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Efficacy of Extreme Temperatures on All Life Stages of the Mediterranean Flour Moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)

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Abstract: In the present study, we examined the effect of extreme temperatures on different life stages of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). The individuals were exposed to different temperatures ranging between 5 and −18 °C for cold treatment, and between 30 and 55 °C for heat treatment, and different exposure intervals, ranging from minutes to days. Complete control was achieved at −10 and −15 °C for cold treatment and 50 and 55 °C for heat treatment at all exposure intervals. Considering the efficacy of extreme temperatures for the control of *E. kuehniella*, our study provides specific temperature exposure modules that can be effective for the control of this species.

Keywords: cold; heat; moth; stored products; temperature; exposure time

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1. Introduction

The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is one of the most important stored product moths in different storage facilities, causing serious losses in a wide range of commodities [1,2]. For the control of stored product moths, incl. this species, several measures have been taken in different facilities and commodities such as contact insecticides [3], impregnated nets [4], mating disruption [5], trapping [2,6], cold treatment [7,8] and heat treatment [9,10]. For instance, Javanshir et al. [11] tested the effect of cypermethrin and dichlorvos with high temperatures that range from 45 to 65 °C and found that the combination of a low concentration of both insecticides with high temperatures can be effective for the control of eggs, third instar larvae and adults of *E. kuehniella*. Moreover, Sieminska et al. [5] tested mating disruption for a long duration of monitoring *E. kuehniella* and found that the reduction was lower in the first year than in the second.

The use of extreme temperatures is a promising alternative to traditional chemicals for the disinfestation of stored products [12,13]. The principle of using extreme temperatures (heat or cold) is based on the exposure of short durations, i.e., less than 24 h for heat treatment and no longer than 7 days, for cold treatment [13,14]. Extreme temperatures have been tested in a wide range of stored product insects, including *E. kuehniella*, with good results against different life stages [15,16]. Andreadis et al. [16] tested the supercooling point of larvae, pupae and adults of *E. kuehniella* and found that temperatures below −12.5 °C are effective for the control of all tested life stages. Moreover, Ben-Ialli [17] tested the effect of heat treatment on the eggs of *E. kuehniella* and found that for complete mortality at temperatures below 50 °C, long exposures are needed, suggesting that temperatures that are lower than that threshold may be ineffective when a short exposure protocol is planned.

The use of heat treatment is based on increasing and maintaining the temperature around 50 to 60 °C in the treated area to kill stored product insects [15,18,19]. In a recent study, Sakka et al. [13] tested heat treatment against stored product insect populations

which were resistant and susceptible to phosphine and found that short exposures to temperatures higher than 50 °C were effective for complete control, even for the resistant populations. Studies published earlier have underlined the need to achieve uniform heat distribution in the treated area to achieve complete mortality [13,18,19].

The application of cold treatment can be an effective method for the control of different stored product insects [8,20]. As in the case of heat treatment, the effectiveness of cold treatment depends on temperature, exposure time, and the different life stages of the species. For instance, Athanassiou et al. [12] tested the eggs, larvae, pupae and adults of the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), and the eggs, larvae and adults of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) at different temperatures ranging from 0 to −15 °C and different exposure times from 2 h to 7 days and found that *O. surinamensis* were more tolerant to cold than *T. confusum*. Moreover, Arthur et al. [14] tested all the life stages of the cabinet beetle, *Trogoderma inclusum* (LeConte) (Coleoptera: Demestidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) at −18 °C and found that the eggs of *T. inclusum* were much more tolerant than the eggs and larvae of *T. castaneum*. All the above studies clearly underline the importance of long exposure periods in order to control the eggs of major stored product insects that may exist in deeper layers of the commodities that are to be treated.

