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Microwave Radiation and Heat Against Immature Stages of The Chinese Beetle *Callosobruchus Chinensis* (Coleoptera: Chrysomellidae)

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ABSTRACT

The present study aimed to investigate the effect of certain physical methods (heat and microwave radiation) as non-pesticidal ways to control the Chinese beetle *Callosobruchus chinensis* (Coleoptera: Chrysomellidae). Exposure of 100 infested faba bean seeds implying different immature stages of *C. chinensis*, (egg, larvae or pupae) to a high level of temperature of 50° C for 15, 30, 45, 60, 75, 90, 105 and 120 min. showed that all stages were significantly sensitive at the highest exposure period of 120 min. Eggs were more tolerant to the tested level of high temperature of 50°C than larvae and/or pupae. The germination of the exposed broad faba been seeds to the same high temperature (50°C) for different periods was significantly affected and it was found that as the time of exposure increased the germination percentages decreased as compared with control.

Moreover, the exposure of the infested faba bean seeds with different immature stages of *C. chinensis* to low temperature levels (25, 10 and -14 ± 1° C) for 24h revealed that all these stages were highly sensitive to the lowest temperature of -14 ± 1° C. Decrease in temperature level resulted in a decreased the average of the emerged adults and consequently increased the reduction percentage of the produced progeny. The reduction of the progeny (emerged adults) post the treatment with the lowest tested temperature (-14° C) showed that the reduction percentage reached 100% for the treated eggs, larvae and pupae.

Results also illustrated that after 24 h post - treatments, the calculated percentages of seeds germination percentage was 46.67% due to their previous exposure to the lowest tested temperature (-14° C), which is less than the half level of full or complete germination of normal healthy faba bean seeds.

Exposure of the infested faba bean seeds with different developing stages of *C. chinensis* to microwave (900W) for different periods of 5, 10, 15, 20, 25 and 30seconds was found to be effective against all the immatures of insect-pest especially after a period of 30 seconds. These treatments also affected the germination of seeds; the percentages of germination were decreased as the exposure period to microwave radiation increased. The results suggested that both the tested physical methods of heat and microwave radiation could be regarded as suitable ecofriendly and non-insecticidal methods or tools for controlling the developing or the harboured and trapped immature stages of *C. chinensis* within the infested seeds.

Key words: Heat, Microwave, Chinese beetle *Callosobruchus chinensis*, immature stages, emerged adults, seed germination

INTRODUCTION

Legumes (pulses) are considered to be the second most important group of crops worldwide for their content of proteins, minerals and vitamins and therefore they invaded by a series of beetles damaging seeds and causing weight loss. Infestation commonly begins in the field, where eggs are laid on mature pods (Singh, 1997; Nahdy *et al*., 1999). The pulse crops are attacked by more than 150 insect pests. Among the insects which infest various pulses are *Callosobruchus chinensis* (Linn.), *C. maculatus* (Fab.) and *C. analis* (Fab.) causing both quantitative and qualitative damage to stored grains.

The cowpea seed beetle (Chinese beetle), *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), is the most important storage insect-pest of cowpea throughout the tropics. Moreover, *C. chinensis* is a major insect-pest of chickpeas (Pandey and Singh, 1997), lentils, green gram, broad beans, soybean (Srinivasacharyulu and Yadav, 1997; Yongxue *et al*., 1998) adzuki bean and cowpeas in various tropical regions. The eggs are cemented to the surface of pulses and are smooth, domed structures with oval and flat bases. The larvae and pupae are normally only found in cells bored within the seeds of pulses (Chavan *et al*.,1997).

The control of stored grain pests stands mostly on broad action insecticides and fumigants. These chemicals have been used for a long time to protect stored products from insect pests (White and Leesch, 1996; Daglish, 2006), but due to environmental concerns and because stored-product insects are developing resistance to insecticides, such chemicals have been under increasingly restrictive policies over

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the past years (Donahaye, 2000; Zettler and Arthur, 2000; Kljajic and Peric, 2005; Collins, 2006).

