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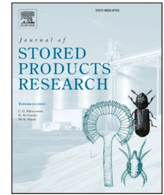

Thermal treatments in controlling *Plodia interpunctella* (Lepidoptera: Pyralidae) on sunflower seeds and their effect on seed vitality

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A R T I C L E I N F O

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A B S T R A C T

This work aimed to assess the potential of thermal treatments for controlling *P. interpunctella* on sunflower seeds, based on its tolerance to high/elevated temperatures and effect on seed vitality. The tests involved mature larvae (L5) and pupae, exposed to 35, 40, 42, 44, 45, 50, 55 and 60 °C, for 15 and 30 min, and 1, 2, 6, 24, 48 h. Larval mortality and survival, Probyt analysis, developmental dynamics, longevity analysis, progeny production and the effect on sunflower seed vitality (germination energy and germination) were assessed. Larval mortality of 75.9% was achieved after 30 min of exposure at 44 °C, 77.0% at 45 °C after 15 min, and 100% after 45 min. At 60 and 65 °C total mortality was achieved already after 15 min of exposure. Lethal exposure time (LT_{50}) needed to kill 50% of larval population was 15 min at 44.2 °C, 30 min at 43.3 °C, 45 min at 42.1 °C or 41.8 °C at 60 min, respectively. LT_{90} can be achieved after 15 min of exposure at 45.2 °C, 30 min at 44.3 °C, 45 min at 43.3 °C, or 60 min at 42.1 °C. Pupal LT_{50} was 15 min at 56.7 °C, 30 min at 53.1 °C, 45 min at 52.4 °C or 60 min at 52.2 °C, while LT_{90} was 15 min at 59.7 °C, 30 min at 56.3 °C, 45 min at 56.1 °C or 60 min at 55.6 °C. The fastest development, and the highest progeny production was in treatments when the parenteral population was exposed to 40 °C for 1, 2 and 6 h. The developmental duration was significantly shortened when parenteral population was exposed to 40 °C for 48 h. The temperature and exposure did affect the duration of the adult life stage only when exposed for 2, 3, 6, 24 and 48 h to 40 °C. The germination of sunflower seed (86.7%) was inhibited by heat at 45 °C when exposed for 24 h.

1. Introduction

Postharvest loss encompasses quantity and quality losses that reduce the economic value of crops and products or make them unsuitable for human consumption. Magnitude of postharvest losses in the food supply chain varies greatly among different crops (commodities), areas, and countries. In developing countries, postharvest losses caused by the lack of knowledge, inadequate technology and/or poor storage infrastructure are high (Kumar and Kalita, 2017). Stored-product insects alone could cause 9–15% of postharvest losses in developed countries, or as high as 20–80% in developing countries (Phillips and Throne, 2010; Sawicka, 2019). Reducing losses of stored products is very important so as to ensure food security and sustainability, mitigate or even eradicate hunger and reduce the usage of agricultural land needed for crop production. Additionally, it contributes to rural development and improvement of farmers' livelihoods.

For decades, usage of chemically synthesized insecticides, including toxic fumigants (methyl bromide, phosphine, etc.) has been the most effective mean of combating stored product pests. However, the management of stored product pests has been rapidly changing from an insecticide-based system to a more integrated approach (Arthur and Phillips, 2003). Concerns over human and animal health, residue hazards, insect resistance, regulatory issues, and restricted use of chemical insecticides (WMO, 1994) have triggered interests in exploring and exploiting alternative and biorational means of controlling stored product insects and disinfesting food storages (Fields, 1992).

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One of the most promising and expanding biorational pest management methods is the application of extreme temperatures. Heat treatments have been studied for decades on a variety of commodities infested with different insects, mites, and microflora (Mullen and Arbogast, 1979; Evans, 1986; Fields, 1992). Back in 1998, Lewthwaite et al. emphasized the potential of application of extreme temperatures as an alternative method for protecting and disinfesting stored commodities against *Plodia interpunctella*. The concept of using thermal energy for disinfection of stored products is not new. Heat treatment is more than one-hundred-year-old technology, and it is safe and effective for management of stored-product insect pests associated with empty bins and grain-processing facilities (Dosland et al., 2006). The first record of using elevated temperatures to control stored-product insects dates to 18th century in western France and was given by Duhamel du Monceau and Tillet (1762) who used a temperature of 69 °C for 3 days to destroy larvae of *Sitotroga cerealella* (Olivier) during its severe outbreak in grain. In practice, heat treatments were first used to control insect pests in flour mills in the early 1900’s (Dean, 1911). When Pepper and Strand (1935) determined that insects can be killed at temperatures between 48.9 and 54.4 °C, they recommended the use of fans to recirculate hot air within a structure to attain uniform temperatures during structural heat treatments. Many studies showed that temperatures during practical heat treatments should be maintained within a range of 50–60 °C for 24 h (Dosland et al., 2006; Subramanyam et al., 2011) while spot treatments of empty storage steel bins can be efficient in 4 h (Tilley et al., 2007).

Time of exposure to high temperatures is an important factor during heat treatments ensuring optimal and uniform dispersion of the heat that must penetrate all cracks in floor or wall spaces and into any processing equipment in order to kill insects in hidden places (Subramanyam et al., 2011). Heat treatments that encompass the use of forced hot air or hot water (dipping technique) have been proposed for a variety of post-harvest insect pests by Burks et al. (2000), but with a remark that long exposure period may cause product damage, especially to seeds and high-value commodities like spices, coffee and tobacco.

One of the most important insect pests of a number of stored products is Indian meal moth, *Plodia interpunctella* (Hübner, 1813). This pest has been well documented in the literature by many authors as a cosmopolitan pest of stored commodities that causes severe damages (Bell, 1975; Johnson and Wofford, 1991; Mohandass et al., 2007; Predojević et al., 2017; Gvozdenac et al., 2018; Vukajlović et al., 2019). It is particularly harmful to high-quality seeds because it feeds on a germinal part of kernels, resulting in reduced germination (Gvozdenac et al., 2018). Several studies have focused on *P. interpunctella* temperature ranges (Cline, 1970; Bell, 1975, 1982; Arbogast, 1981; Nakayama et al., 1983; Johnson and Wofford, 1991; Johnson et al., 1992). However, the research on application of heat as a disinfection tool in controlling of *P. interpunctella* has not been carried out recently, practically on oil crops, in reference to seed quality.