However, there are limited data on the application of cold and heat treatment in the same study, with all life stages of one major stored product insect moth, with the exception of the Indian meal moth, *Plodia interpuctella* (Hubner) (Lepidoptera: Pyralidae) that has been tested in the study of Athanassiou et al. [21] examining only the effect of low temperatures. In this regard and taking into account the data gaps that are illustrated above, the aim of the present study was to evaluate the effect of cold and heat treatment on all the life stages (eggs, larvae, pupae and adults) of *E. kuehniella* under laboratory conditions. Studies available on heat treatment for all the life stages of *E. kuehniella* are limited, leaving gaps regarding the temperature and the exposure time to achieve complete mortality of the species. To our knowledge, this is the first research evaluating both heat and cold treatments on all life stages of this species. Hence, the present study describes laboratory tests to determine the efficacy of five different low temperatures, i.e., 5, 0, −5, −10 and −15 °C, and six exposure times (i.e., 1, 3, 6, 9, 24 and 168 h); and four high temperatures, i.e., 30, 40, 50 and 55 °C, and four exposure times (60 min, 3, 12 and 24 h).

2. Materials and Methods

2.1. Insects

The *E. kuehniella* population used was reared at the Laboratory of Entomology and Agricultural Zoology (LEAZ) at 25 °C, 65% relative humidity (r.h.) and continuous darkness. The diet was composed of 600 g of wheat bran, 400 g cracked wheat, 50 g of brewer's yeast, 80 mL of honey, 80 mL of glycerin and 60 mL of water, and was used as the rearing substrate. Regarding the different life stages used, eggs were 1–2 days old, larvae were used at their last instar, pupae were <5 days old, and adults were <1 week old.

2.2. Cold Tests

Six different temperatures, i.e., 5, 0, −5, −10 and −15 °C, and different exposure intervals, i.e., 1, 3, 6, 9, 24 and 168 h were evaluated. The tests were carried out in a freezer (Artsteel AB-078-G, Artsteel S.A., Lagadas, Thessaloniki, Greece) of controlled temperatures that exist in LEAZ. Plastic cylindrical plastic vials (3 cm in diameter, 8 cm in height, Rotilabo Sample tins Snap on lid, Carl Roth, Karlsruhe, Germany) were used for the tests. Within each vial, 10 individuals were placed (different vials for each life stage), along with a small quantity of food (5 g of wheat flour). Mortality was assessed after 1 day of the application, except eggs and pupae, which were examined for hatch/emergence seven days post-exposure. In addition, separately for each temperature exposure combination, untreated vials were used as the controls and kept at 25 °C and 65% rh. Each temperature

and exposure time was repeated three times, with three replicates and three subreplicates for each combination by preparing a new series of vials each time.

2.3. Heat Tests

Four different temperatures, i.e., 30, 45, 50 and 55 °C, and different exposure intervals, i.e., 60 min, 3 h, 12 h, and 24 h, were evaluated. The experimentation of heat treatment was carried out in a heater (Termaks TS4057, Termaks, Nordic Labtech AB, Bergen, Norway) of controlled temperatures that exist in the facilities of LEAZ. The measurement of mortality, number of replications, etc., were the same as above.

2.4. Data Analysis

The data were submitted to a two-way analysis of variance separately for each life stage (eggs, larvae, pupae and adults), with exposure intervals and temperature as the main effects and mortality as the response variable. All analyses were conducted by using JMP 11 software (SAS Institute Inc., Cary, NC, USA, 2013). The means were separated by the honest significant difference [HSD] test at 0.05.

3. Results

3.1. Cold Tests

All main effects and their associated interactions were significant (Table 1). Mortality on the untreated vials was lower than 5% for all life stages. Mortality of all life stages was high in temperatures lower than 0 °C for 3 h (Figure 1). Mortality reached 100% after exposure to −15 °C for all life stages (Figure 1). Moreover, egg mortality was higher than 40% at all temperatures, after 3 h of exposure (Figure 1). At −10 °C, mortality reached 100%, even after 1 h of exposure (Figure 1). The mortality of larvae was low at −5 °C after 24 h of exposure and reached 100% after 168 h (Figure 1). At −5 °C, mortality reached 20% after 9 h of exposure, but complete mortality was achieved only at 168 h (Figure 1). For pupae, low mortality rates were observed at 0 and −5 °C until the 9 h exposure interval, but at −15 °C, complete mortality was achieved after 1 h of exposure (Figure 1). No mortality was recorded for adults at 5 °C for all the exposures tested (Figure 1). Moreover, about one half of the exposed individuals were dead after 1 h of exposure at all temperatures below 0 °C (Figure 1). Finally, complete mortality was recorded at all temperatures after 168 h of exposure (Figure 1).