Environmentally safe and convenient methods such as the use of inert dusts, plant extracts, oils, leaf powders and pressurized carbon dioxide, temperature management techniques (low and high temperature) and microwave radiation are the growing interest to replace synthetic pesticides (Nasab *et al.*, 2009; Yuya *et al.*, 2009; Loganathan *al.*, 2011). Temperature is the most important factor of the environment, determining the rate of metabolism, growth, development, reproduction, general behaviour and distribution of insect pests. There has been a lot of research on conventional heat treatment for disinfestation of number of stored commodities (Evans, 1986; Dosland *et al*., 2006; Alice *et al*., 2013). Egg, larvae and pupae of bruchids are trapped within the seed and therefore, they are excellent target for management using elevated temperatures. Moreover, microwave is considered to be an eco-friendly method that doesn't cause any hazardous impacts toward humans (Vadivambal *et al*., 2010). The physiological processes of insects are also negatively affected by microwave radiation which leads to a considerable reduction in their reproduction and survival (Webber *et al*., 1980; Wang *et al*., 2003; Vadivambal *et al*., 2008; Valizadegan *et al*., 2009&2011). The microwave has potential applications in pest management and it has been proven to be rapid, nondestructive targeting the incipient insect infestations and would be of benefit to both the producers and consumers of packaged foods (Abdelaal and El-Dafrawy, 2014).

Therefore, the present study was carried out to investigate the ecologically safe physical methods (heat and microwave radiations) to control the Chinese beetle, *Callosobruchus chinensis*. Moreover, the study included also the effect of such treatments on the broad bean seeds germination.

MATERIALS AND METHODS

Insect species tested and culture preparation

A susceptible strain of the azuki bean weevil (the Chinese beetle = the bruchid beetle), *Callosobruchus chinensis* was transferred to a sterilized seeds of faba bean (*Vicia faba*) imported variety that has been used in Egypt for human consumption. Stock and colonies have been cultured using hygrothermically conditioned room at a constant temperature of 28±2° C, a relative humidity of 70 ± 5% and continuous daily darkness of 24 h, where all experimental work was performed in. For culture preparation cleaned and sterilized broad faba bean samples at 70° C for 1h were placed in glass jars separately to reabsorb moisture. Then, amounts of 300g of these sterilized broad bean seeds were added to sterilized culture jars. Adults of the Chinese beetle (200-250 insects) collected from previous culture were added in to each jar; the jars were sealed with muslin and placed in the rearing room. After one week, the insects were sieved out and discarded. Adults of the Chinese beetle of an age of 2-3 days after emergence were used for experimental work.

Heat treatment (high and low temperature levels)

For the treatment of high temperature level, samples of infested seeds containing certain immature stage of the tested insect were treated for different exposure times (15, 30, 45, 60, 75, 90, 105 and 120minutes) at 50° C using a JSR oven. The rates of emerged adults and progeny reduction of *C. chinensis* after the treatmental exposure of the infested broad bean seeds were determined. Seeds infestation was performed by releasing 0-2-day old adults (ten pairs [10 males + 10 females]) on a hundred broad bean seeds replaced in ¼ l glass jar along a period of 24h for oviposition. As the immature development occurs inside the seeds, the tested immature stages were prepared based on the passing period post-oviposition. The tested immature stages included the egg, larvae and pupal stage (2, 8 and 21 days post- oviposition, respectively). Three replicates of each sample were performed. Meanwhile, one hundred infested seeds of each developmental stage in three replicates were exposed to three lower levels of temperature (25, 10 and -14 ± 1°C) for 24 h. The treated seeds were then transferred to controlled conditions of temperature and relative humidity. The emerged adults were counted and progeny reduction of *C. chinensis* was calculated.

Microwave radiation

A hundred of infested seeds of each immature developmental stage in three replicates were exposed to microwave generated at 2450MHz (230 v~50 Hz) (900W) for 5, 10, 15, 20, 25 and 30 seconds.The treated seeds were then transferred to the controlled conditions of temperature and relative humidity. In addition, healthy (uninfested) seeds of faba bean were also subjected to the same performed treatments of heat and microwave radiation and examined by the standard germination tests for checking the embryonic viability of the seeds after treatment. The seeds were first soaked for 24 h in fresh water, and then they were transferred to 9 cm diameter Petri-dishes containing a piece of cotton moistened to saturation with distilled water. Additional water was added when needed and Petri-dishes were covered with clingfilm to minimize water loses; the germination of seeds was checked after 4 days.

