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EFFICACY OF MICROWAVE ENERGY ON: 1- COWPEA WEEVIL, *Callosobruchus maculatus* (F), SOME OF CHEMICAL CONTENTS AND VIABILITY FOR FABA BEAN SEEDS

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ABSTRACT

This study conducted to evaluate the efficacy of microwave energy on all stages of cowpea weevil, *Callosobruchus maculatus* (F.) reared on faba bean seeds exposed to microwave at the lowest energy (17% of 800W.) for the 2,3,4,5, and 6 minutes. Exposure of adults to microwave energy for 5 minutes caused 90% mortality and complete reduction of progeny. When the eggs laid on faba bean seeds were exposed to microwave energy for 5 minutes, the hatchability was 1.9% compared with control (77.7 %). When the larvae inside the seeds were exposed to microwave energy for 5 minutes, complete reduction of progeny was found. Microwave did not affect on pupa stage at all periods of exposure. There was no significant difference in the chemical composition, crude protein, oil and total carbohydrate contents between treated and untreated faba bean seeds to microwave energy. Meantime, results of SDS-PAGE in soluble seed protein revealed a total number of 22 bands (6 common bands were monomorphic while the other were polymorphic). The maximum number of bands (14) appeared with 5 minutes of exposure time but when exposure time decreased to 3 and 4 minutes, it showed the minimum number of bands (10). There was no significant differences in viability and seedling vigor traits of untreated or treated faba bean seeds with microwave energy for 2,3,4, and 5 minutes. Highly significant reduction in the viability and seedling vigor were recorded with exposure time of 6 minutes. Highly significant loss of 100-seed weight was recorded after treatment with microwave energy for 6 minutes compared with other treated and untreated seeds.

INTRODUCTION

In Egypt, faba bean (*Vicia faba* L.) is the most seed legume crop used for human consumption as well as for animal feeding (El-Galfy and Eman A., Mohamed, 2004). The annual losses of cereal grains due to insects and rodents account for about 10% in North America and 30% in Africa and Asia, but higher losses of contamination often occur locally (Hill, 1990). Insect infestation leads to a series of problems such as weight loss, reduced quality and even food safety issues. Chemical fumigation, for example, using methyl bromide and phosphine is commonly applied throughout the world as an effective method of controlling infestations by storehouse pests. However, this method has led to serious environmental damage and hazards to people's health (Zhao *et al*., 2007). Conventional use of phosphine fails to

control insects and certain insects resistant to phosphine (Bell and Wilson., 1995). Microwaves and radiofrequency energy can be used to control insects in stored cereals and cereal products. Microwave heating is based on the transformation of alternating electromagnetic field energy into thermal energy by affecting polar molecules of a material. The most important characteristic of microwave heating is that materials absorb microwave energy directly and internally and convert it into heat (Mullin, 1995). Microwave treatment is considered a safe and competitive alternative method to fumigation, and can avoid problems of food safety and environmental pollution (Zhao *et al*., 2007). Use of microwaves to kill insects in stored grain could be an alternative for chemical methods (Vadivambal *et al*., 2010). High-frequency radiation may not only kill insects by the dielectric heat induced within them but may also affect the reproduction of survivors (Hamid *et al*., 1968). The major advantage of using microwave energy is that no chemical residues are left in the food and hence there are no adverse effects on human beings (Hurlock *et al.,* 1979). Meantime, it has no adverse effect on the environment and the insects are unlikely to develop resistance to this treatment (Watters, 1976). Although microwave treatment causes less thermal damage to the test material than general heating methods (Wang *et al.,* 2003), it causes biochemical reactions (Banik *et al.*, 2003) and changes the molecular conformation of starch (Lewandowicz *et al*.,2000) and protein (Gropper *et al*., 1997).

Therefore the present study was carried out to evaluate the efficiency of microwave energy treatments on cowpea weevil, *Callosobruchus maculatus* (F.) and their impact on quality of faba bean seeds.

MATERIALS AND METHODS

Microwaves :

A microwave oven, EM-280 M, Electra, Japan, capacity 28 L and cavity dimensions 21.9 × 35 × 35 was used .The oven was operated at the low energy level i.e. 17% of power out put (output: 800 W). The operating frequency of the oven was 2450 MHz.