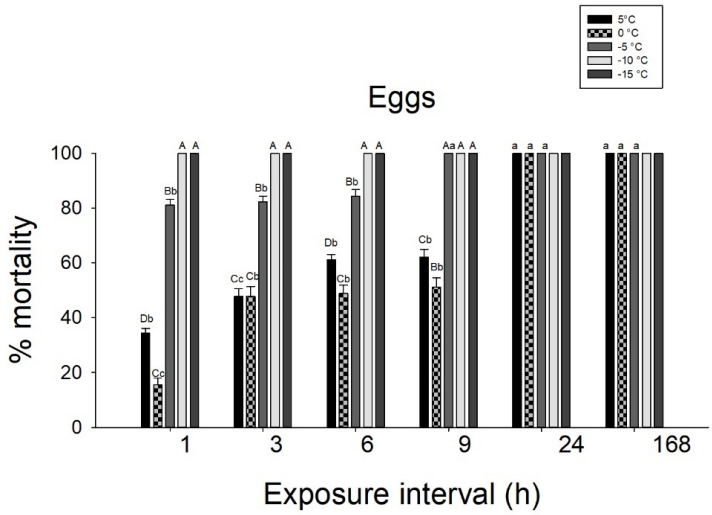


Figure 1. *Cont.*

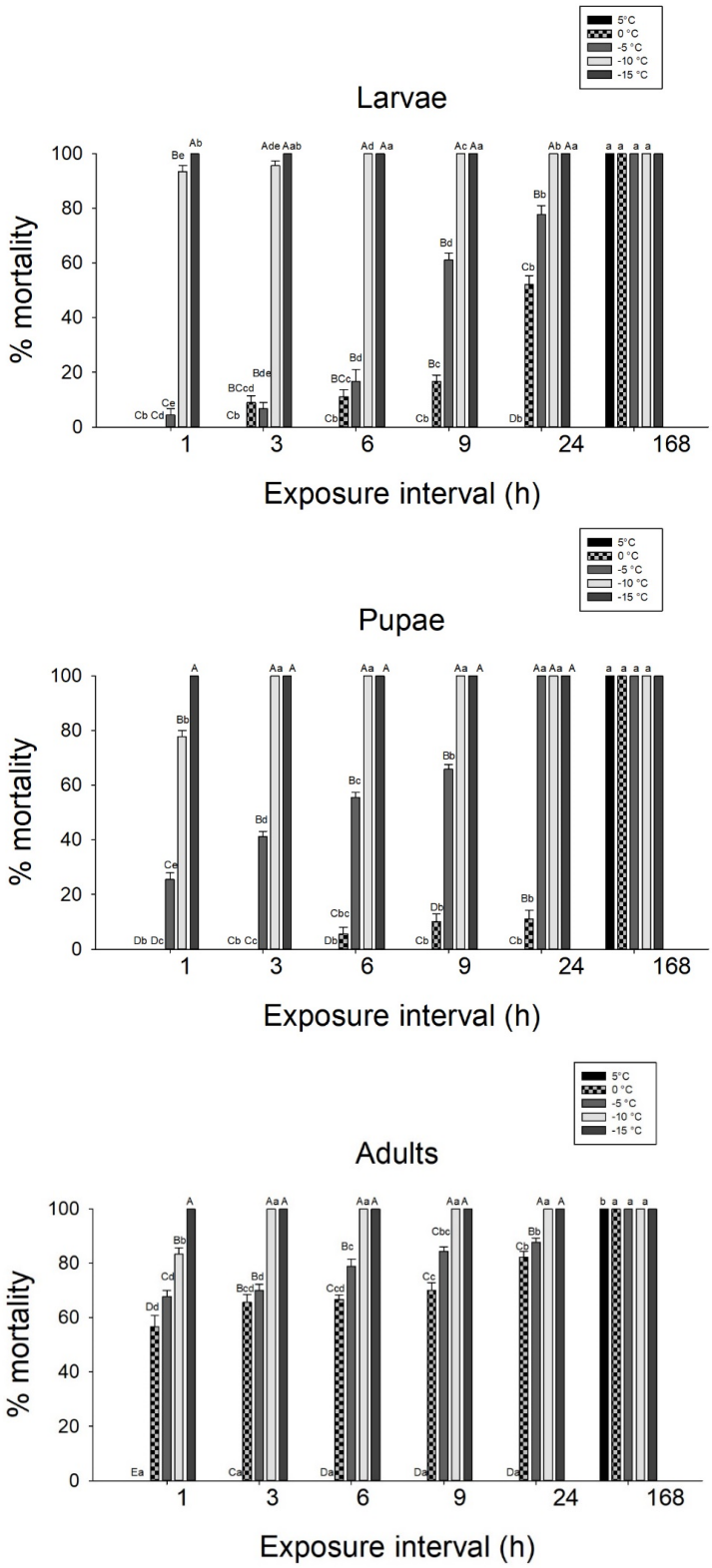


Figure 1. Mortality (%) of *Ephestia kuehniella* eggs, larvae, pupae, and adults after exposure at 5, 0, −5, −10 and −15 °C at six different exposure intervals (1, 3, 6, 9, 24 and 168 h). The means of each exposure interval followed by the same uppercase letters do not differ significantly (in all cases, error *df* = 48; Tukey's HSD test at $p = 0.05$). The means of each temperature followed by the same lowercase letter do not differ significantly (in all cases, error *df* = 40; Tukey's HSD test at $p = 0.05$). Where no letters exist, no significant differences were noted.

Table 1. ANOVA parameters for the main effects on and interactions between all the life stages of *Ephestia kuehniella* (error *df* = 978) receiving cold treatment.

Source	*df*	F	*p*
Model	119	698.9	<0.01
Intercept	1	250,913.8	<0.01
Life stage	3	1985.0	<0.01
Temperature	4	10,227.2	<0.01
Time	5	2599.8	<0.01
Life stage × Temperature	12	612.7	<0.01
Life stage × Time	15	105.7	<0.01
Temperature × Time	20	515.0	<0.01
Life stage × Temperature × Time	60	62.5	<0.01

3.2. Heat Tests

All the main effects and their associated interactions were significant (Table 2). Mortality in the untreated vials was lower than 5% for all life stages. In all life stages at 55 °C after exposure to 24 h mortality reached 100% (Figure 2). At 40 °C, mortality was low in the case of eggs and did not exceed 40% after 1 and 6 h of exposure; nevertheless, at 50 °C, mortality was close to 100% (Figure 2). In contrast, there was no effect on larvae when these were exposed at 30 °C at all = exposure times (Figure 2). After 1 and 3 h of exposure, at 40 °C, larvae mortality did not exceed 20% (Figure 2). At 50 and 55 °C, the complete mortality of larvae was recorded, even after 1 h of exposure (Figure 2). For pupae, mortality was low and did not exceed 60% after exposure to 40 °C for 6 h (Figure 2). Complete pupae mortality was noted after 1 h of exposure at 50 and 55 °C (Figure 2). Adults were not affected at 30 °C and all individuals survived (Figure 2). Low mortality was recorded at 40 °C after 1 and 3 h and did not exceed 10%, but after 9 and 24 h, the mortality was 71 and 100%, respectively (Figure 2).

