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Research Article

EFFECTIVENESS OF HIGH TEMPERATURES AGAINST *CALLOSOBRUCHUS MACULATUS* (Coleoptera: Bruchidae) ADULTS

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Abstract

Pulse beetle, *Callosobruchus maculatus* is a worldwide severe insect pest of pulse grains in the storage conditions. Considering the negative consequences of synthetic chemicals used against *C. maculatus,* effectiveness of various high temperatures was evaluated against it under laboratory conditions. The Classic® 1080 series laboratory oven (HT Company (Ilford) Ltd. UK) was used to get the desired temperatures of 40, 45, 50, 55, and 60ºC. Five pairs of freshly emerged *C. maculatus* adults were placed with twenty cowpea seeds in a petri dish were used for each temperature regime for five exposure times i.e., 10, 20, 30, 40, and 50 minutes. Data on adult mortality were observed after 24-hours. Results confirmed the effective high temperatures against adults of *C. maculatus* as 100% mortality of the targeted adults was recorded within 10, 40 and 50 minutes, at 60°C, 55 and 50 °C, respectively. The LT_{50} values against *C. maculatus* at various exposure times ranged between 43.54°C at 50 minutes exposure to 48.99 °C at 10 minutes exposure, whereas overall minimum and maximum fiducial limits for LT_{50} were ranged between 42.85 to 49.66 °C. No negative impact of high temperatures was also recorded on the germination percentage of cowpea seeds as germination success recorded at various high temperatures was not significantly different from the control cowpea seeds. Thus, the high temperatures 50, 55, and 60 °C was found effective to cause 100 % mortality in *C. maculatus* and can be evaluated at commercial level in large warehouses to optimize their performance.

Keywords: *Callosobruchus*, germination, mortality, pulses, temperature
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1. INTRODUCTION

Callosobruchus maculatus has been found to be more abundant and widely distributed pulse beetle species in various districts of Sindh, Pakistan (Deeba et al., 2006; FAO, 2018). Moreover, it was also found to be very damaging and destructive pest on commonly consumed pulses i.e., cowpea, chickpea, green lentil, and yellow lentil in the region, causing up to 67% losses in the weight of pulses (Bidar et al., 2021). Therefore, considering the wide distribution and huge losses of *C. maculatus* in the province, it become eminent to adopt timely and adequate management strategies to restrict its further spread and losses. Among the management tools, synthetic pesticides are most commonly used and reliable method against *C. maculatus* mainly because it is effective, less costly, and easy to apply (de Andrade Rodrigues et al., 2022). Pyrethroids and organophosphates as protective insecticides and phosphine are the commonly used insecticides against *C. maculatus* (Iturralde-Garcia et al., 2016). However, large-scale, and indiscriminate use of such chemicals has led to the problems for humans and their environment (Pourya et al., 2018; Wakil et al., 2013), besides the development of resistance in *C. maculatus* against most commonly used insecticides

(Malaikozhundan and Vinodhini 2018; Mutlu et al., 2019). Therefore, it has become necessary to evaluate alternative control measures that not only provide sustainable management of *C. maculatus* in the stored pulses, but also safe to humans and their environment (de Andrade Rodrigues et al., 2022).

Among environmental factors, temperature has been reported to play a vital role in the growth, development, and damage potential of insects, including the stored grain pests such as *Callosobruchus* spp. (Omar and Mahmoud, 2020). Accordingly, heat treatment has got the attention in recent years to disinfest stored commodities from the noxious insect pests (Mahroof et al., 2003; Roesli et al., 2003). It is reported that at high temperatures, rate of metabolism is increased, whereas low temperatures slowdown the development along with reduced fecundity (Flinn and Hagstrum 1990; Loganathan et al., 2011; Umoetok Akpassam et al., 2017). Therefore, manipulation in temperatures has been widely practiced controlling the populations of *C. maculatus,* either by covering the pulses with plastic sheets under sunlight (Ajayi et al., 2021; Baoua et al., 2012a, b; Ganiger et al., 2022; Gbaye et al., 2011) or applying various appliances to get the desired temperatures, either low or high (Mahdi et al., 2015; Omar and Mahmoud, 2020; Onur and Tuncer, 2022; Terada et al., 2019). Accordingly, Karimzadeh et al. (2020) while studying variable control methods against *C. maculatus* including higher temperatures found LTi_{50} values of 34, 29, 18, 11 and 8 min at at 45, 48, 50, 52 and 55 °C, respectively. Similarly, significant effect of higher temperature on mortality of various stages of *C. maculatus* was recorded by Loganthan et al. (2011) as eggs and larvae were found to comparatively more susceptible than pupae and adults.