With a growth of human population and thus increased need for food amount, losses caused by pests in warehouses become economically more and more significant. This especially applies to high-quality seeds (pedigreed seeds) that is used for further propagation in agriculture (Marić, 1987). Seed production as a branch of agriculture involves the production and processing of high-quality seeds, and their preservation until sowing. Since more than 2/3 of the total arable land in the world is sown with seeds, which satisfies about 90% of humanity’s needs in food and agro-processing industry (Mirić and Brkić, 2002), a special attention is given to solve the problem of storing, preserving and treating of high-quality seeds. Even though heat treatment is an old technology, there is renewed interest in optimizing the treatments and adapting them for different storage facilities and food processing industry around the world, as a viable insect control alternative to chemical fumigation. Therefore, this work aimed to assess the influence of high temperatures and different exposure periods on mortality, survival, and life-history of *P. interpunctella* well as the effect on the vitality of high-quality sunflower seeds.

2. Material and methods

2.1. Insect bioassay

2.1.1. Insect culture/population

The laboratory population of *P. interpunctella* was reared for ~50 generations on a standard laboratory diet (SLD), as described by Silhacek and Miller (1972). Rearing was carried out in growth chamber (Velp Scinetifica Srl, Italy) at 27 ± 2 °C, 65 ± 5% relative humidity (RH), and 14:10 h L:D photoperiod, in Laboratory for Entomological Research, at Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia.

Prior to the experiment set up, *P. interpunctella* egg hatchability was tested by transferring paired moths into separate glass jars for oviposition and collecting eggs daily. The natural egg hatchability was high and ranged from 92 to 98%. After larvae hatched in separate jars, they were transferred on SLD until the stage L_5 (mature larvae) when they were transferred in Petri dishes for the treatment.

2.1.2. Experimental conditions and protocol

This work tested the tolerance of different *P. interpunctella* developmental stages, mature larvae (L_5) and mature pupae, to elevated temperatures, and to different exposure periods. The larval stage was determined based on head capsule dimensions (Allotey and Goswami, 1990) and “mature” pupae, age-graded based on cuticle pigmentation (Arbogast, 1981). Only those that were dark and hard (25 days old since egg hatch and within 3 days before emergence as pharate adults) were used in the tests. Protective silken cocoons around the pupae were not removed during the exposure to the high temperatures because the intention was to simulate natural conditions for pupal exposure.

Tests were carried out in a laboratory incubator (Binder KB 53) with an accuracy of ±0.5 °C. Twenty larvae (L_5) and pupae of *P. interpunctella,* per replicate, were put in Petri dishes (ø 9 cm) and exposed directly (without any substrate) to the following temperatures: 35, 40, 42, 44, 45, 50, 55 and 60 °C, for 15 and 30 min, and 1, 2, 6, 24, 48 h. The relative humidity at each temperature was 20–22%, simulating common/typical humidity levels during a structural heat treatment of food-processing facilities (Mahroof et al., 2003a, 2003b). A separate control treatment consisted of Petri dishes with *P. interpunctella* larvae or pupae kept at the rearing conditions (27 ± 2 °C, RH 65 ± 5% and photoperiod 14:10, light: dark). Petri dishes in the control treatments were used to determine the natural mortality of insects and were removed from the control chamber and checked twice, once at the beginning and second time at the end of the exposure period in temperature treatments. Each temperature and exposure combination for each developmental stage was performed in six replications.

After the exposure time elapsed, the dishes were removed from the thermostat. Active and alive larvae and all pupae were transferred into the climate chamber, and each specimen was in a separate glass jar on SLD, kept under constant rearing conditions (27 ± 2 °C, RH 65 ± 5% and photoperiod 14:10, light: dark), and daily checked for the survival, i.e. mortality (%) and emergence of adults from the pupae. Developmental dynamics of surviving larvae was recorded and longevity analysis was performed.

2.1.2.1. Mortality and survival. For assessing the efficacy of heat treatments, the mortality of *P. interpunctella* larvae and pupal survival was recorded. The mortality of larvae was based on the number of dead specimens out of the total exposed number (in each of the six replicates). For the pupal mortality, only those that failed to emerge into adults were counted. The larval and pupal mortality was corrected for the mortality in the control (specimens not exposed to the heat treatment) using Schneider-Orelli’s formula:

$$Corrected\ \% = \frac{Mortality\,\%\,in\,treated\,plot - Mortality\,\%\,in\,control\,plot}{100 - Mortality\,\%\,in\,control\,plot} * 100$$

2.1.2.2. Developmental dynamics. Developmental dynamics of *P. interpunctella* was determined for the specimens that survived the treatments. It was based on the time each specimen needed to develop/change into the next stage. Developmental dynamics is presented graphically.

2.1.2.3. Longevity analysis. This analysis enabled the assessment of influence of different heat treatments and exposure period on a duration of each following stage (pupae, adults) as well as of the larval stage (up to L_5) in the second generation.

2.2. Seed bioassay

The effect of heat treatments was assessed also on sunflower seed vitality in germination tests. The sunflower oil hybrid Leone (Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia) was used in the experiment. It is an oil hybrid with high oil (46–48%) and low protein content (20–22%) in seeds.

The seeds were exposed simultaneously with insects and under the same temperature and exposure conditions, except at 35 and 40 °C, because the safe drying of seeds during postharvest is performed with warm air, up to 40 °C (Prole et al., 2011). After the exposure to high temperatures, the effect on seed germination energy (GE - %) and germination rate (G - %) was assessed based on the number of germinated seeds in comparison to total number of placed seeds. A standard germination method in filter paper was performed, as recommended by ISTA (2019). Sunflower seeds (50 seeds) were placed at moistened filter paper towel and wrapped in a roll and incubated in a germination chamber at 25 ± 1 °C and relative air humidity of 95 ± 2%. GE was determined after four days and G after ten, by counting the number of typical seedlings. The experiment was set in four replicates.

2.3. Data analyses

The data from insect bioassays were presented as % of larval mortality, lethal doses (LT_{50} and LT_{90}) for defining the temperature and exposure period needed to achieve 50 and 90% of population mortality for larvae and pupae, as well as mean values ± SD and median (IQR) values for the longevity of a specific life stage (larvae, pupae, and adults).

Mean values ± *SD* were calculated and plotted against the exposure time. Data was subjected to one-way ANOVA and two-way ANOVA, to determine the influence of two factors, temperature (35, 40, 42, 44, 45, 50, 55 and 60 °C), and exposure period (15 and 30 min, and 1, 2, 6, 24, 48 h), as well as their interaction on the mortality and survival of larvae and pupae of *P. interpunctella*. Duncan's multiple range test was used to test the differences in the progeny production between different exposure periods. This test enabled us to obtain homogenous groups that are not statistically significant.

