

## EFFECT OF FREEZING, HIGH TEMPERATURE AND LASER RADIATION FOR CONTROLLING KHAPRA BEETLE, *TROGODERMA GRANARIUM* (EVERTS), ON WHEAT GRAINS

ABDEL-FATTAH, NILLY A. H.<sup>1</sup>; Z. A. HALAWA <sup>1</sup>; ASAMAA Z. ALSHARKAWY<sup>2</sup>;  
AFAF A. ABBAS <sup>2</sup> and H. I. MAHMOUD<sup>3</sup>.

1- Plant Protection Research Institute, ARC, Dokki, Giza.

2- Faculty of Science (Girls), Al-Azhar University.

3- National Institute of Laser Enhanced Sciences, Cairo University - Egypt.

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### Abstract

**K**hapra beetle, *Trogoderma granarium* (Everts) (Coleoptera, Dermestidae) is one of primary insects on stored grains. To control this pest with non-chemical methods, all stages (eggs, larvae, pupae and adults) of the pest were exposed to low and high temperature for different periods. Results revealed that mortality of all stages of *T. granarium* reached 100% after 7 and 5 days freezing at -10 and -15 °C, respectively. Eggs were more sensitive to low temperature than the other stages. On the other hand, high temperatures of 50 and 60 °C caused 100% mortality for all stages of the same pest after 6 hours of exposure. The increase of high temperature to 70 °C, the decrease of exposure period to 4 hours. Both low and high temperatures significantly affected germination and chlorophyll contents of wheat grains. Also laser ray of solid state of diod pump (wave length of 532 nm) reduced egg hatchability and pupation as well as adult emergence of exposed 0-1 day old eggs or 0-1 day old pupae. Laser irradiation significantly reduced germination rate, but there was no effect on chlorophyll content.

### INTRODUCTION

Khapra beetle, *Trogoderma granarium* (Everts), (Coleoptera, Dermestidae) has posed serious threat to global food security and safety. Economic importance of studied beetle is due to its capability to cause huge loss in stored grains through voracious feeding and heating of grains. Its larvae able to withstand starvation for up to 3 years and its ability to live on food with very low moisture content.

Khapra beetle is a primary pest which considers to be one of the most serious pests of grain in the world that may causes losses to stored grain of 5% to 30% and losses have been known to reach as high as 70 % (Ahmedani *et al.*, 2007).

Controlling of pests in stored products, principally cereal grains, by use of chemicals, a common strategy for post harvest loss avoidance, leads to apparition of many problems as pollution of environment, toxicity to human, appear of resistant pest strains and many other damages.

Using of high temperatures is a promising method to provide a rapid, non-chemical alternative to fumigation and other methods of chemical control for stored grain insects. Research and development in heating technology is needed because of:

- Increasing market preference for residue-free grain.
- Appearance of high level of insect resistance to phosphine.
- Current phase-out of methyl bromide.
- Presence of chemical residues is increasingly becoming limiting factor in marketing grain and processed foods.

At temperatures that are not instantly lethal, insects die through heat stress and dehydration. Heat can also make insects more susceptible to other methods of treatment. In the early 1900's, several food processing companies in the United States used heat to successfully control pests.

The use of heat as a disinfestation process in structures has continued up to the present time (Carter, 2000).

The term laser is an acronym for Light Amplification by Stimulated Emission of Radiation. Laser radiation caused mortality, induced sterility, inhibited or prevented reproduction, prolonged or reduced longevity and affected both physiological and biochemical processes.

The importance of food preservation, the low cost of laser light treatment, and the results obtained, suggest that the Laser light (as a new potential method for pest control in preserved foods) may become a practical method for pest control with great value in the future (Elordy, 2010).

## **MATERIALS AND METHODS**

### **1- Tested Insects and Grains:**

The individuals of Khapra beetle, *Trogoderma granarium* Everts, was obtained from laboratory strain maintained by Grains and Stored Product Pest Research Department, Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation in Dokki, Giza, Egypt. This strain was reared for several generations without any insecticide exposure before starting the present study.

Grains of wheat, *Triticum aestivum* L. cultivar Sakha 93 were used in this study. The used grains were sterilized by deep-freezing at -10 °C for two weeks at least before in the experiments.

