *Curr. Sci.* **2010**, *98*, 479–480.
7. Nayak, M.K.; Daglish, G.J. Importance of stored product insects. In *Recent Advances in Stored Product Protection*; Athanassiou, C.G., Arthur, F.H., Eds.; Springer: Berlin/Heidelberg, Germany, 2018; pp. 1–17.
8. Stejskal, V.; Hubert, J.; Li, Z. Human health problems and accidents associated with occurrence and control of storage arthropods and rodents. Insect pest management in stored grain. In *Recent Advances in Stored Product Protection*; Athanassiou, C.G., Arthur, F.H., Eds.; Springer: Berlin/Heidelberg, Germany, 2018; pp. 19–43.
9. Stejskal, V.; Vendl, T.; Aulicky, R.; Athanassiou, C. Synthetic and natural insecticides: Gas, liquid, gel and solid formulations for stored-product and food-industry pest control. *Insects* **2021**, *12*, 590. [CrossRef]
10. Karkanis, A.C.; Athanassiou, C.G. Natural insecticides from native plants of the mediterranean basin and their activity for the control of major insect pests in vegetable crops: Shifting from the past to the future. *J. Pest Sci.* **2021**, *94*, 187–202. [CrossRef]
11. Prakash, A.; Rao, J. *Botanical Pesticides in Agriculture*; CRC Press: Boca Raton, FL, USA, 1997; pp. 1–364.
12. Obeng-Ofori, D.; Reichmuth, C.H.; Bekele, J.; Hassanali, A. Biological activity of 1,8 cineole, a major component of essential oil of *Ocimum kenyense* (Ayobaugira) against stored product beetles. *J. Appl. Entomol.* **1997**, *121*, 237–243. [CrossRef]
13. Weaver, D.K.; Subramanyam, B. Botanicals. In *Alternatives to Pesticides in Stored-Product IPM*; Subramanyam, B., Hagstrum, D.W., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000; pp. 303–320.
14. Huang, Y.; Lam, S.L.; Ho, S.H. Bioactivities of essential oil from *Elletaria ncardamomum* (L.) maton. to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). *J. Stored Prod. Res.* **2000**, *36*, 107–117. [CrossRef]
15. Tunç, I.; Berger, B.M.; Erler, F.; Dagli, F. Ovicidal activity of essential oils from five plants against two stored-product insects. *J. Stored Prod. Res.* **2000**, *36*, 161–168. [CrossRef]
16. Lee, B.H.; Lee, S.E.; Annis, P.C.; Pratt, S.J.; Park, B.S.; Tummaaliia, F. Fumigant toxicity of essential oils and monoterpenes against the red four beetle, *Tribolium castaneum* (Herbst). *J. Asia Pac. Entomol.* **2002**, *5*, 237–240. [CrossRef]
17. El-Bakry, A.M.; Abdel-Aziz, N.F.; Sammour, E.A.; Abdelgaleil, S.A.M. Insecticidal activity of natural plant essential oils against some stored product insects and their side effects on wheat seed germination. *Egypt J. Biol. Pest Control.* **2016**, *26*, 83–88.
18. Ayvaz, A.; Sagdic, O.; Karaborklu, S.; Ozturk, I. Insecticidal activity of the essential oils from different plants against three stored-product insects. *J. Insect Sci.* **2010**, *10*, 21. [CrossRef] [PubMed]
19. Vokou, S.; Kokkini, S.; Bessiere, J.M. Geographic variation of Greek oregano (*Origanum vulgare* ssp. *hirtum*) essential oils. *Biochem. Syst. Ecol.* **1993**, *21*, 287–295. [CrossRef]
20. Pezzani, R.; Vitalini, S.; Iriti, M. Bioactivities of *Origanum vulgare* L.: An update. *J. Photochem.* **2017**, *16*, 1253–1268. [CrossRef]
21. Naquvi, K.J.; Ahamad, J.; Salma, A.; Ansari, S.H.; Najmi, A.K. A critical review on traditional uses, photochemistry and pharmacological uses of *Origanum vulgaris* Linn. *Int. J. Pharm. Res.* **2019**, *10*, 7–11. [CrossRef]
22. Chun, S.S.; Vattem, D.A.; Lin, Y.T.; Shetty, K. Phenolic antioxidants from clonal oregano (*Origanum vulgare*) with antimicrobial activity against *Helicobacter pylori*. *Process Biochem.* **2005**, *40*, 809–816. [CrossRef]
23. Bouyahya, A.; Jamal, A.; Edaoudi, F.; Et-Touys, A.; Bakri, Y. *Origanum compactum* Benth: A Review on Phytochemistry and Pharmacological Properties. *Med. Aromat. Plants* **2016**, *5*, 252. [CrossRef]
24. Soltani, S.; Shakeri, A.; Iranshahi, M.; Boozari, M. A Review of the phytochemistry and antimicrobial properties of *Origanum vulgare* L. and subspecies. *Iran. J. Pharm. Res.* **2021**, *20*, 268–285.