The exposure time + mortality data of each stage were subjected to Probyt analysis for estimating the time required to cause the mortality of 50% and 90% (LT_{exp50} and LT_{exp90}) of the exposed insect specimens at a corresponding temperature. Also, LT_{temp50} and LT_{temp90} were calculated to determine the lethal temperature (causing 50% and 90%), for each exposure period.

In analysis of longevity, the distribution was not normal and Leven's homogeneity test indicated that variances were not homogenous and therefore nonparametric tests were applied. For the purpose of longevity analysis one factor was created with 12 levels which represented combinations of temperatures and exposure periods. Data were presented as mean values ± *SD* and median (IQR). Kruskal-Wallis test was performed and pairwise comparison of treatments with Bonferroni correction was applied.

Statistical package TIBCO Statistica 14.0 and trial version of IBM SPSS Statistics 21, software was used for statistical analysis.

3. Results

3.1. Mortality, developmental dynamics, and longevity analysis of P. interpunctella

The effects of high temperatures and different exposure periods on the mortality, developmental dynamics, and longevity of different life stages of *P. interpunctella* are presented in Tables 1–7.

3.1.1. The mortality of P. interpunctella

The larval mortality was both temperature and exposure-dependent, as was proven in one-way ANOVA (Table 1; Fig. 1).

A significant larval mortality was achieved after 30 min of exposure (75.9%) at 44 °C. The mortality increased with the increase of the exposure period, and after 120 min it reached 100%. The difference in larval mortality between the exposure periods at this temperature was statistically highly significant ($F = 11709.9^{**}$, $P < 0.01$). At 45 °C, the mortality reached 77.0% even after a short exposure of 15 min, while after 45 min it was 100%. Like in the previous treatment, the difference in the mortality depending on the exposure period was statistically highly significant ($F = 1533.6^{**}$, $P < 0.01$). At higher temperatures, high mortality was achieved during short exposure periods (Table 1). At 60 and 65 °C total mortality was achieved already after 15 min of exposure.

When comparing the percentage of larval mortality between the temperatures at the same exposure periods, significant differences existed in treatments after 15, 30, 45, 60 and 120 min (Table 1). 15 min of exposure was sufficient to cause a significant mortality (77.0%) at 45 °C. After 30 min of exposure, a significant mortality occurred at 44 °C, 75.9%, while after 45 min, the temperatures below 42 °C, did not cause significant mortality (0–21.3%). The exposure of 45 min to all temperatures higher than 44 °C resulted in almost total mortality (97.7–100%). The same was observed for the exposure period of 60 and 120 min, after which at 42 °C, the mortality was 76.5 and 92.4%, respectively.

Two-way ANOVA indicated strong influence and effect of both factors (temperature and exposure period) as well as their interaction (temperature and exposure) on the larval mortality ($F = 2394717.0^{**}$; 15249.0^{**}; 37417.0^{**}, $P < 0.01$, respectively). Based on the *SS* values, the temperature was the dominant factor responsible for mortality of larvae (Table 2).

3.1.2. The Probyt analysis

The Probyt analysis presents a regression relationship between the temperature and exposure period, and the mortality of *P. interpunctella* larvae and pupae. The exposure periods needed for larvae and pupae to be subjected to a certain temperature to achieve 50 or 90% of mortality (LT_{exp50} and $LT_{exp\ 90}$ values) are presented in Table 3.

According to the Probyt analysis, larvae need to be exposed for 20.96 min at 44 °C or for 33.37 min at 42 °C, to reduce the population by 50%. To achieve the 90% reduction of the larval population, they should be subjected for 35.67 min to 44 °C or for 67.21 min to 42 °C.

The pupae were more tolerant to high temperatures in comparison to larvae. To achieve 50% of pupal mortality they need to be subjected for 38.91 min to 44 °C or 56.52 min to 42 °C. To achieve 90% mortality, pupae should be subjected for 49.85 min to 44 °C or for 79.83 to 42 °C (Table 3).

This analysis also determined the temperature at which larvae and pupae are to be subjected for a specific exposure period to achieve 50 and 90% of larval and pupal mortality (LT_{temp50} and LT_{temp90} values), as presented in Table 4.

To achieve 50% of larval mortality (LT_{temp50}), the larvae should be subjected for 15 min to 44.2 °C, 30 min to 43.3 °C, 45 min to 42.1 °C or

Table 1
The effect of temperatures and exposure periods on the mortality (%) of *P. interpunctella* larvae.

T (°C)	Exposure (min)							X	F value
	15	30	45	60	120	180	240		
35	0.0 ± 0.0 aA	0.0 ± 0.0 aA	0.0 ± 0.0 aA	0.0 ± 0.0 cA	0.00 ± 0.0 cA	0.0 ± 0.0 aA	0.0 ± 0.0 aA	0	**0.00NS**
40	0.0 ± 0.0 aA	0.0 ± 0.0 aA	0.0 ± 0.0 aA	0.0 ± 0.0 cA	0.00 ± 0.0 cA	0.0 ± 0.0 aA	0.0 ± 0.0 aA	0	**0.00NS**
42	2.9 ± 0.3 dC	45.0 ± 0.2 dB	53.5 ± 0.7 bA	76.5 ± 2.5 bA	92.4 ± 1.0 bA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	60.8	**4907.8****
44	21.3 ± 0.5 cC	75.9 ± 0.2 cB	97.7 ± 0.1 aA	99.8 ± 0.21 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	90.0	**11709.9****
45	77.0 ± 0.3 bB	96.6 ± 2.9 bA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	96.2	**1533.6****
50	79.5 ± 0.5 bB	98.2 ± 0.35 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	96.8	**2035.1****
55	85.5 ± 0.2 abB	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	97.9	**2523.0****
60	100 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100	**0.00NS**
65	100 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100.0 ± 0.0 aA	100	**0.00NS**
X	51.8	68.4	72.4	75.1	76.9	66.67[1]	66.67[1]	/	
F value	**33480.9****	**1941.0****	**198470.8****	**194609.1****	**203448.4****	**N/A**	**N/A**		

T-temperature; Values represent mean mortalities ± *SD*; Values with the same lower letter are at the same level of significance between the rows (temperature); Values with the same capital letter are at the same level of significance between the columns (exposure period); X - mean values within column or row; NS - P > 0.05; * - P < 0.05; ** - P < 0.01; [1] - mean values of the last two columns are not relevant due to extreme values; N/A.

Table 2
The influence of temperature, exposure period and their interaction on the mortality of *Plodia interpunctella* larvae.