Preparation of seeds:

Faba bean *Vicia faba* (L.) var, Giza 716, one month after 2009/10 harvesting season was used. Grains were sterilized by freezing at -18°C for one week to kill any prior insect infestation, then stored in sealed polyethylene bags at 5°C until require for experiments. The moisture contents of these grains were measured by oven-drying duplicate samples each of 5 g at 130°C for 1 hour, then calculated from the following formula:

$$\text{\% Moisture content} = \frac{\text{(Initial grain wt – Final grain wt)}}{\text{Initial grain wt}} \times 100$$

The grains were stored in sealed polyethylene bags in a refrigerator at 5°C till required for experiments.

Insect tested :

Cowpea weevil, *Callosobruchus maculatus* (F.) (Fam: Bruchidae) :

The original strain of tested insect was obtained from the Department of Stored-Product Pests, Plant protection Research Institute, Agricultural Research Center, Dokki, Egypt. Insect species tested and their life cycles were identified according to Badawy and Doraeham, (1991)

Insects were maintained in small glass jars containing 150-200 unsexed adults and approximately 200 g of cowpea seeds each. Jars were covered with muslin and kept in position with rubber bands. The cowpea seeds were used for insect culture. After 7 days the parent adults were removed. Newly emerged adults (0-24 hrs old) were used in the experiments. In order to obtain the adults, the culture medium was sieved to separate the emerged insects and collected for experiments.

Methods :

Ten pairs of adults of *C. maculatus* were confined to 9 cm-diameter petri-dish containing 10g of faba bean seeds. Three replicates were assigned for each treatment.

Adult treatment:

For adult treatment, newly ten pairs of emerged adults (0-24 hrs. old) were confined into 9 cm-diameter petri-dish containing 10g faba bean seeds, and placed inside the oven. The oven was operated at the low energy level i.e. 17% of power out put (output: 800 W). The operating frequency of the oven was 2450 MHz. Exposure periods were 2, 3, 4, 5 and 6 minutes. For each period, the temperature inside the oven was recorded by inserting the bulb of a thermometer inside the seeds. At the end of each specified period, mortality counts were recorded. Similar previous techniques were carried out without exposure to microwave for control treatment.

Egg treatment:

For egg treatment, ten newly pairs of emerged adults (0-24 hrs. old) were confined to 9 cm-diameter petri-dish containing 10g faba bean seeds. The insects were left to lay the eggs for 72 hrs. and were removed. The eggs laid on the seeds were exposed to microwave for 2, 3, 4, 5 and 6 minutes. After exposure, dishes were placed inside the incubator under the laboratory conditions of 26 ± 1°C, 65 ± 5% R.H. until hatching and adult emergence. Similar previous techniques were carried out without exposure to microwave for control treatment. The hatchability were counted as following formula :

$$\%\text{Hatchability} = \frac{\text{No. of egg hatched}}{\text{No. of total egg laid}} \times 100$$

and the reduction of progeny was estimated as following formula :

$$\%\text{Reduction} = \frac{\text{No. of progeny of control - No. of progeny of treatment}}{\text{No. of progeny of control}} \times 100$$

Larvae treatment :

As previous treatment, the eggs laid on the seeds were placed inside the incubator under the laboratory conditions until hatching. After hatch, the larvae inside the seeds were exposed to microwave for 2, 3, 4, 5 and 6 minutes. After exposure, dishes were placed inside the incubator under the laboratory conditions of 26 ± 1°C, 65 ± 5% R.H. until adult emergence. Similar previous method was carried out without exposure to microwave for control treatment. The reduction of progeny from larvae treatment was calculated as following formula:

$$\% \text{ Reduction} = \frac{\text{No. of progeny of control - No. of progeny of treatment}}{\text{No. of progeny of control}} \times 100$$

Pupa treatment:

As previous methods, the eggs laid on the seeds were placed inside the incubator under the laboratory conditions and were left to reach to pupa stage, then placed inside the microwave oven to exposure for previous periods. After exposure, dishes were placed inside the incubator under the laboratory conditions of 26 ± 1°C, 65 ± 5% R.H. until adult emergence. Similar previous method was carried out without exposure to microwave for control treatment. The reduction of progeny from pupa treatment was counted as following formula:

$$\% \text{ Reduction} = \frac{\text{No. of progeny of control - No. of progeny of treatment}}{\text{No. of progeny of control}} \times 100$$

Seeds quality:

100g of faba bean seeds were exposed to microwave without insects for previous periods (2, 3, 4, 5 and 6 minutes) to use for determination of :

Chemical analysis:

Percentage of oil, crude protein and total carbohydrates, were determined where tested seeds were ground to fine powder to pass through 2 mm mesh and determined according to the procedures outlined in AOAC, (2000).