25. Daouk, R.K.; Dagher, S.M.; Sattout, E.J. Antifungal activity of the essential oil of *Origanum syriacum* L. *J. Food Prot.* **1995**, *58*, 1147–1149. [CrossRef]
26. Bouchra, C.; Achouri, M.; Idrissi Hassani, L.M.; Hmamouchi, M. Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea*. *Pers. Fr. J. Ethnopharmacol.* **2003**, *89*, 165–169. [CrossRef]
27. Calmasur, O.; Aslan, I.; Fikrettin, S. Insecticidal and acaricidal effect of three Lamiaceae plant essential oils against *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. *Ind. Crop. Prod.* **2006**, *23*, 140–146. [CrossRef]
28. Soylu, S.; Yigitbas, H.; Soylu, E.M.; Kurt, S. Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum*. *J. Appl. Microbiol.* **2007**, *103*, 1021–1030. [CrossRef] [PubMed]
29. Kordali, S.; Cakir, A.; Ozer, H.; Cakmakci, R.; Kesdek, M.; Mete, E. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. *Bioresour. Technol.* **2008**, *99*, 8788–8795. [CrossRef] [PubMed]
30. Salomone, A.; Scaritto, G.; Sacco, A.; Cabras, G.; Angioni, A. Inhibitory effects of the main compounds of oregano essential oil against some pathogenic fungi. Modern fungicide and Antifungal compounds V. *Dtsch. Phytomedizinische Ges.* **2008**, *44*, 345–360.
31. Sertkaya, E.; Kaya, K.; Soylu, S. Acaricidal activities of the essential oils from several medicinal plants against the carmine spider mite (*Tetranychus cinnabarinus* Boisd.) (Acarina, Tetranychidae). *Ind. Crop. Prod.* **2010**, *31*, 107–112. [CrossRef]
32. Jnaid, Y.; Yacoub, R.; Al-Biski, F. Antioxidant and antimicrobial activities of *Origanum vulgare* essential oil. *Int. Food Res. J.* **2016**, *23*, 1706–1710.

33. Daferera, D.J.; Ziagos, B.N.; Polissiou, M.G. The effectiveness of plant essential oils on the growth of Botrytis cinerea, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. *Crop Prot.* **2003**, *22*, 39–44. [CrossRef]
34. Esen, G.; Azaz, A.D.; Kurkcuoglu, M.; Baser, K.H.C.; Tinnaz, A. Essential oil and antimicrobial activity of wild and cultivated *Origanum vulgare L. subsp. Hirtum* (Link) letswaart from the Marmara region, Turkey. *Flavour Fragr. J.* **2007**, *22*, 371–376. [CrossRef]
35. Kim, S.I.; Yoon, J.S.; Jung, J.W.; Hong, K.B.; Ahn, Y.J.; Kwon, H.W. Toxicity and repellency of Origanum essential oils and its components against *Tribolium castaneum* (Coleoptera: Tenebrionidae). *J. Asia Pac. Entomol.* **2010**, *13*, 369–373. [CrossRef]
36. Çaglar, O.; Çalmasur, O.; Aslan, I.; Kaya, O. Insecticidal effect of essential oil of *Origanum acutidens* against several stored product pests. *Fresenius Environ.* **2007**, *16*, 1395–1400.