Source	*SS*	*df*	MS	F	Significance
Temperature	**293953.46**	**8**	**36744.2**	**2394717**	**,000**
Exposure	14039.1	6	2339.87	15249	,000
Temperature x Exposure	27552.65	48	574.0	37417	,000

SS – sum of squares; *df* – degrees of freedom; *MS* - mean square.

Table 3
Exposure time (minutes) for LT_{exp50} and $LT_{exp\ 90}$ values at corresponding temperatures.

		Temperature	
		44 °C	42 °C
Larvae	LT_{exp50}	20.96	33.37
	LT_{exp90}	35.67	67.21
Pupae	LT_{exp50}	38.91	56.52
	LT_{exp90}	49.85	79.83

Table 4
Temperatures (°C) for LT_{temp50} and $LT_{temp\ 90}$ values in corresponding exposure periods.

		Exposure period			
		15 min	30 min	45 min	60 min
Larvae	LT_{temp50}	44.2	43.3	42.1	41.8
	LT_{temp90}	45.2	44.3	43.3	42.1
Pupae	LT_{temp50}	56.7	53.1	52.4	52.2
	LT_{temp90}	59.7	56.3	56.1	55.6

41.8 °C to 60 min, respectively. The mortality of 90% (LT_{temp90}) can be achieved after 15 min of exposure at 45.2 °C, 30 min at 44.3 °C, 45 min at 43.3 °C, or 60 min at 42.1 °C, as presented in Table 4.

To achieve 50% of pupal mortality (LT_{temp50}) the pupae should be subjected for 15 min at 56.7 °C, for 30 min at 53.1 °C, for 45 min at 52.4 °C or for 60 min at 52.2 °C. For 90% mortality (LT_{temp90}), 15 min of exposure is needed at 59.7 °C, 30 min at 56.3 °C, 45 min at 56.1 °C or 60 min at 55.6 °C.

3.1.3. Developmental dynamics

The surviving larvae (L_5) were allowed to continue their development for another six weeks. The effect of high temperatures and different exposure periods was observed on the survival and life history of the next generation of *P. interpunctella* exposed to 35 °C (Fig. 2) and 40 °C (Fig. 3). The fastest development, and transition in the next stage, as well as the highest progeny production (number of L_1-L_5 larvae in the next generation) was recorded when parenteral population was exposed for 48 h at 35 °C (Fig. 1). The differences in the progeny production were highly significant between this treatment and other exposure periods, as proven by Duncan's multiple range test.

The fastest development, and transition in the next stage, as well as the highest progeny production for the parenteral population exposed at 40 °C, was in the case of 1, 2 and 6 h of exposure (Fig. 2). The differences in the progeny production were significantly higher in these treatments in comparison to the exposures for 24 and 48 h (Duncan's multiple range test), when the emergence of the next generation larvae was delayed.

3.1.4. Longevity analysis

The longevity analysis of *P. interpunctella* larvae, pupae and adults is presented in Tables 5–7

The larval development was significantly shortened when exposed to 40 °C for 6 and 24 h in comparison to the duration when exposed to 35 °C for 1, 2, 6 and 24 h. When exposed to 40 °C for 48 h it was significantly shortened in comparison to all exposure periods at 35 °C as well as at 40 °C for 1, 2 and 3 h (Table 5).

The stage of pupa lasted significantly shorter when exposed to 40 °C for 6 h, 24 and 48 h in comparison to stage duration at 35 °C at 1, 2, 3 and 6 h (Table 6).

The temperature and exposure did affect the duration of the adult life stage only when the specimens were exposed for 2, 3, 6, 24 and 48 h to 40 °C in comparison to the adult longevity at 35 °C for 1 h (Table 7).

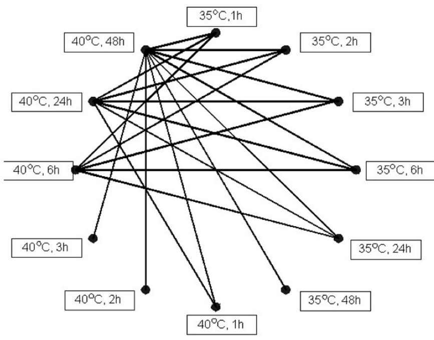
3.2. Seed bioassay

The effect of heating on seed germination energy (GE - %) and germination (G - %) is presented in Tables 8 and 9. High temperatures and long exposure affected germination energy. When seed was exposed to temperatures higher than 40 °C (45, 50 and 55 °C), the germination energy was significantly reduced in comparison to other treatments ($F = 1.11^*, 7.73^*; 1.58^*, P < 0.05$, respectively).

The germination of sunflower seed was negatively influenced by exposure to higher temperatures for a longer period of time, which is particularly noticeable when exposed to a temperature of 45 °C for 24 h. In these treatments, the germination was within the limits of the minimum germination (80%) prescribed by the national regulations on seed quality, but with a reduced possibility of seed trade. However, when observing the average values, a significant difference was not recorded. In the control treatment the germination was 91.3%.

Table 5
Developmental duration of *P. interpunctella* larvae at different temperatures and exposure.

	35ºC 1 h	35ºC 2 h	35ºC 3 h	35ºC 6 h	35ºC 24 h	35ºC 48 h	40ºC 1 h	40ºC 2 h	40ºC 3 h	40ºC 6 h	40ºC 24 h	40ºC 48 h
35ºC 1 h	**17.2 ± 1.3** **17.00 (2.0)**											*
35ºC 2 h	1.00	**17.4 ± 1.2** **17.00 (2.3)**										
35ºC 3 h	1.000	1.000	**17.1 ± 1.4** **17.00 (5.0)**									
35ºC 6 h	1.000	1.000	1.000	**17.1 ± 1.4** **17.00 (6.0)**								
35ºC 24 h	1.000	1.000	1.000	1.000	**17.1 ± 0.8** **17.00 (2.0)**							
35ºC 48 h	1.000	0.321	1.000	1.000	1.000	**16.4 ± 0.9** **16.00 (1.0)**						
40ºC 1 h	1.000	1.000	1.000	1.000	1.000	1.000	**16.8 ± 1.2** **17.00 (2.0)**					
40ºC 2 h	0.212	0.054	0.544	0.341	0.436	1.000	1.000	**16.2 ± 1.1** **16.00 (2.0)**				
40ºC 3 h	0.430	0.119	1.000	0.671	0.843	1.000	1.000	1.000	**16.3 ± 0.8** **16.00 (1.0)**			
40ºC 6 h	0.006	0.010	0.020	0.011	0.015	1.000	0.513	1.000	1,000	**15.8 ± 1.1** **16.00 (2.0)**		
40ºC 24 h	0.000	0.000	0.000	0.000	0.000	0.079	0.104	0.275	0.164	1.000	**14.4 ± 1.1** **14.00 (1.5)**	
40ºC 48 h	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.001	0.120	1.000	**13.7 ± 0.8** **13.5 (2.0)**