Statistical analysis

The results were analyzed and subjected to CoStat computer program ver. 6.303, CoHort software (CoStat,

2005) to determine the significant difference between the means. Comparisons among the means of the various treatments were performed, using the revised least significant different (L. S. D) at < 0.05 level.

RESULTS AND DISCUSSION

Efficacy of heat against different immature stages of *Callosobruchus chinensis*

High heat level (50ºC)

The effect of exposing 100 infested bean seeds with different immature stages of *C. Chinensis* (either eggs, larvae or pupae) for different durations of 15, 30, 45, 60, 75, 90, 105 and 120 minutes to a high temperature level of 50° C and the determination of emerged progeny of adults in these infested samples and the progeny reduction percentages after treatment are shown in Table 1. Generally, all the tested immature stages are affected by that high temperature level of 50° C treatment and the most affected stages are larvae and pupae, while eggs were more tolerant to high temperature level (50° C). Herein, Bhalla *et al*. (2008) revealed that all stages of *C. maculatus* are sensitive to an exposure period of 6h at 50° C. In addition, Marijana *et al*. (2011) indicated that short-term exposure of weevils from *Sitophilus* genus at the temperature of 50° C adversely affects their survival and progeny production, as well as that there is a potential for its successful use as a physical measure in control of storage pest insects. Mansoor *et al*. (2017) found that temperature had great influence on *S. oryzae* and its life span as an insect of stored product. High effect was exhibited at high temperature in comparison with low one.

The mean numbers of emerged adults after the treatment of 100 seeds containing different immature stages were decreased with the increase of exposure time. At the highest time of exposure (120 min), the reduction percentages of those emerged adults resulted from the treated seeds containing different immature stage of the tested insect were 94.11, 100.00 and 100.00 for eggs, larvae and pupae treatments, respectively. Statistical analysis showed that there were significant differences among the mean numbers of the emerged adults due to the heat treatment at different exposure periods ($P < 0.05$). It could be noticed that at the exposure time of 75 min and more, all the different tested stage started to be strongly affected and the longer time as 75 min might be a critical one for the tested insect since the effect was pronounced.

Figure 1 illustrated the effect of the high temperature level of 50° C on seed germination after treatment for different periods (15, 30, 45, 60, 75, 90, 105 and 120 min). The germination of the seeds was significantly affected and it was found that as the time of exposure increased the germination percentages decreased as compared with the control.

Table 1.Efficacy of high heat level (50° C) against different tested immature-stages of *Callosobruchus chinensis*

Time (min)	Mean No. of emerged adults after the treatment of 100 seeds containing different immature stages					
	Eggs	Reduction** %	Larvae	Reduction %	Pupae	Reduction %
15	161.33^{a*}	I (103.36)	81.33^{a}	I (2.5)	48.33^{bc}	39.07
30	80.00^{bc}	I (0.84)	71.33^{abc}	10.08	45.33^{bc}	42.86
45	99.33^{ab}	I (25.21)	43.00^{cd}	45.79	67.67^{ab}	14.69
60	75.00^{bcd}	5.46	45.00^{bcd}	43.27	19.00^{cd}	76.05
75	21.00^{cde}	73.50	18.67^{de}	76.46	10.33^{d}	86.98
90	23.67^{cde}	70.16	4.67^{e}	94.11	7.67^{d}	90.33
105	16.67^{de}	78.99	3.33^{e}	95.80	0.00^{d}	100.00
120	4.67^{e}	94.11	0.00^{e}	100.00	0.00^{d}	100.00
Control	79.33^{bcd}		79.33^{ab}		79.33^{a}	
$LSD_{0.05}$	62.87		35.06		30.29	

*Means followed by the same letter (s) in a column are not significantly different at 0.05 level of probability.