## **2- Rearing Technique of Stock Cultures:**

Stock culture of *T. granarium* was reared on wheat grains in glass jars of 1 liter capacity (each contained suitable quantities of the prepared material). One hundred adults were introduced to each rearing jar for 24 hours to lay eggs, then the adults were removed. The jars were covered with muslin cloth, secured with rubber bands and kept under constant conditions in the incubator at 30 °C and 70% R.H. When the new adults emerged, they were collected and used to initiate new cultures. The rearing was continued for several generations before using insects in any experiment. To obtain large number of eggs of the same age after one day, all insects were removed from the media to collect the eggs. Pupae and adults of *T. granarium* were segregated by sex according to its size. (Khalifa, 2002).

## **3- Effect of Freezing and High Temperatures on Insect's Mortality:**

Three replicates were used for each treatment. Each contained one hundred of each the developmental stages (eggs, larvae and pupae) as well as adults of the tested insect species. They were exposed to two low temperatures of -10 °C and -15 °C in deep-freezers for 1, 3, 5 and 7 days after that, the treated grains were incubated at 30 °C and 70 % R.H. for 7 days of treatment to record all dead individuals.

On the other hand, three degrees of high temperature of 50, 60 and 70 °C were tested against the developmental stages (eggs, larvae and pupae) and adults of the tested insect. The periods of insect exposure to the tested high temperatures were 2, 4 and 6 hour. Three replicates were used for each treatment. Each contained one hundred of the same stage. The grains were inspected after treatment by 7 days to record mortalities.

## **4- Effect of Freezing and High Temperature on Wheat Grains:**

These tests were carried out in laboratory to estimate effect of grains exposure to high and low temperatures on germination ratio after 7 days from planting and its chlorophyll contents after 10 days from planting.

### **a- Germination test:**

Samples of 20 seeds ( in 3 replicates) were placed separately on a surface of a layer of cotton in Petri dish (6 x 1 cm). Cotton layers were wetted thoroughly with tap water every day, (Awadalla, 2006). Germination of seeds was determined after one week of plantation by counting the viable seeds and the germination percentages were calculated for each sample.

### **b- Chlorophyll content:**

Chlorophyll content of the seedlings leaves of grains of the same previous treatments was recorded after 10 days of plantation according to Witham *et al.* (1971).

### **5- Laser Radiation:**

Irradiation was carried out at Department of Environmental, Chemical and Agriculture Applications, National Institute of Laser Enhanced Sciences, Cairo University, Giza, Egypt.

#### **Type of laser and irradiation technique:**

Type of laser radiation used in this study was solid state diod pump (SSDP) laser with wave length of 532 nanometers. The laser beam coming from the used instrument was dropped vertically by hand on Petri dish containing either 0-1-day old eggs or pupae of insect.

The two tested stages were exposed to different energy doses of laser radiation to determined sub-lethal doses (LD<sub>30</sub> and LD<sub>50</sub>) and lethal dose (LD<sub>99</sub>) (Khalifa, 2002) as follows:

**Energy (joules) = power (milli watt) x time (second)**

**Where power density =  $P / A$  ,  $A = \pi r^2$**

**P = power of laser instrument.**

**A= area of radiation field (circle).**

**$\pi$  = constant = 3.14**

**r = radius of circle.**

Power of used SSDP instrument was 250 mw and times which applied in this study were 30, 45, 60 and 75 seconds for egg stage and 30, 45, 60, 75 and 90 seconds as a primary experiment to record the mortality rates. The resulting data were analyzed by computer program (probit program) to calculate the time required to the sub-lethal doses (LD<sub>30</sub> and LD<sub>50</sub>) and lethal dose (LD<sub>99</sub>).

Stock culture and treatments were kept at constant conditions in an incubator at 30 °C and 70% R.H. to determine effect of irradiation of eggs and pupae. Also, the newly emerged adults of Khapra beetle emerged from 0-1 day old pupae treated with different doses of laser were examined using a stereoscopic microscope for deformities.

### **6- Effect of Laser Rays on Wheat Grains Germination and Chlorophyll Content:**

Grains exposed to tested doses of laser were germinated as mentioned before. Chlorophyll content of irradiated germinated grains was estimated as mentioned before.

## 7- Statistical Analysis:

Data were statistically analyzed using the SAS program by Duncan grouping according to SAS Institute (1985). Probit analysis was used to calculate the times  $LT_{30}$ ,  $LT_{50}$  and  $LT_{99}$  to determine  $LD_{30}$ ,  $LD_{50}$  and  $LD_{99}$  of insect- tested laser rays.