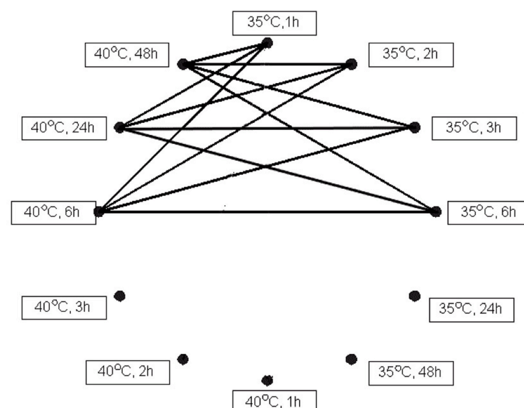
* On the diagonal mean values ± SD and median (IQR) of the data are given, and p - values are presented under the diagonal. On the graph nested in the table, p - values less than 0.05 are represented by lines. These lines represent levels of different temperatures and exposure periods that are statistically significantly different in the duration of a certain life stage.

4. Discussion

During last decades, the Indian meal moth (*P. interpunctella*) has been reported in several studies as the species that causes huge damages to durable and processed stored commodities. However, in the context of increasing moth population due to climate changes (Deutsch et al., 2018), a lack of available insecticides (Karabelas et al., 2009) as well as increasing damages like reduction of nutritional value and quality, germination rate, and safety of a commodity for human and animal consumption (Hubert et al., 2018; Zhao et al., 2022), there is a growing need of improving and optimizing means of controlling this pest. Heat treatments have been widely used to control different insect pests on stored grains (Kim et al., 2017). However, such treatments may result in unsatisfactory efficacy due to heat tolerance of the target insects. Also, there is limited published information on responses of *P. interpunctella*, exposed to elevated temperatures typically used during heat treatments. In a recent and extensive review, Stejskal et al. (2019) presented the results on lower temperature development thresholds of different stored product pests that define temperature zones for safe storage. Our research, on the other hand, assessed the potential of heat treatment in controlling different life stages of *P. interpunctella*, in relation to mortality, survival, development duration, progeny production but also effect on seed vitality on which population developed. According to the results of our study, a significant larval mortality was achieved after 30 min of exposure (75.9%) to 44 °C and it increased with the exposure period, reaching 100% after 120 min. The temperature of 45 °C caused 77.0% mortality after 15 min, while at 60 and 65 °C it was total after this exposure period. The results are in accordance with those presented by Mahroof and Subramanyam (2006) who noted that the mortality of each stage increased with increasing temperature and exposure period. In general, according to Mahroof and Subramanyam (2006), the fifth-instar larvae were the most heat-tolerant stage at all temperatures tested. The exposure of 34 min at 50 °C caused 99% of larval mortality, while 100% mortality was achieved for all developmental stages in less than 20 min at 54.6 °C in their research. In our experiment, at 55 °C, the mortality of mature larvae after 15 min was 85.5% and after 30 min it was 100% which is in concordance with the presented results of Mahroof and Subramanyam (2006). The research of Nakayama et al. (1983) supports our findings as they found no infestation by *P. interpunctella* life stages on dried peaches after 30 min exposure to temperatures above 50.8 °C. Kim et al. (2017) reported that heat treatment at 44 °C for 1 h caused a significant mortality to all developmental stages, while the

Table 6
Developmental duration of *P. interpunctella* pupae at different temperatures and exposure.

	35ºC 1 h	35ºC 2 h	35ºC 3 h	35ºC 6 h	35ºC 24 h	35ºC 48 h	40ºC 1 h	40ºC 2 h	40ºC 3 h	40ºC 6 h	40ºC 24 h	40ºC 48 h
35ºC 1 h	**8.4 ± 1.5** **8.00 (2.0)**											*
35ºC 2 h	1.000	**7.9 ± 1.6** **8.00 (2.0)**										
35ºC 3 h	1.000	1.000	**7.6 ± 1.1** **7.50 (1.3)**									
35ºC 6 h	1.000	1.000	1.000	**7.7 ± 1.2** **7.50 (2.0)**								
35ºC 24 h	1.000	1.000	1.000	1.000	**7.5 ± 1.4** **7.00 (3.0)**							
35ºC 48 h	0.695	1.000	1.000	1.000	1.000	**7.3 ± 0.9** **7.00 (3.0)**						
40ºC 1 h	0.076	1.000	1.000	1.000	1.000	1.000	**7.1 ± 0.8** **7.00 (2.0)**					
40ºC 2 h	0.056	1.000	1.000	1.000	1.000	1.000	1.000	**7.1 ± 0.9** **7.00 (2.0)**				
40ºC 3 h	0.467	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**7.2 ± 0.8** **7.00 (1.0)**			
40ºC 6 h	0.000	0.002	0.007	0.003	0.143	0.261	1.000	1.000	0.392	**6.5 ± 0.6** **7.00 (1.0)**		
40ºC 24 h	0.020	0.049	0.017	0.050	0.765	1.000	1.000	1.000	1.000	1.000	**6.4 ± 0.7** **6.00 (1.0)**	
40ºC 48 h	0.000	0.002	0.006	0.003	0.087	0.148	1.000	1.000	0.213	1.000	1.000	**6.6 ± 0.6** **6.00 (1.0)**



* On the diagonal mean values ± SD and median (IQR) of the data are given, and p - values are presented under the diagonal. On the graph nested in the table, p - values less than 0.05 are represented by lines. These lines represent levels of different temperatures and exposure periods that are statistically significantly different in the duration of a certain life stage.

late-instar larvae exhibited the highest tolerance, as also proved in our work. The results of our research are also in accordance with results presented by Arbogast (1981) showed that 3-h heat treatment at 45.8 °C completely controlled *P. interpunctella* pupae, although the efficient exposure at this range of temperatures was shorter in our work. Nonetheless, as suggested by Mahroof and Subramanyam (2006) heat treatments aiming to control specific instars should be able to control all other stages of *P. interpunctella*.