Viability traits:

Percentage of germination, seedling vigor and the electrical conductivity (EC) of leached from four replicates of 50 seeds weighed and soaked in 250 ml of distilled water for 24 hours was measured in u-mhos/gm seed using a conductivity meter, were carried out under optimum conditions according to the international rules (ISTA, 1993). At the final count, ten of normal seedlings from each replicate were taken randomly to measure the shoot and radical length in (cm). After that, the seedlings were dried in a hot air oven at 85°C for 12 hours to obtain the seedling dry weight which was determined according to the procedures reported in the seed vigor testing handbook (AOSA,1991).

SDS-Protein electrophoresis:

Sodium dodecy sulfate - polyacylamide gel electrophoresis (SDS-PAGE) was used to study the banding patterns of varieties under study according to the method of Laemmli, (1970) as modified by Studier, (1973).

Statistical analysis:

Statistical analysis of data was carried out according to Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Insecticidal activity of microwave energy on cowpea weevil, *Callosobruchus maculatus* (F):

Adult treatment:

When the adults of *C. maculatus* were exposed to microwave, results recorded in Table (1) revealed that the short periods (2 and 3 minutes) gave 22 and 60% mortality, respectively, while the long periods (5 and 6 minutes) gave 90 and 100% mortality, respectively. The reduction in progeny was 55.3% at 2 minutes period, but reached 100 % at 5 minutes (Table1).

Table (1) : Effect of microwave on adults of *C. maculatus* on faba bean seeds

Exposure time(minute)	% Mortality	% Reduction of progeny
control	0.0 f	0.0 e
2	22.0 e	55.3 d
3	60.0 d	86.0 c
4	70.0 c	91.1 b
5	90.0b	100.0 a
6	100.0 a	100.0 a

In a column ,means followed by the same letter are not significant at the 5% level.

Egg treatment:

Results obtained in Table (2) showed that there was a gradual reduction in hatchability with increasing the exposure periods. When the eggs were exposed for 2, 3 minutes, the hatchabilities were 35.2% and 22.3%, respectively. The long periods (5 and 6 minutes) gave 1.9**%** and 0 % hatchability, respectively (Table2).The reduction of progeny was 69.4**%** and 90.0**%** at periods, of 2 and 3 minutes, but gave complete reduction at periods, of 5 and 6 minutes, respectively.

Larvae treatment:

When the larvae exposed to microwave, results recorded in (Table 3) showed that the exposure for 2 and 3 minutes gave 67.5 and 86.1% reduction of progeny, respectively, but gave complete reduction at 5 and 6 minutes (Table 3).

Pupae treatment:

Results obtained in (Table 3) showed no effect of microwave on pupa stage.

Table (2): Effect of microwave on eggs of *C. maculatus* on faba bean seeds

Exposure time(minute)	% Hatchability	% Reduction of progeny
control	77.7 a	0.0 e
2	35.2 b	69.4 d
3	22.3 c	90.0 c
4	14.6 d	94.6 b
5	1.9 e	100.0 a
6	0.0 f	100.0 a

In a column, means followed by the same letter are not significant at the 5% level.

Table (3): Effect of microwave on larvae and pupae of *C. maculatus* inside faba bean seeds.

Exposure time(minute)	% Reduction of progeny of larvae	% Reduction of progeny of pupae
control	0.0 f	0.0 a
2	67.5 d	0.0 a
3	86.1 c	0.0 a
4	92.7 b	0.0 a
5	100.0 a	0.0 a
6	100.0 a	0.0 a

In a column, means followed by the same letter are not significant at the 5% level.

These results are in agreement with those of Vadivambal, *et al*, (2010) when they use microwave energy at power level of 300, 400, 500 and 600 W to kill larval and adult stages of three stored grain insects, *Sitophilus zeamais, Tribolium castaneum* and *Plodia interpunctella* in corn. They obtained complete mortality of *S. zeamais* and *T. castaneum* larvae and adults at 600 W for 14 seconds or at 500 W for 28 s. For *P. interpunctella* larvae and adults, one hundred percent mortality was obtained at 500 W for 14 s or at 400 W for 28 s. Also, the results obtained by Vadivambal, *et al*., (2007) showed that the mortality of *T. castaneum* was 45% by use microwave energy at power level of 250W and an exposure time of 28 s, and the mortality increased to 58%, 85%, and 100% at power levels of 300, 400, and 500 W, respectively at exposure time of 28 s. when the exposure time was increased to 56 s, 100% mortality was achieved at a power of 400 W. (Bhalla *et al*., 2008) revealed that the exposure of cowpea and green gram seeds infested with different stages of *C. maculatus* to microwaves generated at frequency 2450 MHz for 70 seconds was effective against all the stages of pest.