## RESULTS AND DISCUSSION

### I- Effect of Freezing and High Temperatures on Insect Mortality:

#### a- Freezing:

Results presented in Table (1) show the effect of two freezing temperature (-10 and -15 °C) as a safe control method against different stages of *T. granarium*. Results, showed that, at -10 °C, the mortality of different developmental stages of *T. granarium* reached 100 % after 3 days for eggs and 7 days for the other stages, except with the pupae which reached 100 % mortality after 5 days. This indicated that eggs was the most susceptible stage followed by pupal stage then adults and larvae. But at -15 °C, the time required to attain a complete mortality was slightly decreased than those recorded -10 °C. Mortality percentages recorded 100 % after 3 and 5 days of exposure for egg and the other tested stages, respectively.

#### b- High temperatures:

Three degrees of high temperature (50, 60 and 70 °C) were tested as a safe control method against all different stages of *T. granarium*. The results obtained were tabulated in Table (2) and reveal that the mortality of different stages of *T. granarium* increased with increment of exposure period. Mortality reached 100 % at 50 and 60 °C after 6 hours of exposure for all tested stages of *T. granarium*. But, at 70 °C, all stages recorded 100% mortalities after 4 hours of exposure. High and low temperatures had an effect on increasing susceptibility of all life stages. For each treatment of high temperature, the increment of exposure period, the increment of mortality percentages of all stages, but for each stage of beetle, the increment of high temperature, the decrement of exposure periods. .

The use of high temperature is a well known technique to control stored product pests. For example, temperatures above 40 °C are lethal for most stored food pests (Sallam, 2007). Adult emergence of *Sitotroga cerealella*, *Sitophilus oryzae* and *Rhyzopertha dominica* can be totally suppressed after exposing their pupae to 45 °C for 72 hours. However, low

temperature treatment of grains may also provide a degree of control (Sharma *et al.*, 1997). However, in the application of high temperature treatments for disinfestation, in which acute thermal stresses are applied, heat shock protein may play a very important role in this respect. Research by Yocum and Denlinger (1992) showed that a mild heat treatment of 40°C for 2 h. to the flesh fly, *Sarcophaga crassipalpis*, conferred thermal tolerance to a subsequent normally lethal heat treatment of 90 min at 45°C. This thermal tolerance decayed over time, but lasted 72 h., beyond the time over which the originally induced were degraded (24 h.), this was an excellent example of how pre-conditioning of an insect can confer thermal tolerance to a subsequent higher thermal treatment, but this effect is not necessarily being related to heat shock proteins; other factors may participate in thermal tolerance. Cuticle is also sensitive to temperature changes. The wax layer of the cuticle is important in protecting insects from external environment and maintaining water balance. High temperatures can alter the wax complex to become more fluid and may lead to desiccation (Hepburn, 1985). Effects of high temperature on insect mortality in low humidity circumstances may be compounded with desiccation stress (Beament, 1959). However, a high temperature treatment in a highly saturated environment may lead to drowning, primarily due to the loss of cuticular protection of spiracles leading to tracheoles.

Table (1). Mortality percentages of *T. granarium* stages exposed to freezing for different periods.

Stage	Exposure period (day)	Mortality (%) at	
		-10 °C	-15 °C
Eggs	1	54	61
	3	100	100
New larvae	1	0	0
	3	36	70
	5	72	100
	7	100	-
Old larvae	1	0	0
	3	0	20
	5	40	100
	7	100	-
Pupae	1	0	0
	3	62	73
	5	100	100
Adults	1	12	0
	3	72	85
	5	86	100
	7	100	-

Table (2). Mortality percentages of *T. granarium* stages exposed to high temperatures for different periods.