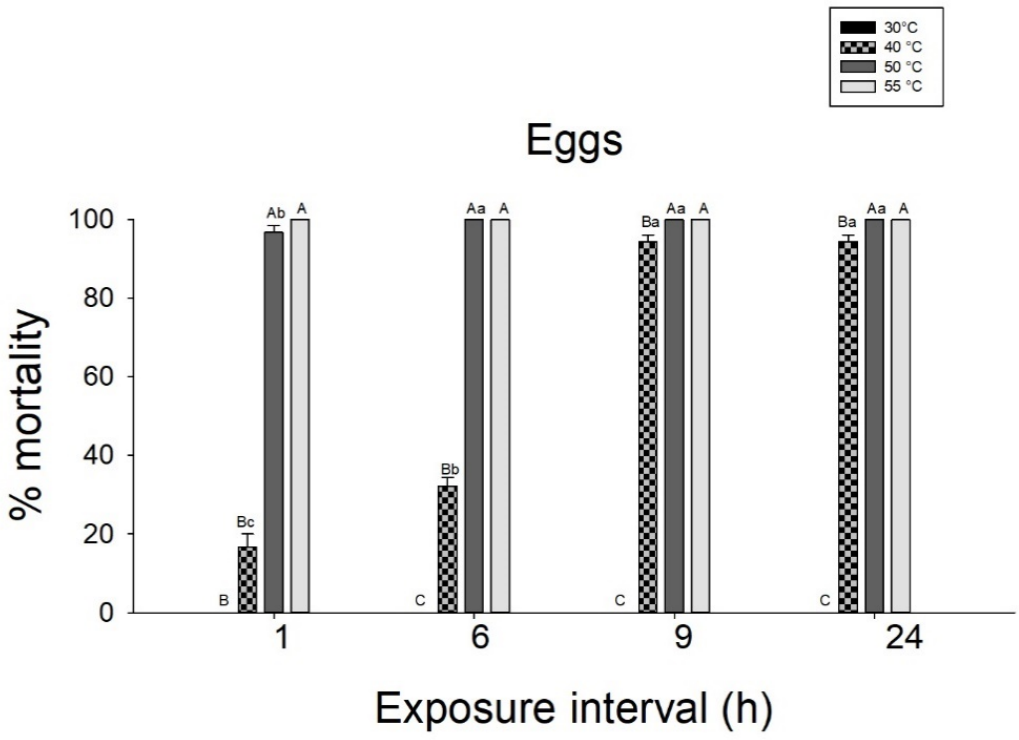
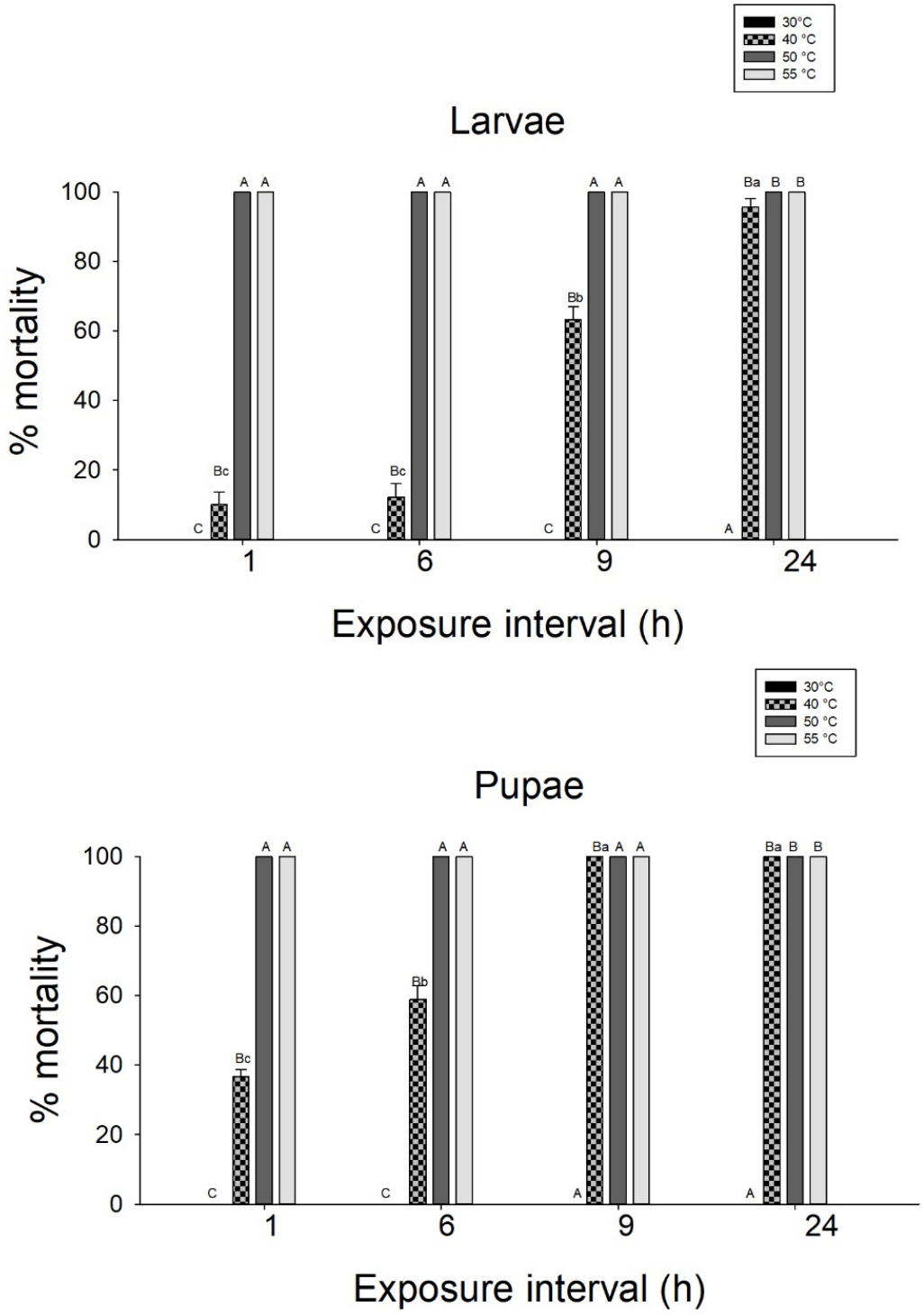