** Reduction percentage of the emerged adults due to the treatments; I = increase of progeny as compared with control.

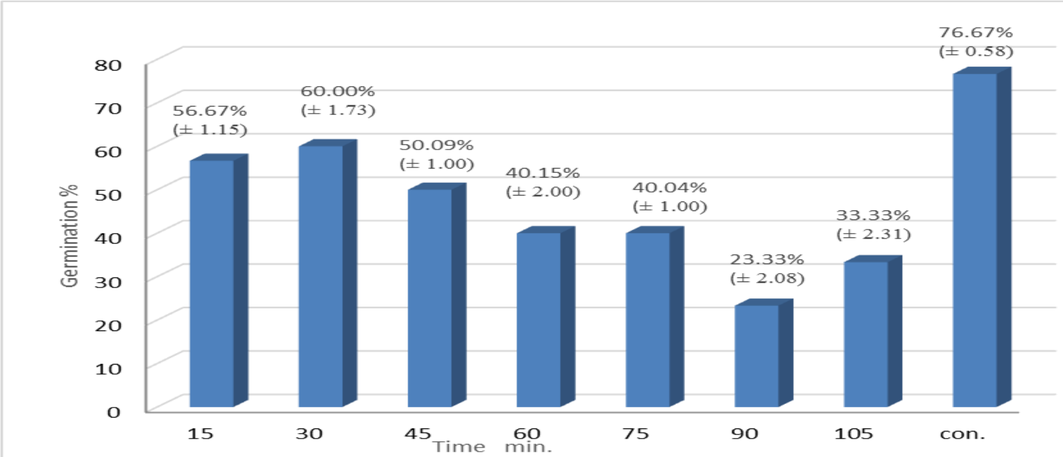

Fig. 1. Germination percentages (± SD) of broad bean seeds after exposure to high heat level (50° C) for different times

Efficacy of low heat levels against different stages of *C. chinensis*

Exposure of 100 bean seeds infested with a known stage of *C. chinensis* (eggs, larvae or pupae) to low levels of temperature 25, 10 and -14 ± 1° C for 24 hrs revealed that all the stages were highly sensitive to the cooled temperature of -14 ± 1° C. Data presented in Table 2 show that eggs were more sensitive than the other two stages when exposed to low temperature. Decrease in temperature level resulted in the decrease of the average of the emerged adults and meanwhile increasing the reduction percentage of the produced progeny. The present results are in agreement with those reported by Bhalla *et al*. (2008) who revealed that all stages of *C. maculatus* are sensitive to a temperature of - 14 ± 1° C. Also, Maharjan *et al*. (2017) showed that low temperature treatments have significant effects on the different stages of *C. chinensis*.

The reduction of the progeny (emerged adults) post the treatment with the lowest tested temperature (-14° C) showed that the reduction percentage reached 100% for the treated eggs, larvae and pupae. This could be explained by the effect of such low temperature on the water and protein within the egg, larva and/or pupa which eventually die and therefore there were no emerged new individuals. It is well known that temperature is a principal factor influencing egg development in insects (Jaworski and Hilszczański, 2013). The tested insect as a stored product insect has no ability to survive the lowest temperature (-14° C). The low temperature is affecting also the enzymes activity and function and these might lead to the failure of hatching and the death of larvae and pupae of the Chinese beetle *C. chinensis* due to lethal freezing and therefore there were no new progeny (Bhalla *et al*., 2008).

Table 2.Efficacy of low temperature levels for 24 hours exposure against different immature stages of *C. chinensis*

Temp. (°C)	Mean No. of emerged adults after the treatment of 100 seeds containing a certain immature stage					
	Eggs	Reduction** %	Larvae	Reduction %	Pupae	Reduction %
25	44.33 [b]	44.12	48.00 [a]	39.49	55.33 [a]	30.25
10	31.33 [b]	60.51	67.33 [a]	15.13	53.67 [a]	32.34
-14	0.00 [c]	100.00	0.00 [b]	100.00	0.00 [b]	100.00
Control	79.33 [a]		79.33 [a]		79.33 [a]	
LSD $_{0.05}$	26.43		33.51		40.84	

*Means followed by the same letter (s) in a column are not significantly different at 0.05 level of probability. ** Reduction percentage of the emerged adults due to the treatments; I = increase of progeny as compared with control.

Figure 2 show the effect of the tested levels of temperature (30, 25, 10 and -14° C) on broad bean seeds germination as they exposed to those levels for 24 hrs. Results illustrated that after 24 hrs of heat treatments, the germination percentage was 46.67% when the seed were previously exposed to lowest tested temperature (-14° C), which is less than the half level of full germination of faba bean seeds. It could be seen that germination percentage did not progressively increased when the seeds were kept for 24 hrs at temperature above freezing. Meanwhile, the tested seeds kept at 10° C for 24 hrs before germination recorded a higher germination percentage of 60.00 compared with 66.67 and 76.67 .0% for those seeds kept at 25 and 30° C which has been as control. Vadivambal *et al*. (2007) cleared that high temperature affects the germination capacity of the seeds.