Stage	Exposure period (hours)	Mortality (%) at		
		50 °C	60 °C	70 °C
	2	0	13	69
Eggs	4	61	77	100
	6	100	100	-
	2	0	12	86
New larvae	4	76	87	100
	6	100	100	-
	2	0	11	66
Old larvae	4	70	83	100
	6	100	100	-
	2	0	20	79
Pupae	4	88	91	100
	6	100	100	-
	2	18	25	79
Adults	4	79	88	100
	6	100	100	-

## II- Effect of Freezing and High Temperatures on Wheat Grains:

### a- Germination:

Effect of freezing grains at -10 and -15 °C for a week as well as exposure to 50 and 60 °C for 6 hours and 70 °C for 2 hours on germination of wheat grains is presented in Table (3). Data showed that the differences in % germination of wheat grains exposed to different low and high temperatures were statistically significant. Grains exposed to low temperature of -10 and -15 °C showed significantly high percentages of germination (81.7 and 70.7, respectively) which significantly varied with that of untreated grains of 91.7%. Also, the high temperatures of 50 and 60 °C gave significantly moderate % germination of 65 and 53.3, whereas exposure to 70 °C for 2 hrs nearly inhibited germination recording significantly the lowest percentage of germination of 1.7. Respecting abnormal germination (%) all treatments significantly recorded high abnormality germination. The highest percentage of abnormal germinated grains (23.3) was observed at 60 °C, which insignificantly differed with those of -10, -15 and 50 °C (11.7% abnormality for each). The lowest abnormal germinated grains of 6.7 % was insignificantly investigated in untreated grains and that exposed to 70 °C for 2 hours.

### b- Chlorophyll content:

Effect of freezing and high temperatures on chlorophyll contents given in Table (3). The obtained results showed that chlorophyll contents in case of freezing treatments (-10 and -15 °C) insignificantly were 17.1 and 16.5 mg/gm, respectively. While, those obtained with high temperature treatments of 50 and 60 °C significantly were 19.4 and 17.8 mg/gm, respectively. Chlorophyll contents of untreated planting grains (17.9 mg/gm) insignificantly varied with those obtained with freezing at -10 °C and heating to 60 °C, but it significantly differed with those of -15 °C (the lowest value) and 50 °C (the highest one). The results showed that the tested low temperature had low significant effect on seed germination, while the later significantly affected by the used high temperature which recorded the lowest percentage at 70 °C. This may be due to DNA is particularly sensitive to heat damage (Warner and Brizgys, 1987). Heat can alter the structure of proteins and nucleic acids by destabilizing weak interactions. Ribosomal RNA, being shorter in length, is more susceptible to changes in temperature than longer polymers. These lesions are easily repaired once the cells are returned to normal temperatures. However, if temperatures remain elevated, DNA repair enzymes might not be able or available to repair the damage.

The obtained results are in harmony with the findings of many investigators as Awadallah (2006) who evaluated efficacy of some safety control methods of low and high temperatures for stored products insects. On other side, germination of seeds was affected by high temperature and this may be due to that, temperature should not exceed 43 °C for cereal seeds and 35°C for legumes. Higher temperatures (up to 60 °C) can be used to dry cereals of consumption (Sallam, 2007).

Table (3). Effect of different low and high temperatures on germination and chlorophyll content of wheat grains.

Parameter	Freezing for a week at		Heating for			Control
			6 hrs		2 hrs	
	-10 °C	-15 °C	50 °C	60 °C	70 °C	
Germination (%)	81.7 b	70 c	65 c	53.3 d	1.7e	91.7 a
Abnormal germination (%)	11.7ab	11.7ab	11.7ab	23.3 a	6.7 b	6.7 b
Chlorophyll (mg/gm dry weight)	17.1 bc	16.5 c	19.4 a	17.8 b	–	17.9 b

Mean following by same letters in each row insignificantly differed



### **III- Laser Radiation:**

#### **a- Effect of different laser doses on 0-1 day old eggs:**

Effect of diod pump laser with wave length of 532 nanometer on 0-1 day old eggs of *T. granarium* with different doses of 23.85, 19.1, 14.31 and 9.54 J/cm<sup>2</sup> were assessed. Survival rate of eggs and some successive stages resulted from irradiated eggs are presented in Table (4). Results showed that hatchability of irradiated eggs decreased as the dose increased recording 0, 37.3, 63 and 82.6 % for the above mentioned compared to 88.3 % for untreated eggs, respectively. Pupation resulted from irradiated eggs occurred by 0, 35.6, 59.3 and 78 % compared to 87.3 % at doses of 23.85, 19.1, 14.31 and 9.54 J/cm<sup>2</sup> as well as that recorded with check, respectively. Adults emerged from irradiated eggs gradually increased with the decrease of doses to record (for the abovementioned doses) 33.3, 56.3 and 73.3 % compared to check of 85.6 %, respectively. Eggs of 0-1 day old exposed to laser irradiation produced malformed adults by 35.7, 18.9 and 7.7% of total emerged adults at 19.1, 14.31 and 9.54 J/cm<sup>2</sup>, respectively. While deformities occurred in emerged adults of untreated individuals recorded only 1.5%. It is obviously to observe the increment of irradiation dose, the increment of malformed adults. The calculated lethal times (LT) to cause 99, 50 and 30 % mortality were 112.03, 47.16 and 38.78 sec. which equivalent to 35.62, 15.00 and 12.3 J/cm<sup>2</sup> doses for 0-1 day old eggs of *T. granarium*, respectively.