Seeds quality:

Chemical analysis:

Chemical compositions of tested faba bean seed as affected by microwave energy treatment are shown in Table (4). Analysis of variance was performed between the control sample and treated samples for crude protein, oil and total carbohydrate contents, showed no significant differences in the chemical composition of treated samples and control sample. Walde *et al*., (2002) studied the microwave drying characteristics of wheat and reported that there was no change in the total protein content of microwave treated

wheat samples but the structural and functional characteristics of wheat protein - gluten were changed. Also, Vadivambal, *et al*. (2007) concluded that there was no significant difference in the quality on grain protein of the wheat subjected to microwave energy.

Table (4): Percentages of chemical composition of tested faba bean seed on dry matter basis, as affected by microwave energy treatments.

Item (%)	Control	Time of treatments (minute)				
		2	3	4	5	6
Crude protein	25.99 a	25.93 a	25.64 a	25.09 a	26.10 a	25.74 a
Oil	02.59 a	02.17 a	02.45 a	02.03 a	02.40 a	02.47 a
Total carbohydrate	64.88 a	63.71 a	63.20 a	63.17 a	64.19 a	62.76 a

In a row, means followed by the same letter are not significant at the 5% level.

SDS-Protein electrophoresis:

The SDS-PAGE for water soluble seed protein was used to investigate the genetic differences among the faba bean seed treated with microwave energy. Table(5) and Fig.(1) show the electrophoresis banding patterns of protein extracted from the faba bean seed treated. The band pattern shows wide differences among the tested seeds in number and position of the bands.

Table (5): Densitometry analysis for SDS seed protein (water soluble fraction) of the faba bean treated by different time of microwave.

series	M.W	Control	2min.	3min.	4min.	5min.	6min.
1	287.576	-	-	-	-	-	-
2	215.758	+	+	+	+	+	+
3	146.868	-	-	-	-	+	-
4	129.334	+	-	-	-	-	-
5	117.158	-	-	-	-	-	+
6	114.562	-	+	+	+	+	+
7	109.441	-	-	-	-	+	+
8	104.252	+	+	+	+	-	-
9	98.558	-	-	-	-	-	+
10	91.150	-	+	-	-	-	+
11	87.477	+	+	+	+	+	+
12	86.296	+	+	+	+	+	-
13	85.496	-	+	+	-	+	-
14	84.856	-	+	-	+	+	+
15	83.832	+	+	+	+	+	+
16	80.764	+	+	+	+	+	+
17	49.527	-	-	-	-	-	-
18	48.702	+	+	+	+	+	+
19	30.162	+	-	-	-	+	-
20	22.140	+	+	-	-	+	-
21	14.614	+	+	+	-	+	-
22	11.132	+	-	-	+	-	-
Total of bands		12	13	10	10	14	12

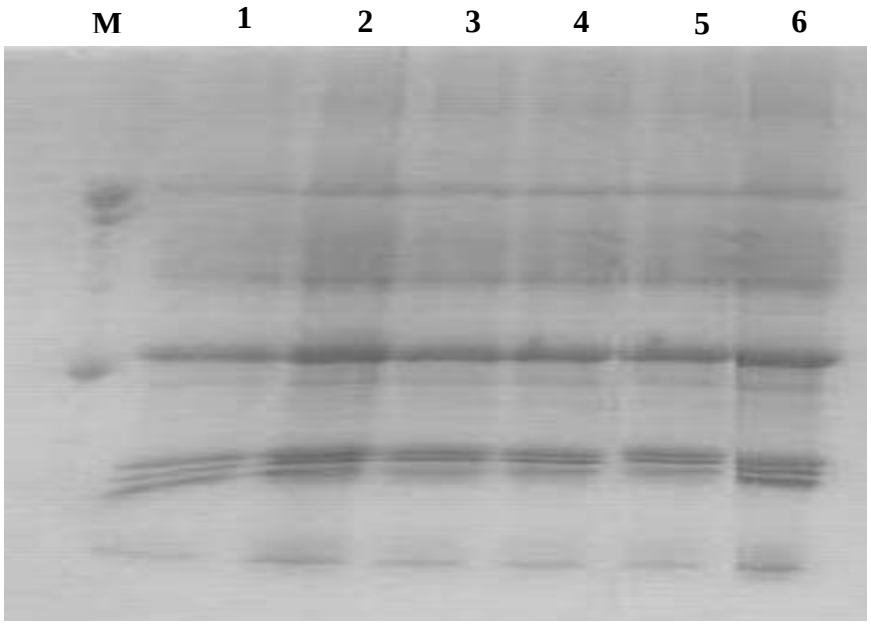

Fig (1): SDS – PAGE protein banding patterns of tested faba bean Seeds as affected by microwave treatment.