Figure 2. *Cont.*



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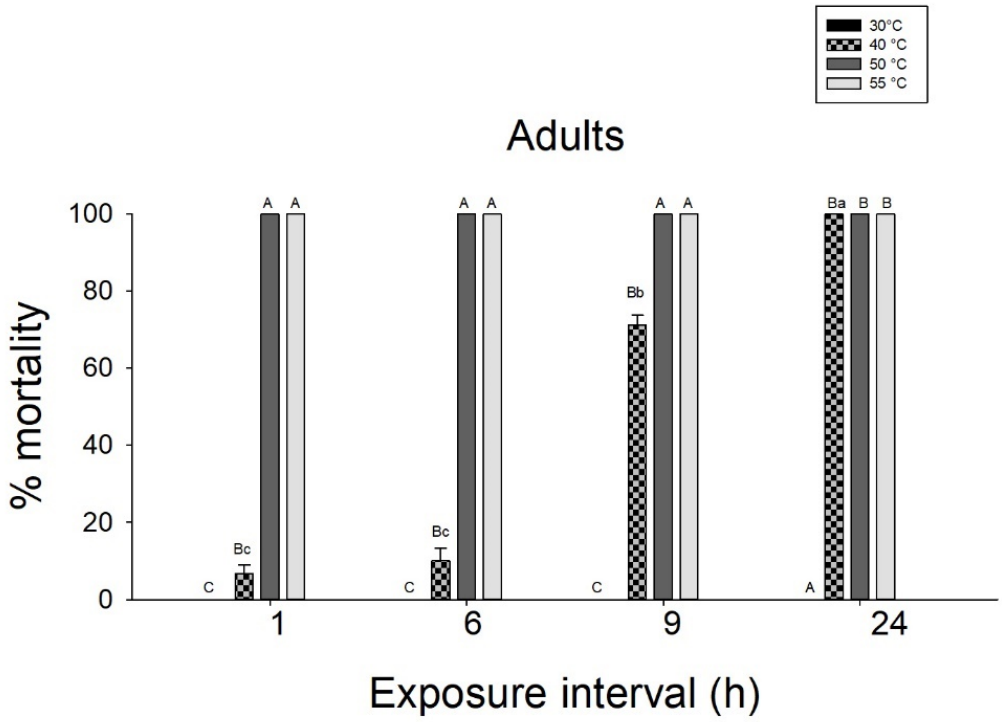