#### **b- Effect of different doses of laser on 0-1 day old pupae:**

Results presented in Table (5) show effect of different lethal doses of diod laser radiation on adult emergence of, *T. granarium* irradiated as 0-1 day old pupae. Adult emergence of irradiated pupae were 0, 0, 36, 57.6 and 73 % for laser doses of 28.6, 23.85, 19.10, 14.31 and 9.54 J/cm<sup>2</sup>, respectively. The increment of irradiation dose, the decrement of emerged adults, where the percentages of treated adult emergence were littler than that of check individuals (98.3%). LT<sub>99</sub>, LT<sub>50</sub> and were 112.16, 43.75 and 35.39 sec. which equivalent to doses of 35.66, 13.91 and 11.25 J/cm<sup>2</sup>, respectively.

Table (4). Effect of different doses of diod laser radiation (532 nm) on certain biological aspects of *T. granarium* resulted from irradiated 0-1 day old eggs.

Energy	Egg	Pupation	Adult	
dose (J/cm <sup>2</sup> )	hatchability (%)	(%)	% emergence	% malformation
23.85	0	-	-	-
19.10	37.3	35.6	33.3	35.7
14.31	63	59.3	56.3	18.9
9.54	82.6	78	73.3	7.7
Control	88.3	87.3	85.6	1.5

Table (5). Effect of different doses of laser radiation (532 nm) on adult emergence of *T. granarium* as a result of 0-1 day old pupae irradiated.

Energy	Adult	
dose (J/cm <sup>2</sup> )	% emergence	% malformation
28.60	0	-
23.85	0	-
19.10	36	6.4
14.31	57.6	3.4
9.54	73	1.8
Control	98.3	0.7

### c- Malformation:

The malformation in adults of *T. granarium* resulted from pupae irradiated as 0-1 day old pupae increased gradually as the exposure times of laser radiation increased (table, 5). The obtained malformations were morphologically illustrated as shown in figs. (1-6).

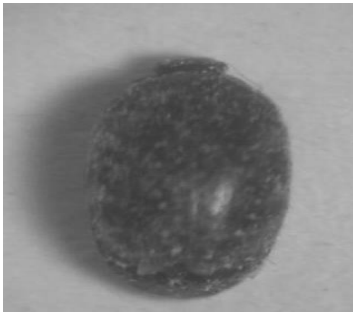


Fig. (1): Normal adult.

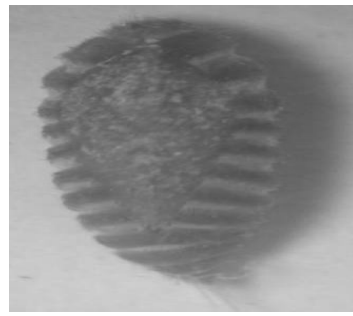


Fig. (2): Normal pupa.

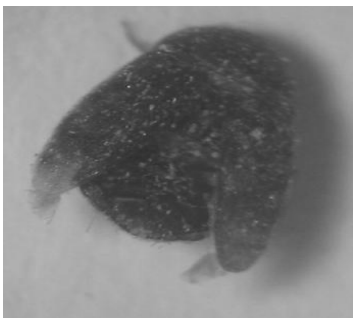


Fig. (3) Adult with crumpled wings.

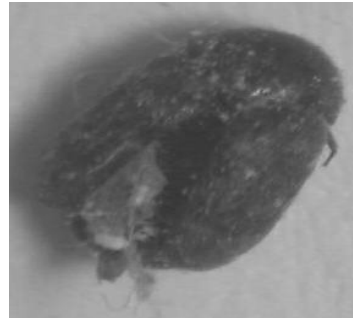


Fig. (4) Adult with deformities in abdomen.