Therefore, laboratory studies were carried out to determine the impact of high temperatures on the mortality of *C. maculatus* as the result obtained could help to reduce the pest infestation using the temperature with less dependence on synthetic insecticides.

2. Materials and Methods

The study was conducted in the Research Laboratory, Department of Plant Protection, Sindh Agriculture University, Tando Jam. The Classic® 1080 series laboratory oven (HT Company (Ilford) Ltd. UK) (Figure- 1) was used in the experiment to get the desired temperatures of 40, 45, 50, 55, and 60 ºC.

The culture of *C. maculatus* was maintained in the laboratory on cowpea seeds maintaining temperature and relative humidity at 30±2 ºC and 60±5 %, respectively. Five pairs of freshly emerged 24-horus old *C. maculatus* adults were used along with twenty cowpea seeds in a petri dish and placed in the oven. The adults at the individual temperature regimes were exposed for 10, 20, 30, 40, and 50 minutes. Separate petri dishes were used for the individual treatment combination.

After the treatment of adults, they were taken out immediately from the oven and kept under the standard laboratory

Figure 1: Experimental set-up of high temperatures against *Callosobruchus maculatus* adults

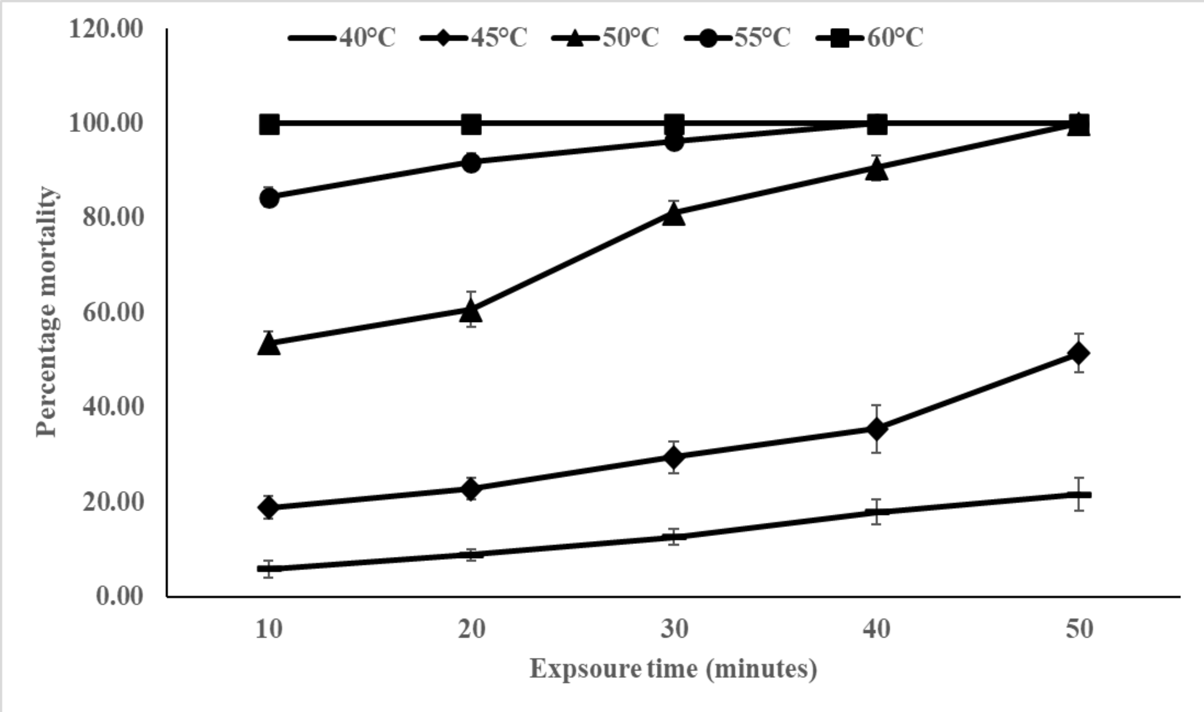

Figure 2: Effect of high temperatures applied at various exposure times on percentage mortality of *Callosobruchus maculatus* adults in cowpeas
*Means followed by same letters are not significantly different from each other at individual (LSD = 5.9138, P < 0.05)