Where: M = Standard marker, 1 = Control, 2 = treated by 2 min., 3 = treated by 3 min., 4 = treated by 4 min., 5 = treated by 5 min., 6 = treated by 6 min.

The presence and absence of bands were assessed (+) and (-), respectively. The results of SDS-PAGE revealed a total number of 22 bands in faba bean seed with molecular weights (MW) ranging from 287.576 to 11.132 KDa, which were not necessary to be presented in all treatments. Data showed six common bands (monomorphic) in faba bean seeds, while the other bands were polymorphic.

The maximum number of bands (14) appeared in the treatment of 5 min. by microwave, and the minimum number of bands (10) appeared in the treatment of 3 and 4 min.

The above results indicated that SDS-PAGE of seed protein used in this study made clear identification of the treated faba bean seeds. These results are in agreement with Abd-el-Tawab *et al.*, (1993) who confirmed that SDS-PAGE was a highly successful technique in cultivar identification.

Viability traits:

The effect of microwave energy treatment on seed viability and seedling vigor of tested faba bean are shown in Table (6). Results showed that after 6 minutes of microwave treatments, the germination percentage was 79.0%, which is less than the recommended level of germination (85%) of faba bean seeds by Ministry of Agricultural.

Meantime, tested seeds treated for 5 minutes recorded the highest germination percentage (99.0%) compared with 97.0% for untreated seeds.

The data in this table cleared that the percentage of germination of faba bean seed exposed for 2, 3, 4, 5 minutes and untreated seeds were moderated from 90.0 to 99.0% which is higher than the level of germination determined by Egypt's Ministry of Agricultural (85%). The decrease in germination at 6 minutes of exposure time (79.0%) was due to the increase in temperature of the samples as the result of the increasing exposure time. Vadivambal, *et al*., (2007) cleared that high temperature affects the germination capacity of the seeds. Campana, *et al*., (1993) studied the physical, chemical and baking properties of wheat dried with microwave energy, and concluded that germination capacity was neggatvely affected by exposure to microwave energy. The decrease in germination capacity was related to the final temperature and the initial moisture content of the grains.

Table(6): Percentages of viability and seedling vigor of tested faba bean seeds, as affected by microwave energy treatments.

Item	Control	Time of treatments (minute)				
		2	3	4	5	6
Germination (%)	97.00 a	91.00 b	90.00 b	93.00 b	99.00 a	79.00 c
Shoot length (cm)	25.90 b	25.00 b	23.90 c	24.20 c	27.80 a	18.80 d
Radical length (cm)	09.60 b	10.20 b	10.60 ab	09.90 b	11.70 a	08.80 c
Seedling length (cm)	35.50 b	35.20 b	34.50 b	34.10 b	39.50 a	27.60 c
Seedling growth rate	02.54 b	02.51 b	02.46 b	02.44 b	02.82 a	01.97 c
Seedling vigor index	34.44 b	31.68 c	31.50 c	31.71 c	39.11 a	21.80 d
Seedling dry weight (mg)	293.0 a	296.0 a	283.0 b	284.0 b	301.0 a	264.0 c
E.C (u-mhos, 1 gm seed)	27.80 b	27.50 b	28.00 b	28.80 b	24.70 c	38.40 a
100-sed weight (gm)	92.30 a	29.60 a	92.50 a	92.70 a	92.80 a	90.80 b

In a row, means followed by the same letter are not significant at the 5% level.
E.C = Electrical conductivity (u-mhos/gm seed).

The highest significant values of seed parameters (shoot length, radical length, seedling growth rate, seedling vigor index and seedling dry weight) except electrical conductivity were obtained after microwave treatments for 5 minutes as shown in Table (6), while the previous parameters of seed viability and seedling vigor showed opposite trend (decreased) after 6 minutes of microwave treatments and recorded the lowest significant values.