The decrease of germination (46.67%) of those seeds stored for 24 hrs at the lowest tested temperature level (-14° C) (below-freezing) was due to chilling and freezing abiotic stresses that play critical roles in germination. The decrease in germination capacity was related to the final temperature and the initial moisture content of the seeds. Temperature can affect the germination capacity through its effects on seed deterioration, loss of dormancy and the germination process itself (Roberts, 1988). Germination is one of the biological processes mediated by the coordination of the metabolic processes catalyzed by different enzymes (Renaut *et al*., 2006). The exposure of the seeds for 24 hrs to a temperature below-freezing (-14° C) might disrupt such enzymes.

Microwave radiation

Exposure of bean seeds infested with different stages of *C. chinensis* (eggs, larvae and pupae) exposed to microwaves (900 W) for 5, 10, 15, 20, 25 and 30 seconds revealed that all the stages were sensitive to a microwave radiation. The greater the duration of exposure to microwave radiation, the greater the rate of inhibition of immature stages within the seeds or reduction of adults emergence. Data in Table 3 show that eggs and larvae were more susceptible and more affected by microwave radiation than pupae. The results of the present study indicate that the tested high power of microwave radiation treatment (900 W) cause death of the immature stage preventing them from completing their development and reducing the numbers of the emerged individuals that cause seeds reinfestion or such radiation cause severe physiological effects on the stored product insect-pest *C. chinensis* especially at the high exposure periods (15-30 sec).

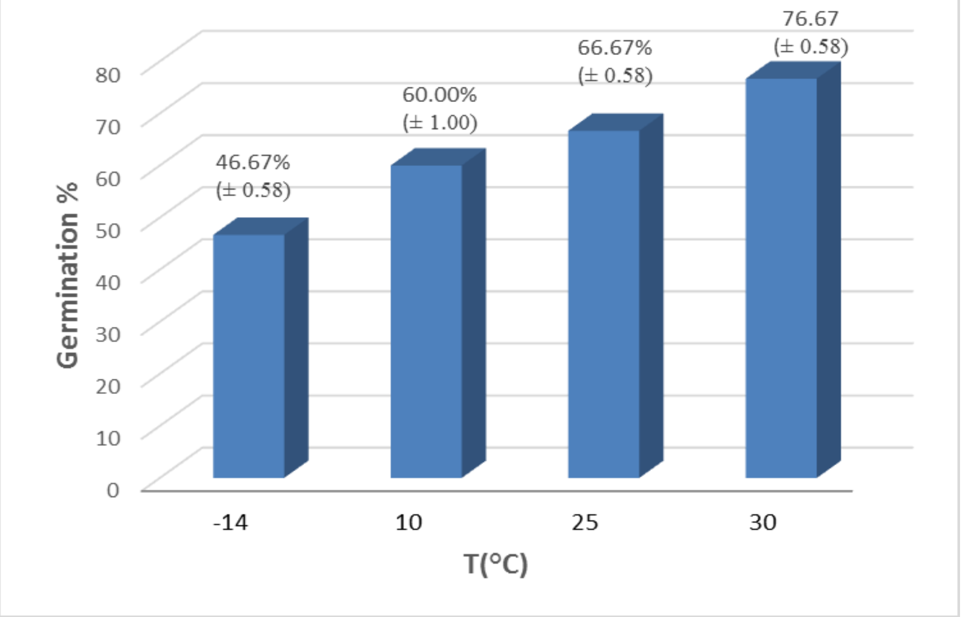

Fig. 2. Percentages (± SD) of faba bean seeds germination after exposure to different temperature for 24 hours

Table 3.Efficacy of microwave (900W) against different immature stages of *Callosobruchus chinensis*