conditions (30±2ºC temperature and 60±5% relative humidity). A control treatment with five pairs of adult *C. maculatus* was also maintained under the standards laboratory conditions to record the natural mortality. The mortality of adults was recorded after 24-hours of the exposure to desired temperatures and mortality percentage was calculated using the following formula:

$$\textbf{Adutl mortality \%} = (\textbf{Dead adults /Adults treated})\ \textbf{100}$$

A separate experiment was also conducted to determine the effect of various high temperatures mentioned above for different exposure times on the germination capability of the cowpea seeds. Twenty cow pea seeds were exposed to desired temperatures and exposure times along with a control treatment maintained under the standard laboratory conditions. Afterwards, these seeds were planted under natural

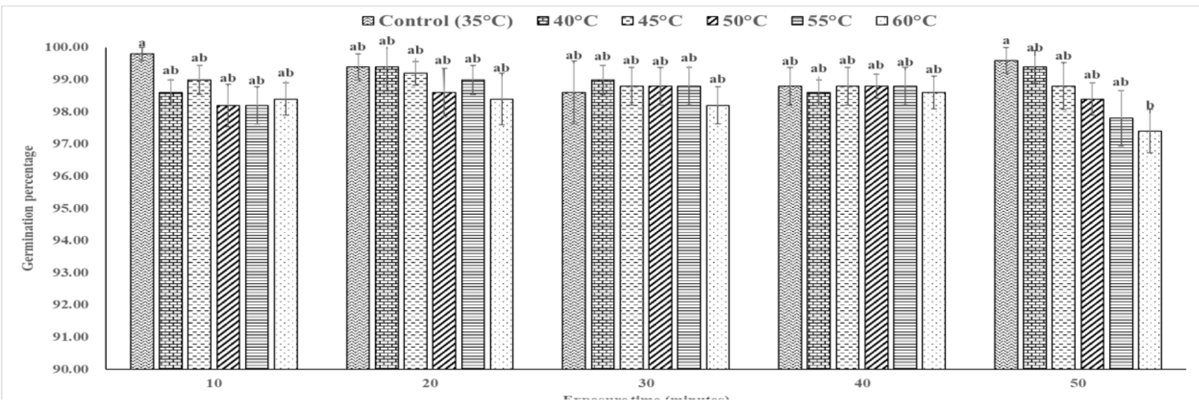

Figure 3: Effect of high temperatures applied at various timings applied against *Callosobruchus maculatus* on germination percentage of cowpeas
*Means followed by same letters are not significantly different from each other at individual (LSD = 1.6198, P < 0.05)

conditions to record the germination percentage using the following formula:

Germination (%) = (Seeds germinated / Total seeds planted) X 100

Both the experiments were arranged in a Completely Randomized Design as five replications were maintained for the individual treatment combinations of temperatures and exposure timings. Analysis of Variance was used for the data analysis, whereas the Least Significant Test (LSD) at 5% probability was used to determine the means with significant differences. The STATISTIX 8.1 computer software was used for the data analysis.

3. Results

Figure- 2 shows the results of impact of various high temperatures applied at various exposure times against the adult *C. maculatus*. A highly significant difference ($F = 13.68$, $P < 0.001$) was observed in the mortality of *C. maculatus* adults due to the applications of various high temperatures at different time intervals. Although, mortality of adult *C. maculatus* was observed at all the temperatures applied at various time intervals, however the mortality percentage increased with increasing temperatures and exposure timings. Accordingly, the 100% mortality of the targeted *C. maculatus* adults was recorded when they were treated with 60 °C temperature for 10 minutes, whereas it took 40 minutes to get 100% mortality of *C. maculatus* when they were treated with 55 °C. Moreover, 100% mortality of adult *C. maculatus* was also recorded when they were treated with 50 °C temperature for 50 minutes.