The temperature and exposure period, as well as their interaction, had strong influence and effect on larval mortality, which is in accordance with Lewthwaite et al. (1998) and Yu et al. (2011). Other factors that affect success of heat treatments and influence insects' response to thermal treatments are insect species (Beckett and Morton, 2003), life stage (Mahroof et al., 2005), method of application (Fields, 1992; Brokerhof and Banks, 1993), acclimation and relative humidity (Howe, 1967). Additionally, factors such as diet, population density, developmental stage and gender may affect temperature hardiness of individual species (Johnson and Wofford, 1991; Fields, 1992; Brokerhof and Banks, 1993). Generally, stored product insects are more tolerant to temperature treatments than other insects because their habitats are less subjected to sudden temperature changes (Mullen and Arbogast, 1979). By the definition, the mortality of an insect stage at high temperatures is a function of temperature and exposure time. At any given temperature, mortality increases with exposure time (Mahroof et al., 2003b; Boina and Subramanyam, 2004). The Probyt analysis revealed that susceptibility to elevated temperatures differed depending on the life stage, with pupae being more tolerant in comparison to larvae. In research performed by Mbata et al. (2004), comparison of LT_{99} shows that *P. interpunctella* mortality responses varied with egg age, temperature, and treatment duration. Aside from lethal effect, high temperature can also cause sublethal effects such as reduced fecundity and progeny production (Mahroof et al., 2005), which was also proven in our experiments.

Mahroof et al. (2003a) conducted a laboratory experiment to

Table 7
Developmental duration of *P. interpunctella* adults at different temperatures and exposure.

	35ºC 1 h	35ºC 2 h	35ºC 3 h	35ºC 6 h	35ºC 24 h	35ºC 48 h	40ºC 1 h	40ºC 2 h	40ºC 3 h	40ºC 6 h	40ºC 24 h	40ºC 48 h
35ºC 1 h	**7.2 ± 0.8 7.00 (0.0)**											
35ºC 2 h	1.000	**6.9 ± 1.1 7.00 (2.0)**										
35ºC 3 h	1.000	1.000	**6.6 ± 0.7 7.00 (1.0)**									
35ºC 6 h	1.000	1.000	1.000	**6.8 ± 0.8 7.00 (1.0)**								
35ºC 24 h	1.000	1.000	1.000	1.000	**6.9 ± 0.7 7.00 (1.0)**							
35ºC 48 h	1.000	1.000	1.000	1.000	1.000	**6.8 ± 0.9 7.00 (1.0)**						
40ºC 1 h	1.000	1.000	1.000	1.000	1.000	1.000	**6.7 ± 0.6 7.00 (1.0)**					
40ºC 2 h	0.036	1.000	1.000	1.000	1.000	1.000	1.000	**6.4 ± 0.8 6.00 (1.0)**				
40ºC 3 h	0.002	0.190	1.000	0.565	0.120	0.774	0.881	1.000	**6.2 ± 0.7 6.00 (1.0)**			
40ºC 6 h	0.002	0.154	1.000	0.415	0.098	0.616	0.700	1.000	1.000	**6.2 ± 0.8 6.00 (3.0)**		
40ºC 24 h	0.058	0.919	1.000	1.000	0.697	1.000	1.000	1.000	1.000	1.000	**6.1 ± 0.7 6.00 (1.3)**	
40ºC 48 h	0.050	1.000	1.000	1.000	0.818	1.000	1.000	1.000	1.000	1.000	1.000	**6.1 ± 0.9 6.00 (2.0)**

* On the diagonal mean values ± SD and median (IQR) of the data are given, and p - values are presented under the diagonal. On the graph nested in the table, p - values less than 0.05 are represented by lines. These lines represent levels of different temperatures and exposure periods that are statistically significantly different in the duration of a certain life stage.

determine time-mortality relationships for different life stages (eggs, young larvae, old larvae, pupae, and adults) of *T. castaneum*, and revealed that young larvae (first instars) were the most heat-tolerant stage, thus the success of a heat treatment should be gauged based on the mortality of the young larvae. In our case, larvae were more susceptible to high temperatures thus the treatment should be oriented towards suppressing pupae as more tolerant stage. However, additional tests are needed to assess the tolerance of eggs to high temperatures.

In the current study, the effect of high temperatures and different exposure periods was observed on the survival and life history of the next generation of *P. interpunctella* exposed to 35 °C and 40 °C. The fastest development, and transition in the next stage, as well as the highest progeny production was recorded when parenteral population was exposed to 35 °C for 48 h. The fastest development, and transition in the next stage, as well as the highest progeny production for the parenteral population exposed to 40 °C, was in the case of 1, 2 and 6 h of exposure. The temperature and exposure did affect the duration of the adult life stage only when the specimens were exposed to 40 °C longer than 2 h in comparison to the adult longevity at 35 °C for 1 h. A general theory of the relationship between insect development and temperature has been proposed by Gilbert and Raworth (1996) which also sets the upper limits of growth and survival. They show almost a linear relationship between growth rate and temperature up to about 28 °C, followed by a very sharp decline, with several exceptions. As recorded by Fields (1992) and Dosland et al. (2006), the temperature increase above the optimum (32 °C) initiates three-stage insect responses. In the first stage (40–45 °C), egg laying declines, hatching and adult emergence are difficult to complete, fecundity is declining, and adult longevity is shorter, which all results in population decline. In the second stage (45–55 °C), individuals survive several hours experiencing water stress, but high environmental humidity can prolong and extend survival. The last stage (>55 °C), there is rapid mortality and entire population is dead in minutes to seconds. This theory was supported by the results of our study in terms of mortality and progeny production as well as developmental duration of *P. interpunctella*.

Another important consideration to be taken into account when using thermal treatments is the type of the commodity. This refers especially to valuable goods such as high-quality seeds and/or seedling material. Our results indicate that high temperatures and long exposure affected germination energy of sunflower seeds. When seeds were