Time (S)	Mean No. of emerged adults after the treatment of 100 seeds containing a certain immature stage					
	Eggs	**Reduction** %**	**Larvae**	**Reduction %**	**Pupae**	**Reduction %**
5	52.33[b]	34.04	68.33[ab]	13.87	99.67[a]	I (25.64)
10	22.00[c]	72.27	32.00[bc]	59.66	63.00[c]	20.58
15	6.67[d]	91.59	3.67[c]	95.37	17.33[d]	78.15
20	0.67[d]	99.15	0.33[c]	99.58	6.00[de]	92.44
25	0.33[d]	99.58	0.00[c]	100.00	2.67[e]	96.63
30	0.33[d]	99.58	0.00[c]	100.00	0.00[e]	100.00
Control	79.33[a]		79.33[a]		79.33[b]	
LSD $_{0.05}$	10.42		40.14		13.92	

*Means followed by the same letter (s) in a column are not significantly different at 0.05 level of probability.
** Reduction percentage of the emerged adults due to the treatments; I = increase of progeny as compared with control.

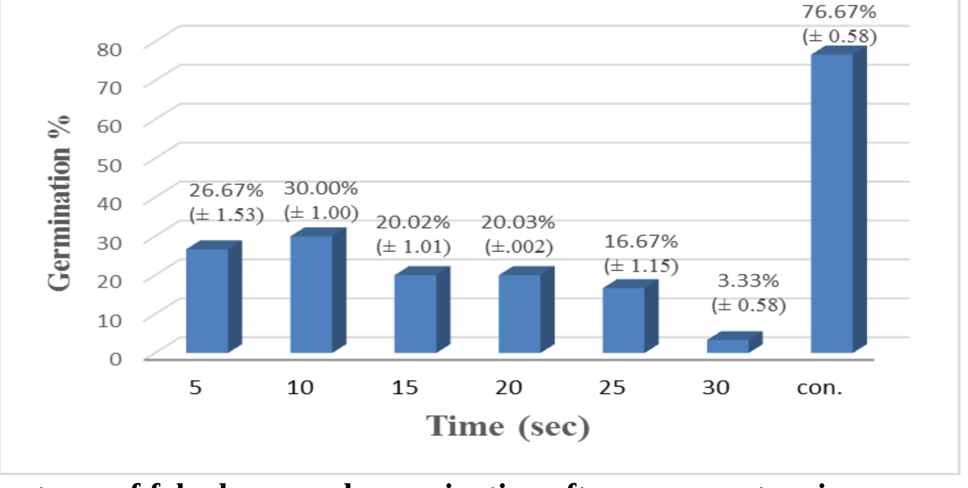

Fig. 3. Percentages of faba bean seeds germination after exposure to microwave radiation at different times

The present results are in agreement with those reported by Bhalla *et al*. (2008) who revealed that all stages of *C. maculates* were controlled by microwave radiation and the pest mortality increased with the increase of exposure period. Also, Purohit *et al*. (2013) found that the mortality of all life stages (eggs, young larvae, old larvae, pupae and adults) reached 100% when they were exposed to 400 W power level for 28 s. Eggs were the most susceptible and adults were the least susceptible life stages to microwave treatments. Mung bean seeds temperature increased, and germination decreased as the power level or exposure time increased. There has been an increasing trend of microwave use not only in food industry but also in household kitchen as well.

The effect of microwave radiation (900 W) on broad bean germination after 2 and 4 days post-treatment is shown in Fig. (3). The recorded germination percentages showed significant differences between all treatments as compared with control. The germination due to the microwave exposure for 5, 10, 15, 20, 25 and 30 s decreased from 76.67%% (in control) to 26.67, 30.00, 20.02, 20.03, 16.67 and 3.33% for the exposure periods of 5, 10, 15, 20, 25 and 30 s, respectively. The presented results agreed with the results of Halverson *et al*. (1996) who found that the percentages of germination were decreased as the exposure period to microwave radiation increased. Also, Vadivambal *et al*. (2010) mentioned that the germination of corn was decreased with the increase of microwave power or exposure time or both.

The presented results are also in agreement with those of Vadivambal *et al*. (2008) who reported that germination of rye was lowered after treatment with microwave energy. It was also noticed that there was a temporary increase in the seeds temperature with the increase of exposure time of microwave radiation power lasted only for limited time.