The maximum mortality of *C. maculatus* adults recorded at 40 and 45 °C was 21.60±3.56 and 51.40±4.13 %, respectively, both recorded at 50 minutes exposure timing.

Table-1 illustrates the results regarding LT_{50} temperature required to cause mortality of the 50% of the targeted *C. maculatus* at various exposure timings. It was observed that application of high temperatures was found effective at various exposure timings i.e., 10, 20, 30, 40, and 50 minutes with no great variation among the time required at various temperatures to obtain 50% mortality of the targeted *C. maculatus*. Thus, the LT_{50} temperatures against *C. maculatus* at various exposure timings ranged between 43.544 °C (at 50 minutes exposure time) to 48.994 °C (at 10-minute exposure time), whereas overall minimum and maximum fiducial limits for LT_{50} temperatures at various exposure timings were also narrow within the range of 42.845 to 49.655°C.

Results given in Figure-3 show the germination percentage of cowpea seeds treated with various temperatures for different exposure timings. It was observed that no significant ($F = 0.43$, $P = 0.9844$) effect of various temperatures and exposure timings was recorded on the germination capability of the treated cowpea seeds. Thus, the mean germination percentage recorded among various treatments ranged between 97.40±0.68% to 99.80±0.20% that were recorded in 60 °C temperature applied for 50-\minutes and control treatments, respectively.

Table 1: Lethal temperature [LT_{50} (°C)] calculated at different exposure timings against *Callosobruchus maculatus*

Exposure time (minutes)	LT_{50} (°C)	FL 95%	Slope ± SE	χ^2	Probability
10	48.994	48.333-49.655	21.423±0.689	77.526	0.282
20	47.897	47.256-48.535	21.483±0.692	74.580	0.056
30	46.186	45.552-46.810	22.661±0.754	78.113	0.000
40	44.936	44.936-44.148	24.476±0.871	131.658	0.000
50	43.544	42.845-44.221	27.508±1.097	118.225	0.000

4. Discussion

Temperature has been considered as the key factor that can regulate the growth and development of living organisms, especially insects. Hence, its manipulation in controlled warehouse environments could be of great importance in regulating the population of noxious insect pests including *Callosobruchus* spp. Thus, treatment of adult *C. maculatus* with higher temperatures i.e., 40, 45, 50, 55, and 60°C for 10, 20, 30, 40, and 50 minutes were found effective to cause significant mortality as the mortality percentage increased with increasing temperatures and exposure timings. Accordingly, 100% mortality of the targeted adult *C. maculatus* was recorded at 60°C within 10 minutes of exposure, whereas it took 40 and 50 minutes to kill 100% of the adults at 55 and 50°C, respectively.

In continuation of the above findings, Lale and Vidal (2000) reported that when eggs, first and late instar larvae along with adults of *C. maculatus* and *C. subinnotatus* were exposed to 50°C in an incubator, resulted in complete suppression of adults of both species. Moreover, 100% mortality of 1st instar larvae and adults was recorded when they were exposed for 2-hours and 20 minutes, respectively. Overall, *C. maculatus* adults were found more susceptible to the applied temperature than *C. subinnotatus.* An increase in the average mortality of *C. maculatus* and *C. confusum* was recorded when they were treated with 50, 55, 60, 65, 70 and 75°C for 10, 15, 20, 25, 30, 35, and 40 minutes (Ahmady et al., 2016). They also reported that percentage loss of wheat flour and adult emergence were significantly decreased with increased temperatures and exposure timings with no promising effect of heating on the germination of cowpea seeds used in the study (Ahmady et al., 201 6). Simialrly, Sahadia and Aziz (2011) also found significant mortality of immature stages of *Callasobruchus* spp. when they were exposed to temperature in the regime of 60 to 65°C within few minutes. Osman et al. (2015) also found *C. maculatus* did not complete its development when exposed to 40°C and above temperatures.