Figure 2. Mortality (%) of *Ephestia kuehniella* eggs, larvae, pupae and adults after exposure to heat treatment at 30, 40, 50 and 55 °C at four different exposure intervals (1, 6, 9 and 24 h). The means of each exposure interval followed by the same uppercase letters do not differ significantly (in all cases, error *df* = 32; Tukey's HSD test at $p = 0.05$). Where no letters exist, no significant differences were noted. The means of each temperature followed by the same lowercase letter do not differ significantly (in all cases, error *df* = 32; Tukey's HSD test at $p = 0.05$).

Table 2. ANOVA parameters for the main effects on and interactions between all the life stages of *Ephestia kuehniella* (error *df* = 512) receiving heat treatment.

Source	*df*	F	*p*
Model	63	1252.6	<0.01
Intercept	1	160,102.6	<0.01
Life stage	3	106.4	<0.01
Temperature	3	21,893.8	<0.01
Time	3	960.0	<0.01
Life stage × Temperature	9	107.8	<0.01
Life stage × Time	9	16.5	<0.01
Temperature × Time	9	942.4	<0.01
Life stage × Temperature × Time	27	15.9	<0.01

4. Discussion

Different studies have been carried out on the tolerance of a wide range of species and life stages to extreme temperatures and the challenge that such an application creates in stored product protection [16,17,19–25]. Our results provide specific combinations of temperatures and exposure intervals that can provide 100% mortality against all the life stages of *E. kuehniella*. Nevertheless, extreme temperatures may have a different target application scenario that can be commodity-, species-, life stage- or even facility-mediated. For instance, heat is mostly used for the disinfestation of processing facilities, such as flour mills, etc., while cold is used, to a large extent, directly on the commodity, such as flour, etc. [8,12,22]. These two dissimilar approaches should be taken into account when an extreme-temperature-based strategy is planned. Hence, despite the fact that generalizations should be avoided, cold treatments should be selected in the case of commodity treatments,

while heat treatments are more prone to be applied in space treatment, such as in food processing facilities. There are cases, however, where these techniques can substitute each other in specific application scenarios, especially in the case of heat, that can be applied directly at different facilities that may have some quantities of different durable products [13].

A series of previously published reports provide a wide range of data on the susceptibility of different life stages of *E. kuehniella*, with often contradictory results. Andreadis et al. [16] tested all the life stages of *E. kuehniella* at temperatures ranging from −5 to −12.5 °C and for exposure intervals between 30 and 120 min and found complete mortality after 120 min at −12.5 °C for larvae and adults. Athanassiou et al. [12] tested larvae, and eggs of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and found that eggs were more cold-tolerant than larvae. In our study, larvae and pupae seem to be the most cold-tolerant life stage than eggs and adults. This discrepancy underscores the variability in cold tolerance across different insect species and potentially different experimental conditions. Practically, the inability of low temperatures to control the eggs of different stored product insects may create undetectable infestation foci, that can easily create a rapid population rebound after the termination of the cold treatment.

Interestingly, when comparing the cold/heat susceptibility of *E. kuehniella* to other Lepidoptera like *P. interpunctella*, our findings show both similarities and differences. Athanassiou et al. [21] reported that eggs were the least susceptible life stage of *P. interpunctella* to cold, while similar results have been reported in the case of other major stored product insects and mites [26,27]. Our tests for *E. kuehniella* have shown that eggs were the least susceptible life stage, given that after 9 h of exposure, mortality at −5 °C was 100%, while for the other three life stages, at this temperature, complete mortality required considerably longer intervals. Our findings suggest that *E. kuehniella* eggs were less cold-tolerant than larvae and pupae, with complete mortality at shorter exposure times and higher temperatures. These parallels suggest a common trend among Lepidoptera regarding the relative cold tolerance of their eggs. However, the degree of tolerance and specific temperature thresholds can vary, reflecting the importance of species-specific studies. In principle, the results of the present study demonstrate that the eggs of *E. kuehniella* are more susceptible to low temperatures than the eggs of stored product beetle species [8,12,14].