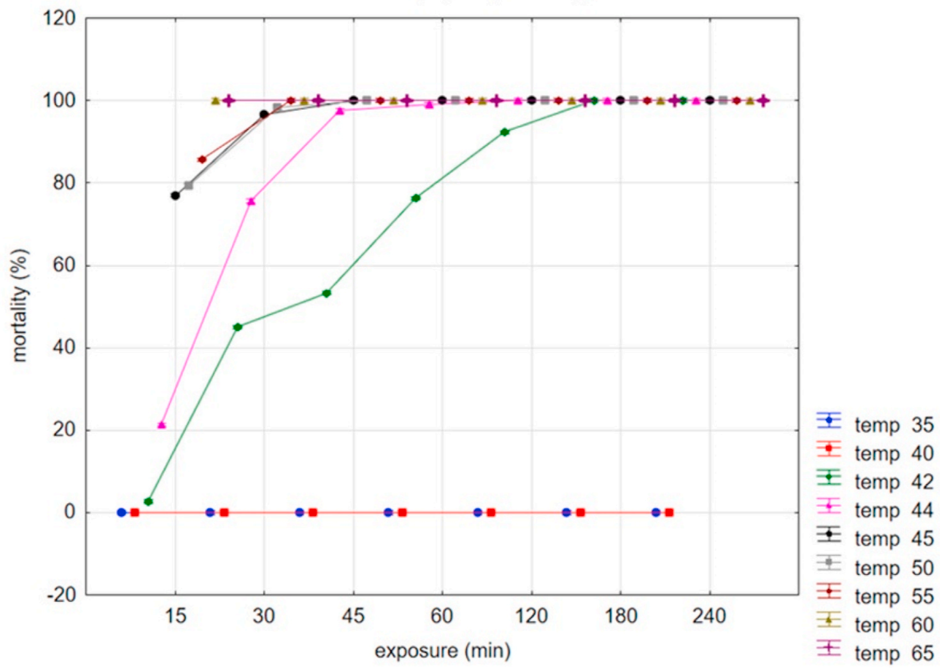


Fig. 1. Interaction of temperature and exposure period, $F^{48,126} = 3741.0$, $P = 0.000$.

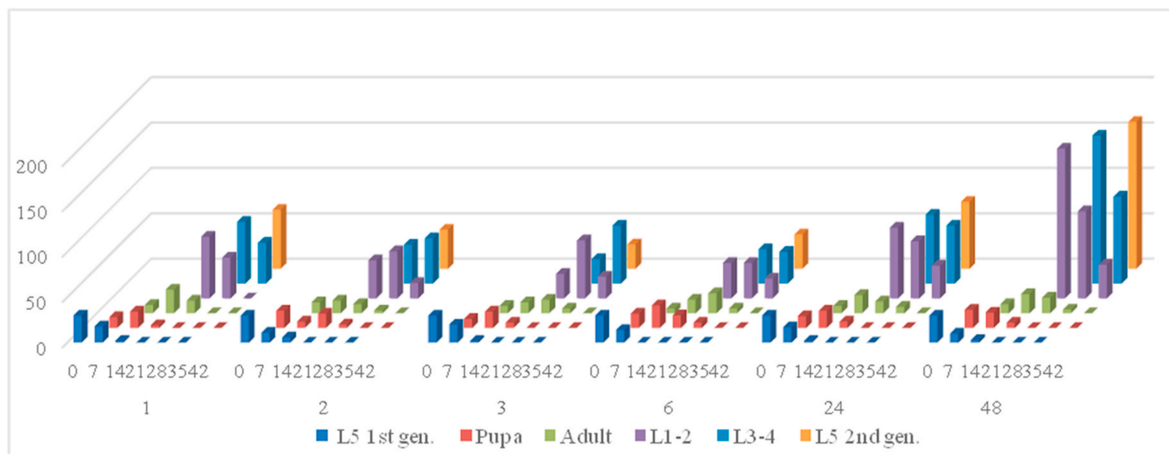


Fig. 2. Distribution of life stages of *P. interpunctella*, after the exposure at 35 °C for 1–48 h during six weeks (0–42 days).

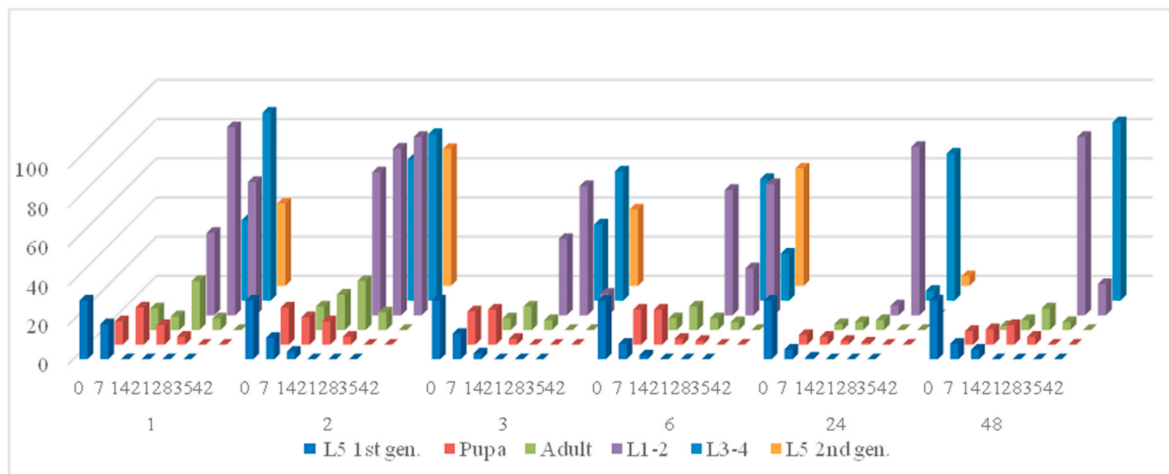


Fig. 3. Distribution of life stages of *P. interpunctella* after the exposure at 40 °C for 1–48 h during six weeks (0–42 days).

Table 8
The effect of high temperatures and exposure on germination energy of sunflower seeds.

T (°C)	Exposure							X average	F value
	30 min	1h	2 h	3h	6h	24h	48h		
35	/	/	/	93.3 ± 1.1 a	94.7 ± 3.1 a	93.3 ± 4.7 a	94.0 ± 3.5 a	**93.8**	0.11NS
40	/	/	/	89.3 ± 5.0 a	92.0 ± 5.3 a	93.3 ± 2.3 a	92.7 ± 6.1 a	**91.8**	0.38NS
45	91.3 ± 4.2 a	92.7 ± 2.3 a	90.0 ± 2.0 a	88.0 ± 6.0 a	92.0 ± 4.0 a	86.0 ± 5.3 aB	/	**90.0**	1.11*
50	92.7 ± 3.0 a	87.3 ± 6.4 a	90.7 ± 3.1 a	92.0 ± 0.0 a	89.3 ± 9.0 a	/	/	**90.4**	7.73*
55	89.3 ± 4.2 a	92.0 ± 3.5 a	94.0 ± 5.3 a	91.3 ± 2.3 a	94.7 ± 5.0 a	/	/	**92.3**	1.58*
60	94.7 ± 4.2 a	94.7 ± 1.2 a	90.0 ± 4.0 a	96.0 ± 2.0 a	92.7 ± 3.0 a	/	/	**93.6**	1.71NS
65	90.7 ± 5 0.8 a	95.3 ± 1.2 a	88.0 ± 4.0 a	94.0 ± 0.0 a	93.3 ± 2.3 a	/	/	**92.3**	2.30NS
X average	**91.7**	**92.4**	**90.5**	**91.9**	**92.7**	**90.9**	**93.3**	/	
F value	**0.65NS**	**2.42NS**	**0.97NS**	**2.20NS**	**0.41NS**	**2.95***	**0.08NS**		

T – temperature in degrees Celsius; Values represent means ± *SD*; Values with the same lower letter are at the same level of significance between the rows (temperature); Values with the same capital letter are at the same level of significance between the columns (exposure period); X average – average values within column or row; NS – $P > 0.05$; * - $P < 0.05$; ** - $P < 0.01$.