Accordingly, findings of all above-mentioned studies reported a significant impact of increasing temperature on the mortality of various developmental stages of *C. maculatus*, hence supported the results obtained in our study. Another study on exposure of cowpea seeds infested with different stages i.e., egg, larva, pupa, and adults of *C. maculatus* at 50°C for 2, 4, and 6 hours indicated that an exposure period of 6-hours at 50°C was found effective against all the stages, whereas 100% adult mortality was achieved at the said temperature within twelve minutes of exposure (Bhalla et al., 2008). However, findings of Loganathan et al. (2011) were partially supported our results as they found pupae and adults equally tolerant to the tested higher (42 °C) and lower (0 °C) temperatures, having LT_{50} values of 78-hours and 10 days, and 71-horus and 4-days at 40 and 0°C, respectively. In the same study, eggs and larvae of *C. maculatus* were found comparatively more susceptible with LT_{50} values 3-days and 18-hours, and 8-days and 57-horus, respectively at 0 and 42°C (Loganathan et al., 2011). Therefore, LT_{50} values of 4-days for adults recorded at 40°C partially supported our findings as the same values for adults recorded at 40°C when they were exposed for 5-hours, whereas the mortality percentage increased with increasing temperatures and exposure timings.

Another study on the impact of four temperatures i.e., 25, 30, 35, and 40°C and three relative humidity levels 30, 60, and 90% on oviposition and development of *C. maculatus* and *C. subinnotatus* found that egg laying and growth of both species were relatively more effected by temperature than relative humidity as no population developed at 40°C (Lale and Vidal, 2003).As compared to findings of Lale and Vidal (2003), 40°C temperature was found so much effective in our study as compared to higher temperatures of 50°C and above.

The reason for such higher moralities recorded in that study at 40°C may be because of the difference in the geography, environmental conditions and temperature requirements of the particular strain used in the study at Nigeria, whereas we used local population of Pakistan, where temperatures remained quite high during most of the year.

It was also observed in this study that treatment of cowpea seeds at higher temperatures for various exposure timings did not have any effect on their germination capability as percentage germination recorded in various treatments was not significantly different from the germination in the control. Similar type of results regarding germination was recorded by Lale and Vidal (2000) when they tested various high temperatures against *C. maculatus* and *C. subinnotatus* in cowpea seeds, reported no promising effect of heating on the germination of cowpea seeds used in the study. Similarly, Murdock and Shade (1991) also did not find no significant impact of the temperatures and exposure times on the germination of cowpea seeds. Moreover, Karimzadeh et al. (2020) also found same trend in germination of cowpea seeds as all the seeds treated with various high temperatures (40, 45, 47, 50, 53, 55, 57, 60 and 65°C) germinated and reached seedling stage. While evaluating the higher temperatures against *C. confusum* and *C. maculatus* in cowpea seeds, Ahmady et al. (2016) also observed that exposure to these temperatures did not affect the germination of the seeds. Therefore, germination results obtained in this study are continuation of the above-mentioned studies that suggested that higher temperatures can be applied to cowpea seeds for disinfestation from insect pests as they did not affect their germination capability. EL-Sawy et al. (2022) and Maharjan et al. (2017) also found no evidence of the application of temperature treatment on the germination of cowpea seeds.

5. Conclusion

Application of high temperatures against adult *C. maculatus* cause significant impact as 100% mortality of the targeted adults was recorded within 10 minutes at 60°C, whereas it took 40- and 50-minutes to kill 100% of the adults at 55 and 50°C, respectively. No negative impact of high temperatures was also recorded on the germination percentage of cowpea seeds as germination success recorded at various high temperatures was not significantly different from the control cowpea seeds. Therefore, higher temperatures i.e., 50, 55, and 60 °C could be further evaluated at commercial level in large warehouses against stored grain pests, especially *C. maculatus* to optimize their performance.

6. Conflict of Interest

Authors do not have any conflict of interest.

7. Acknowledgments

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8. Author’s contributions:

AAP conducted the research trials and wrote the rough draft of the manuscript. AAG and LBR Conceive the idea and planning of research work. JGMS helps in statistical analysis, NK and AAG finalize the manuscript.

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