37. Karabörklü, S.; Ayvaz, A.; Yilmaz, S.; Akbulut, M. Chemical composition and fumigant toxicity of some essential oils against *Ephestia kuehniella*. *J. Econ. Entomol.* **2011**, *104*, 1212–1219. [CrossRef] [PubMed]
38. Abdelgaleil, S.A.M.; Mohamed, M.I.E.; Shawir, M.S.; Abou-Taleb, H.K. Chemical composition, insecticidal and biochemical effects of essential oils of different plant species from Northern Egypt on the rice weevil, *Sitophilus oryzae* L. *J. Pest Sci.* **2016**, *89*, 219–229. [CrossRef]
39. Plata-Rueda, A.; Zanuncio, J.C.; Serrão, J.E.; Martínez, L.C. *Origanum vulgare* essential oil against *Tenebrio molitor* (Coleoptera: Tenebrionidae): Composition, insecticidal activity, and behavioral response. *Plants* **2021**, *10*, 2513. [CrossRef] [PubMed]
40. Yang, F.L.; Liang, G.W.; Xu, Y.J.; Lu, Y.Y.; Zeng, L. Diatomaceous earth enhances the toxicity of garlic, *Allium sativum*, essential oil against stored product pests. *J. Stored Prod. Res.* **2010**, *46*, 118–123. [CrossRef]
41. Athanassiou, C.G.; Kavallieratos, N.G.; Evergetis, E.; Katsoula, A.; Haroutounian, S.A. Insecticidal efficacy of silica gel with *Juniperus oxycedrus* ssp. oxycedrus (Pinales: Cupressaceae) essential oil against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* **2013**, *106*, 1902–1910. [CrossRef] [PubMed]
42. Islam, M.Z.S.; Hasan, M.; Chaoliang, L.Z.; Mucha-Pelzer, T.; Mewis, I.; Ulrichs, C. Direct and admixture toxicity of diatomaceous earth and monoterpenoids against the storage pests *Callosobruchus maculatus* (F.) and *Sitophilus oryzae* (L.). *J. Pest Sci.* **2014**, *83*, 105–112. [CrossRef]
43. Skoufos, I.; Giannenas, I.; Tontis, D.; Bartzanas, T.; Kittas, C.; Panagakis, P.; Tzora, A. Effects of oregano essential oil and attapulgite on growth performance, intestinal microbiota and morphometry in broilers. *S. Afr. J. Anim. Sci.* **2016**, *46*, 77. [CrossRef]
44. Barr, M.; Arnista, E.S. Adsorption Studies on Clays I. *J. Am. Pharm. Assoc. (Sci. Ed.)* **1957**, *46*, 486–489. [CrossRef]
45. Huo, C.L.; Yang, H.M. Synthesis and characterization of ZnO/palygorskite. *Appl. Clay Sci.* **2010**, *50*, 362–366. [CrossRef]
46. Rosendo, F.R.G.V.; Pinto, L.I.F.; de Lima, I.S.; Trigueiro, P.; Honório, L.M.D.C.; Fonseca, M.G.; Silva-Filho, E.C.; Ribeiro, A.B.; Furtini, M.B.; Osajima, J.A. Antimicrobial efficacy of building material based on ZnO/palygorskite against gram-negative and gram-positive bacteria. *Appl. Clay Sci.* **2020**, *188*, 105499. [CrossRef]
47. Hui, A.P.; Dong, S.Q.; Kang, Y.R.; Zhou, Y.M.; Wang, A.Q. Hydrothermal fabrication of spindle-shaped ZnO/palygorskite nanocomposites using nonionic surfactant for enhancement of antibacterial activity. *Nanomaterials* **2019**, *9*, 1453. [CrossRef] [PubMed]
48. Hui, A.P.; Yan, R.; Wang, W.B.; Wang, Q.; Zhou, Y.M.; Wang, A.Q. Incorporation of quaternary ammonium chitooligosaccharides on ZnO/palygorskite nanocomposites for enhancing antibacterial activities. *Carbohydr. Polym.* **2020**, *247*, 116685. [CrossRef] [PubMed]
49. Hui, A.; Yang, F.; Yan, R.; Kang, Y.; Wang, A. Palygorskite-based organic–inorganic hybrid nanocomposite for enhanced antibacterial activities. *Nanomaterials* **2021**, *11*, 3230. [CrossRef] [PubMed]
50. Liu, J.; Gao, Z.; Liu, H.; Pang, L.; He, X.; Hui, A.; Wang, A. A study on improving the antibacterial properties of palygorskite by using cobalt-doped zinc oxide nanoparticles. *Appl. Clay Sci.* **2021**, *209*, 106–112. [CrossRef]
51. Barala, P.; Hooda, V. Hydrophilic polymers in agriculture and the use of nanoadditives to improve their capabilities. *J. Soil Sci. Plant Nutr.* **2022**. [CrossRef]
52. Mahanthi, V. Management of stored grain pests of maize using safer grain protectants. *Pestology* **2006**, *30*, 23–31.