The highly significant reduction of viability and seedling vigor traits of tested faba bean seed exposed to microwave energy for 6 minutes were due to the increase in temperature of the tested seeds as the result of increasing exposure time. Bhaskara *et al*., (1998) showed that eradication of the pathogen increased with the total microwave energy, but the seed viability and seedling vigor decreased accordingly.

The data in Table (6) showed that, after 6 minutes of microwave treatments a highly significant reduction ($P<0.05$) in the weight of 100-seed weight occurred and recorded the lowest weight (90.8 g /100-seed) compared with other treatments. This reduction of tested 100 faba bean seeds may be attributed to the great losses of moisture from the seed as a result of the great heat produced after 6 minutes of microwave treatments compared with other exposure time.

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تأثير طاقة الميكروويف على :

1- حشرة خنفساء اللوبيا وبعض التركيب الكيماوي وحيوية بذور الفول

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***قسم بحوث تكنولوجيا البذور- معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية**

****قسم بحوث آفات المواد المخزونة – معهد بحوث وقاية النباتات – الدقي – مصر**

أجري البحث لدراسة تأثير موجات الميكروويف على حشرة خنفساء اللوبيا في جميع أطوارها من حشرة كاملة وبيض ويرقات وعذارى والتي ربيت على بذور الفول البلدي وكذا تأثير هذه الموجات على بعض الصفات التكنولوجية مثل التغير في التركيب الكيماوي للبذور والتأثير على الإنبات.

تم تعريض جميع أطوار الحشرة والمرباة على بذور الفول البلدي إلى موجات الميكروويف عند أقل مستوى من الطاقة (17% من 800 وات) لمدد 2،3،4،5،6 دقائق وتم تسجيل نسبة موت للحشرة الكاملة ونسبة فقس البيض وكذا نسبة الخفض في النسل الناتج.

أظهرت النتائج أن التعريض لموجات الميكروويف لمدد 5،6 دقائق كان أكثر تأثيرا في خفض النسل من التعريض لمدد 2،3 دقائق ويمكن تلخيص النتائج كالآتي : -

- تعريض الحشرات الكاملة لمدة 5 دقائق أدى إلى موت 90% منها وأدى إلى خفض كامل في تعداد النسل الناتج .

- أدى تعريض بيض الحشرات لمدة 5 دقائق إلى خفض نسبة الفقس إلى 1.9% مقارنة بالكنترول (77%) عند معاملة البيض الموضوع على بذور الفول البلدي, كما أدى إلى الخفض الكامل في تعداد النسل الناتج من معاملة هذا البيض.
- عند تعريض اليرقات التي تعيش داخل بذور الفول البلدي لمدد 3،2 دقائق فإن أعداد النسل الناتج منها انخفضت بنسبة 67.5 و 86.1 % على التوالي بينما انخفضت أعداد النسل انخفاضاً كاملاً عند تعريض اليرقات لمدة 5 دقائق.
- وعند تعريض عذارى الحشرات لم تتأثر أعداد النسل الخارجة منها عند أي مدة من مدد التعريض المذكورة.
- لا توجد فروق معنوية في التركيب الكيماوي (% البروتين والزيت والكربوهيدرات الكلية) بين بذور الفول التي تعرضت لمدد من 2 إلى 6 دقائق لموجات الميكروويف والتي لم تتعرض.
- باستخدام التفريد الكهربائي(الإلكتروفيريزس) حدث تنشيط للبروتينات الذائبة وأمكن تسجيل عدد 22 رابطة (وزن جزيئي) نتيجة تعرض بذور الفول للمعاملة بالميكروويف في المدد من 2 : 6 دقائق.
- تم تسجيل عدد 6 روابط متشابهة بين المعاملات و 14 رابطة مختلفة.
- سجلت المعاملة عند مدة الخمس دقائق أعل تنشيط للبروتين الذائب وسجلت عدد 14 رابطة (وزن جزيئي) وسجلت النتائج أقل عدد (10 رابطة) عند مدد 3 و4 دقائق.
- سجلت البذور التي تعرضت لموجات الميكروويف عند خمس دقائق أعلى نسبة إنبات وأعلى قيم لقوة البادرات بينما سجلت البذور عند 6 دقائق أقل القيم في الحيوية.
- انخفض معنويا وزن الـ 100 بذرة نتيجة التعرض لموجات الميكروويف عند 6 دقائق مقارنة بالمعاملات الأخرى.

قام بتحكيم البحث

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