Fig. (5): Adult partially emerged With head and thorax.

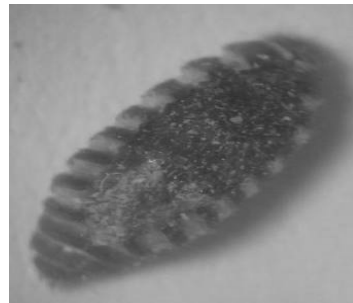


Fig. (6): Pupal - adults Intermediate.

In this respect, our findings agree with those obtained by many investigators who studied the effect of different sources of laser radiation on some insect species as Khalifa (2002) on both *T. granarium* and *T. castaneum* and Selman and Hasan (1995) on some *Tribolium* species. Based on LD<sub>99</sub> values of both insect species treated as eggs or pupae with laser radiation, it is clear from these values that *T. granarium* was less susceptible

than *T. castaneum* and in the same insect species the pupal stage was less susceptible than the egg stage by this diod laser radiation. The differential susceptibility between the two stages in the same species of insect to laser radiation may be due to the differences in body size (Khalifa, 2002).

On the other hand, diod laser (green light) similar to argon laser which possessed on the lowest absorption coefficients for water so, its beam is preferentially absorbed by tissue pigments such as chromophores melanin and other dark pigments, penetration into the living tissue is in the range 1 mm and is determined by the degree of competition of the dark pigments. Light absorption is converted to heat which vaporizes the pigment (Abd El-Sadek, 1999).

#### **VI-Effect of Different Doses of Laser on Wheat Grains:**

##### **a- Germination:**

Data compiled in table (6) showed effect of different doses of diod laser radiation on wheat grains germination. Germination affected by time of exposure where germination percentages decreased as the time increased recording 78.3, 73.3, 45 and 45 % at doses 11.25, 12.3, 35.62, 35.66 J/ cm<sup>2</sup> (compared to control of 93.3 %), respectively. Whereas abnormal germination percentages increased as exposure time decreased to record 15, 13.3, 5.3 and 3.7 % at previously mentioned doses compared to control of 5 %, respectively. Statistical analysis of variance proved that the differences in both normal and abnormal germination percentages were significant.

##### **b- Chlorophyll content:**

Results in Table (6) showed that there was insignificantly low effect of laser doses on chlorophyll content of wheat grains that ranged between 17.5 – 17.7 mg/gm for the treated grains compared to 17.9 mg/gm for untreated ones.

The obtained results clearly indicated that of LD<sub>30</sub> of diod laser had low effect with significant decrement on germination percentage of wheat grains. These findings disagree with the observation of Savel'ev (1981) who found that the treatment of spring wheat grains with laser radiation increased the germination by (10-15) %. Also, Kasperovich *et al.* (1984) irradiated wheat seeds with laser rays and stated that Laser irradiation increased germination percentage, respiration rate and catalase activity in seeds. The same findings were reported by Khalifa (2002) who found that irradiation of grains with argon laser had no effect on the germination.

On the other hand, these results revealed that irradiation of wheat grains by LD<sub>30</sub> and LD<sub>99</sub> of diod laser radiation had no pronounced effect on chlorophyll content, these findings are in harmony with Jiang (1981) irradiated wheat grains by 5w/cm<sup>2</sup> per min laser; showed that no adverse effect on the germination of the wheat. The treated seeds were not damaged and retained their normal colour through after three exposures; moisture content had decreased by 1.7%. Khalifa (2002) who found that there are no effect on nutritive values (chemical constituents) of wheat grain and food products when irradiated by argon or visible spectrum.

Table (6). Effect of different doses of diod laser radiation (532 nm) caused 30% and 90% mortality of both eggs and pupae on germination and chlorophyll content of wheat grains.