53. Fields, P.; Korunic, Z. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. *J. Stored Prod. Res.* **2000**, *36*, 1–13. [CrossRef]
54. Athanassiou, C.G.; Vayias, B.J.; Dimizas, C.B.; Kavallieratos, N.G.; Papagregoriou, A.S.; Buchelos, C.T. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae) on stored wheat: Influence of dose rate, temperature and exposure interval. *J. Stored Prod. Res.* **2005**, *41*, 47–55. [CrossRef]
55. Rumbos, C.I.; Sakka, M.; Berillis, P.; Athanassiou, C.G. Insecticidal potential of zeolite formulations against three major stored-grain insects: Particle size effect, adherence ratio and effect of test weight on grains. *J. Stored Prod. Res.* **2016**, *66*, 93–101. [CrossRef]
56. Eroglou, N.; Emekci, M.; Athanassiou, C.G. Applications of natural zeolites on agriculture and food production. *J. Sci. Food Agric.* **2017**, *97*, 3487–3499. [CrossRef]
57. Evergetis, E.; Michaelakis, A.; Papachristos, D.P.; Badieritakis, E.; Kapsaski-Kanelli, V.N.; Haroutounian, S.A. Seasonal variation and bioactivity fluctuation of two *Juniperus* sp. essential oils against *Aedes* (Stegomyia) *albopictus* (Skuse 1894). *Parasitol. Res.* **2016**, *115*, 2175–2183. [CrossRef] [PubMed]
58. Sokal, R.R.; Rohlf, F.J. *Biometry, the Principles and Practice of Statistics in Biological Research*; Stony Brook University: Stony Brook, NY, USA, 1995.

59. Subramanyam, B.; Roesli, R. Inert dusts. In *Alternatives to Pesticides in Stored-Product IPM*; Subramanyam, B.H., Hagstrum, D.W., Eds.; Kluwer Academic Publishers: Boston, MA, USA, 2000; pp. 321–380.
60. Zeni, V.; Baliota, G.V.; Benelli, G.; Canale, A.; Athanassiou, C.G. Diatomaceous earth for arthropod pest control: Back to the future. *Molecules* **2021**, *26*, 7487. [CrossRef] [PubMed]
61. Bell, C.H.; Wilson, S.M.; Banks, H.J. Studies on the toxicity of phosphine to tolerant stages of *Trogoderma granarium* everts (Coleoptera: Dermestidae). *J. Stored Prod. Res.* **1984**, *20*, 111–117. [CrossRef]
62. Bell, C.H.; Wilson, S.M. Phosphine tolerance and resistance in *Trogoderma granarium* everts (Coleoptera: Dermestidae). *J. Stored Prod. Res.* **1995**, *31*, 199–205. [CrossRef]
63. Edde, P.A.; Eaton, M.; Kells, S.A.; Phillips, T.W. Biology, behavior, and ecology of pests in other durable commodities. In *Stored Product Protection*; Hagstrum, D.W., Phillips, T.W., Cuperus, G., Eds.; Kansas State University: Manhattan, KS, USA, 2012; pp. 45–62.