In some of the combinations tested here during our cold treatment experiments, we observed higher survival at lower temperatures compared with higher temperatures for the same exposure interval. For instance, egg survival was higher at −5 than at 0 °C at some of the short exposures tested here. Although the causes for this paradox have not been investigated, "stress" due to exposure to cold may be linked with both negative and positive results in terms of insect survival. When comparing our results with those obtained for other insect species, significant differences emerge. In a previous study, Athanassiou et al. [28] found that short exposures to low temperatures of *O. surinamensis* and *T. confusum* provided dissimilar results, underlining the importance of acclimation. Such comparisons highlight the complex interplay of species-specific traits, developmental stages, and environmental conditions in determining insect cold tolerance. Egg age is also an important characteristic that determines the efficacy of different methods. For example, gases, such as phosphine and sulfuryl fluoride, were not equally effective when applied for the control of eggs of different ages [29,30]. Acclimation to cold was also an important parameter in mortality of eggs of the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) [30]. Moreover, Bell [31] reported that diapausing the larvae of *Ephestia elutella* was much more tolerant for cold than the non-diapausing larvae. For this species, diapause seems to play a key role in certain biological traits that are linked with tolerance to several extreme conditions, incl. extreme temperatures.

The available data for heat under laboratory conditions for *E. kuehniella* are relatively few and definitely less than those for cold. Sadeghi et al. [10] tested the effect of microwave heating on larvae of *E. kuehniella* and found complete mortality at 900 W for 50 s. In a recent study, Javanshir et al. [11] tested different life stages of *E. kuehniella* at elevated temperatures

in combination with two different insecticides (cypermethrin and dichlorvos) and found that susceptibility to high temperatures can be influenced by life stage. In that study, the authors reported that pupa may be the most heat-tolerant life stage [32]. This is particularly important, as pupa is a cryptic life stage that is not directly exposed to extreme conditions, and thus, heat may not be effective in a building with structural complexity, such as cracks and crevices, where pupation is likely to take place. Nevertheless, from our results, larvae and eggs were the most heat-tolerant life stages, given that at 40 °C, we recorded some survival for both of these life stages, even at the longer exposure interval, in contrast with pupae and adults, where mortality after 24 h at this temperature was 100%.

Not surprisingly, we found that exposure to 30 °C did not result in a considerable mortality of the different *E. kuehniella* life stages, which can be considered expectable given that this temperature is suitable for the development of this species [11,32]. However, the "jump" from 30 to 40 °C resulted in a high mortality, which was close to 100% for eggs and larvae, and 100% for pupae and adults, provided that the exposure is 24 h. Given that the usual target temperature and exposure level for commercial heat treatments is 50 °C [13,22,23], our data show that *E. kuehniella* is likely to be more susceptible to heat than the other stored product insects, which can be considered in commercial heat treatments. This may be true also when heat treatments are based on temperature levels that are lower than 50 °C.

5. Conclusions

In summary, the current data compared the effect of cold/heat treatment in different life stages of *E. kuehniella*. Both techniques can be considered effective against all the life stages of *E. kuehniella*, but there were noticeable differences in the susceptibility level among the four life stages tested. Hence, in the case of cold, larvae and pupae can be considered the most cold-tolerant life stage compared to adults and eggs. In contrast, for heat treatment, larvae and eggs were the most heat-tolerant. The current temperature exposure combinations tested here can be further elaborated in designing algorithms in order to predict the expected mortality of this species following specific extreme temperature combinations at a commercial scale. In light of our findings, both techniques are effective for the control of this species, and their utilization can be prioritized according to the target application scenario. Our results provide additional data that can be further adopted in commercial treatments, when non-chemical treatments are planned.

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Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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