Thermal treatment has been extensively investigated by several researchers as an alternative method of controlling insects. In the current study, high (50° C at different exposure times) and low temperature degrees (for 24 hrs) were evaluated in lab conditions for the management of the stored product insect- pest *C. chinensis*. Insects die by exposing to high temperature degrees, because of their limited physiological capacity to thermoregulate (Fields, 1992). With regard to the results of this study it is evident that high temperature (50°C) had effective role in the management of stored product pests. Therefore, the results presented here would lead to a reduction in the economic loss associated with infestation of *C. chinensis*, and minimize injury to stored beans in the storage. Also, it was found that C. *chinensis* are sensitive to a temperature of - 14 °C. Therefore, it is feasible to utilize low and high temperatures to control *C. chinensis* in storage.

Moreover, in the present study it was found that eggs were more tolerant to high temperature (50° C), in contrast, Jian-Feng *et al*. (2013) found that heat tolerance of pupae is higher than the other three life stages (eggs, larvae and adults).

The use of microwave heating has the advantage of saving time and energy. The treatment of infested seeds by microwave radiation appears to be a reliable alternative to conventional post-harvest insect control methods in the near future, either with stationary or mobile applicators on the farm or quarantine purposes during the loading process (pre- or post-shipment) before seed storage.

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الملخص العربي

أشعاع الميكروويف و الحرارة ضد الأطوار غير الكاملة لخنفساء الفول الصينية

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استهدفت الدراسة الحالية معرفة تأثير بعض العوامل الفيزيائية (الحرارة العالية والمنخفضة ، أشعاع الميكروويف) كطريقة لمكافحة خنفساء الفول الصينية. وقد أوضحت النتائج عند تعريض ١٠٠ من بذور الفول المصابة بالأطوار المختلفة لخنفساء الفول الصينية (بيض ، يرقات ، عذاري) لدرجة الحرارة العالية (٥٠°م) لفترات مختلفة (١٥ ، ٣٠ ، ٤٥ ، ٦٠ ، ٧٥ ، ٩٠ ، ١٠٥ ، ١٢٠ دقيقة) تأثرت كل الأطوار المعاملة نتيجة التعريض لمدة ١٢٠ ق حيث كان البيض هو أكثر الأطوار غير الكاملة تحملاً مقارنة باليرقات أو العذاري. كذلك عند دراسة تأثير الحرارة العالية المختبرة والأزمنة المختلفة للتعريض علي إنبات بذور الفول وجد أن الإنبات تأثر معنوياً وأن زيادة زمن التعرض أدي إلي إنخفاض نسبة الإنبات بالمقارنة بالكنترول.

كذلك أتضح من تعريض بذور الفول المحتوية علي الأطوار غير الكاملة المختلفة من خنفساء الفول الصينية (البيض ، اليرقات ، العذاري) لدرجات الحرارة المنخفضة (٢٥ ، ١٠ ، -١٤ ± ١°م) لمدة ٢٤ ساعة الحساسية العالية للأطوار غير الكاملة لدرجة الحرارة الأكثر إنخفاضاً (-١٤ ± ١°م) حيث أدي ذلك الإنخفاض في درجة الحرارة إلي منع أو نقص معدل خروج الحشرات الكاملة بعد المعاملة حيث أدت المعاملة إلي موت جميع الأطوار غير الكاملة في البذور المعاملة (١٠٠%). كذلك اوضحت النتائج أيضاً أن نسبة إنبات البذور إنخفضت إلي ٤٧,٦٧% بعد المعاملة بدرجة الحرارة المنخفضة (-١٤ °م).

كما أظهرت معاملة حبوب الفول المصابة بالأطوار الغير كاملة لإشعاع الميكروويف (٩٠٠ وات) لفترات مختلفة (٥ ، ١٠ ، ١٥ ، ٢٠ ، ٢٥ ، ٣٠ ثانية) فعالية تلك المعاملة بدرجة كبيرة ضد كل الأطوار المعاملة خاصةً التي تم تعريضها لمدة ٣٠ ثانية. وقد أثرت كل المعاملات السابقة علي إنبات البذور حيث إنخفضت بزيادة فترة التعرض لإشعاع الميكروويف. وتشير نتائج هذه الدراسة إلي إمكانية تطبيق وإستخدام العوامل الفيزيائية بكفاءة وفعالية كبيرة لمكافحة الأطوار غير الكاملة لحشرة خنفساء الفول الصينية المتواجدة داخل بذور الفول المصابة مع التحفظ إلي إستخدام هذه البذور كتقاوي.