64. Athanassiou, C.G.; Kavallieratos, N.G.; Boukouvala, M.C.; Mavroforos, M.E.; Kontodimas, D.C. Efficacy of alpha-cypermethrin and thiamathoxam against *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) on concrete. *J. Stored Prod. Res.* **2015**, *62*, 101–107. [CrossRef]
65. Kousar, N.; Riaz, T.; Feroz, A.; Shakoori, A.R.; Shakoori, F.R. 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*. *Pak. J. Zool.* **2021**, *53*, 2183–2193. [CrossRef]
66. Pavela, R.; Morshedloo, M.R.; Lupidi, G.; Carolla, G.; Barboni, L.; Quassinti, L.; Bramucci, M.; Vitali, L.A.; Petrelli, D.; Kavallieratos, N.G.; et al. The volatile oils from the oleo-gum-resins of ferula assa-foetida and ferula gummosa: A comprehensive investigation of their insecticidal activity and eco-toxicological effects. *Food Chem. Toxicol.* **2020**, *140*, 111312. [CrossRef]
67. Kavallieratos, N.G.; Boukouvala, M.C.; Ntalli, N.; Skourti, A.; Karagianni, E.S.; Nika, E.P.; Kontodimas, D.C.; Cappellaccie, L.; Petrellie, R.; Cianfaglionef, 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]
68. 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]
69. Burges, H.D. Diapause, pest status and control of the khapra beetle, *Trogoderma granarium* Everts. *Ann. Appl. Biol.* **1962**, *50*, 614–617. [CrossRef]
70. Wilches, D.M.; Laird, R.A.; Floate, K.D.; Fields, P.G. A review of diapause and tolerance to extreme temperatures in dermestids (Coleoptera). *J. Stored Prod. Res.* **2016**, *68*, 50–62. [CrossRef]
71. Wilches, D.M.; Laird, R.A.; Floate, K.D.; Fields, P.G. Effects of acclimation and diapause on the cold tolerance of *Trogoderma granarium*. *Entomol. Exp. Appl.* **2017**, *165*, 169–178. [CrossRef]
72. Shivananjappa, S.; Fields, P.; Laird, R.A.; Floate, K.D. Contributions of diet quality and diapause duration to the termination of larval diapause in khapra beetle, *Trogoderma granarium* (Coleoptera: Dermestidae). *J. Stored Prod. Res.* **2020**, *85*, 101535. [CrossRef]
73. Hole, B.D.; Bell, C.H.; Mills, K.A.; Goodship, G. The toxicity of phosphine to all developmental stages of thirteen species of stored product beetles. *J. Stored Prod. Res.* **1976**, *12*, 235–244. [CrossRef]
74. Gourgouta, M.; Agrafioti, P.; Athanassiou, C.G. Insecticidal effect of phosphine for the control of different life stages of the khapra beetle, *Trogoderma granarium* (Coleoptera: Dermestidae). *Crop Prot.* **2021**, *140*, 105409. [CrossRef]
75. Campolo, O.; Giunti, G.; Russo, A.; Palmeri, V.; Zappalà, L. Essential oils in stored product insect pest control. *J. Food Qual.* **2018**, *2018*, 6906105. [CrossRef]
76. Saroukolai, A.T.; Moharramipour, S.; Meshkatalsadat, M.H. Insecticidal properties of *Thymus persicus* essential oil against *Tribolium castaneum* and *Sitophilus oryzae*. *J. Pest Sci.* **2010**, *83*, 3–8. [CrossRef]
77. Koutsaviti, A.; Antonopoulou, V.; Vlassi, A.; Antonatos, S.; Michaelakis, A.; Papachristos, D.P.; Tzakou, O. Chemical composition and fumigant activity of essential oils from six plant families against *Sitophilus oryzae* (Col: Curculionidae). *J. Pest Sci.* **2018**, *91*, 873–886. [CrossRef]
78. Goswami, A.; Roy, I.; Sengupta, S.; Debnath, N. Novel applications of solid and liquid formulations of nanoparticles against insect pests and pathogens. *Thin Solid Films* **2010**, *519*, 1252–1257. [CrossRef]
79. Debnath, N.; Das, S.; Seth, D.; Chandra, R.; Bhattacharya, S.C.; Goswami, A. Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). *Pest Sci.* **2011**, *84*, 99–105. [CrossRef]
80. Lampiri, E.; Agrafioti, P.; Levizou, E.; Athanassiou, C.G. Insecticidal effect of *Dittrichia viscosa* lyophilized epicuticular material against four major stored-product beetle species on wheat. *Crop Prot.* **2020**, *132*, 105095. [CrossRef]
81. Arthur, F.H. Evaluation of a new insecticide formulation (F2) as a protectant of stored wheat, maize, and rice. *J. Stored Prod. Res.* **2004**, *40*, 317–330. [CrossRef]
82. Eroglu, N.; Sakka, M.K.; Emekci, M.; Athanassiou, C.G. Effects of zeolite formulations on the mortality and progeny production of *Sitophilus oryzae* and *Oryzaephilus surinamensis* at different temperature and relative humidity levels. *J. Stored Prod. Res.* **2019**, *81*, 40–45. [CrossRef]
83. Athanassiou, C.G.; Arthur, F.H. (Eds.) Bacterial Insecticides and Inert Materials. In *Recent Advances in Stored Product Protection*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 83–93.