Table 9
The effect of high temperatures and exposure periods on germination of sunflower seeds.

T (°C)	Exposure							X average	F value
	30 min	1h	2 h	3h	6h	24h	48h		
35	/	/	/	94.7 ± 1.1 aA	94.7 ± 3.1 aA	93.3 ± 4.7 aA	94.0 ± 2.3 aA	**94.2**	0.11NS
40	/	/	/	90.7 ± 3.1 aA	92.0 ± 5.3 aA	94.0 ± 2.0 aA	92.7 ± 6.1 aA	**92.3**	0.29NS
45	92.7 ± 3.1 aA	92.7 ± 2.3 aA	90.0 ± 2.0 aA	88.7 ± 7.0 aA	92.0 ± 4.0 aA	86.7 ± 4.2 aB	/	**90.4**	1.06NS
50	93.3 ± 2.3 aA	91.3 ± 5.0 aA	92.0 ± 2.0 aA	92.0 ± 0.0 aA	90.0 ± 8.0 aA	/	/	**91.7**	0.85NS
55	89.0 ± 4.2 aA	92.7 ± 4.2 aA	93.0 ± 0.0 aA	92.7 ± 2.3 aA	94.7 ± 5.0 aA	/	/	**92.6**	1.52NS
60	95.3 ± 3.0 aA	94.7 ± 1.2 aA	93.5 ± 2.0 aA	96.0 ± 2.0 aA	93.3 ± 2.3 aA	/	/	**93.9**	2.35NS
65	91.3 ± 6.4 aA	95.3 ± 1.2 aA	94.0 ± 1.0 aA	94.0 ± 0.0 aA	93.3 ± 2.3 aA	/	/	**92.8**	1.06NS
X average	**92.5**	**93.3**	**91.2**	**92.7**	**92.9**	**91.3**	**93.3**	/	
F value	**0.91NS**	**0.79NS**	**0.71NS**	**1.89NS**	**0.38NS**	**3.47***	**0.23NS**		

T – Temperature in degrees Celsius; Values represent means ± *SD*; Values with the same lower letter are at the same level of significance between the rows (temperature); Values with the same capital letter are at the same level of significance between the columns (exposure period); X average – average values within column or row; NS – $P > 0.05$; * - $P < 0.05$; ** - $P < 0.01$.

exposed to 45, 50 and 55 °C, the germination energy was significantly reduced in comparison to other treatments. The germination of sunflower seed was negatively influenced by temperatures of 45 °C and higher, when exposed for 24 h. Although after the treatment the germination was above the limit stipulated by the Rulebook on quality of seeds of agriculture plants (1987), it still did not exceed the norm required for trading high-quality seeds, which is usually 90% or more. However, when observed the average values, significant differences were not recorded. Due to their specific properties, sunflower seeds are very sensitive to air temperature for drying, which is most often performed artificially, by blowing warm or cold air. Previous research, as well as the experience of processors, have shown that for the safe drying of seeds, and therefore the preservation of its quality, the maximum temperature during drying with warm air must not exceed 40 °C (Gay et al., 1991; Khalifa et al., 2000; Prole et al., 2011; Huang et al., 2021). This especially applies to germination energy as an important parameter of seed quality, which is often not given enough attention. The importance of high germination energy is particularly evident in unfavorable environmental conditions, which make it difficult for seeds to germinate and sprout.

5. Conclusion

At time when there is increasing need for reducing pesticide use and the implementation of integrated pest management strategy in storages, it is appropriate to consider how major pest such as *P. interpunctella* can be controlled using physical measures. Our study provides baseline data for successful use of elevated temperatures for management of different *P. interpunctella* life stages, associated with high-quality seeds. Based on the results, we can conclude that heat treatments could be effectively used for controlling different life stages of *P. interpunctella*, but since pupal stage is the least susceptible one, the heat treatments should be designed towards suppressing pupae. Our results also indicate that high temperatures and long exposure affected germination energy of sunflower seeds. When using thermal treatments, the temperature and exposure periods should be adapted in the way that they do not influence germination processes in high-quality seeds, moreover they should be refined for a specific commodity.

Since the early attempts to control insects by thermal methods, the applicable technologies have progressed in both design and theory. Advances in instrumentation now provide accurate measurements of all treatment variables (temperature, moisture etc.) and techniques have improved in precision and replication. The future application of thermal treatments is promising because of the extensive methods that can be used to produce and control heat.

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CRediT authorship contribution statement

Sonja M. Gvozdenac: Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dejan M. Prvulović:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Zagorka Lozanov-Crvenković:** Writing – review & editing, Writing – original draft, Software, Methodology, Data curation, Conceptualization. **Ivana V. Štajner-Papuga:** Writing – review & editing, Writing – original draft, Software, Methodology, Data curation, Conceptualization. **Jelena S. Ovuka:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Miloš V. Krstić:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis, Data curation. **Snežana T. Tanasković:** Writing – review & editing, Writing – original draft, Validation, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Filip N. Vukajlović:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Sonja M. Gvozdenac reports financial support was provided by Republic of Serbia Ministry of Science, Technological Development and Innovation. Zagorka Lozanov-Crvenkovic reports financial support was provided by Republic of Serbia Science, Technological Development and Innovation. Ivana V. Stajner-Papuga reports financial support was provided by Republic of Serbia Ministry of Science, Technological Development and Innovation. Jelena S. Ovuka reports financial support was provided by Republic of Serbia Ministry of Science, Technological Development and Innovation. Milos V. Krstic reports financial support was provided by Republic of Serbia Ministry of Science, Technological Development and Innovation. Filip N. Vukajlovic reports financial support was provided by Republic of Serbia Ministry of Science, Technological Development and Innovation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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