Energy (J/ cm <sup>2</sup> )	LC 30		LC 90		Control
	11.25	12.33	35.62	35.66	
Parameter					
Germination (%)	78.3 b	73.3 b	45 c	45 c	93.3 a
Abnormal germination (%)	15 a	13.3 a	5.3 b	3.7 c	5 b
Chlorophyll (mg/gm dry weight)	17.5	17.7	17.6	17.7	17.9

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## تأثير التجميد، الحرارة المرتفعة واشعة الليزر فى مكافحة خنفساء الصعيد "الخابرا" التي تصيب حبوب القمح

نيللى أحمد حسن عبد الفتاح<sup>١</sup> ، زغلول عبد الفتاح حلاوة<sup>١</sup> ، أسماء نوز الهمة الشرقاوى<sup>٢</sup> ،  
عفاف عبد الوهاب عباس<sup>٢</sup> ، هشام إمام محمود<sup>٣</sup>

١- معهد بحوث وقاية النباتات - مركز البحوث الزراعية

٢- كلية العلوم فرع النبات - جامعة الأزهر

٣- معهد الليزر - جامعة القاهرة

أجرى هذا البحث بغرض تقييم بعض العوامل البيئية الأمانة مثل درجة الحرارة  
و أشعة الليزر (ضوء) على مكافحة الحشرة واختبار جودة الحبوب بعد إجراء هذه  
المعاملات عليها.

وتم اختبار حبوب القمح سخا ٩٣ لإجراء هذه المعاملات وذلك لأهميتها  
الاقتصادية.

وتم دراسة تأثير المعاملات على جميع الأطوار الحشرية لنوع من أهم أفات الحبوب  
والمواد المخزونة وهى: خنفساء الصعيد (الخابرا) *Trogoderma granarium* وهى أفة  
أولية.

وكانت النتائج المتحصل عليها كالتالى:-

أ- مكافحة الحشرة عن طريق الحرارة:

المكافحة الأمانة لافات الحبوب المخزونة عن طريق استخدام:

أ- التجميد (١٥- و -١٠م)

ب- الحرارة العالية (٥٠ ، ٦٠ و ٧٠م) وقد أختبرت هذه الدرجات على جميع أطوار  
الحشرة.

أظهرت النتائج أن نسبة الموت وصلت الى ١٠٠% فى مدة لا تزيد عن ٧ أيام  
بواسطة مكافحة عن طريق التجميد لجميع المراحل المختلفة وكذلك وصلت نسبة الموت  
الى ١٠٠% فى مدة لا تزيد عن ٦ ساعات عن طريق الحرارة العالية لجميع المراحل  
المختلفة.

نسبة الإنبات وتقدير نسبة الكلوروفيل:

أوضحت البيانات أن مكافحة بالحرارة العالية والمنخفضة تعتبر من أكثر  
الطرق الأمانة لمكافحة الحشرات إضافة الى الإحتفاظ بجودة الحبوب حيث أن اختبار  
الانبات للحبوب بعد معاملتها بدرجات حرارة منخفضة أو حتى عالية والتي لا تزيد عن  
٦٠ م° فانها لا تؤثر بطريقة كبيرة على جودة الحبوب من حيث الانبات بينما على  
درجة ٧٠ م° فان لها تأثير مدمر على إنبات الحبوب.

كما دلت النتائج على أن المحتوى الكلوروفيللى للحبوب المعاملة بدرجات الحرارة المنخفضة أقل من المعاملة بدرجات الحرارة العالية.

#### ب-تأثير أشعة الليزر:

تم دراسة استخدام أشعة الليزر بجرعات مختلفة على كل من طور البيض عمر صفر الى ١ يوم وطور العذراء عمر صفر الى ١ يوم وكذلك على حبوب القمح حيث تم تعيين الجرعة المميتة والتي تسبب ٩٩ % موت وأيضا تم تعيين الجرعة النصف مميتة التي تسبب ٥٠ % موت والجرعة تحت مميتة والتي تسبب ٣٠ % موت. تم تسجيل وتصوير بعض التشوهات الناتجة من تعريض العذارى لأشعة الليزر.

أ- حشرة بالغة ذات اجنحة مجعدة (غير مفروده).

ب - حشرة بالغة مع وجود تشوهات فى الاجنحة والصدر والبطن.

ج - جزء من الحشرة البالغة خارج من غلاف العذراء.

د- مرحلة وسطية بين العذراء والحشرة الكاملة.

#### نسبة الإنبات و تقدير نسبة الكلوروفيل:

كلما زادت جرعة أشعة الليزر تتخفف نسبة الانبات فى الحبوب المعرضة كما دلت النتائج على أن المحتوى الكلوروفيللى للحبوب المعاملة بأشعة الليزر لا تختلف معنويا باختلاف جرعات الليزر.