



EFFECT OF GERMINATION AND IRRADIATION TREATMENTS ON QUALITY AND STORABILITY OF CLOVER SPROUT

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ABSTRACT

Sprouts are most often consumed raw; thus cooking is not used to prevent contamination from pathogens. High microbial counts on clover seeds and its sprouts are the primary factor for a short shelf life of products and potentially present pathogens may cause human illness outbreak.

In this study, clover seeds were germinated with four treatments. T1 (Dipping in 20g/L Calcium hypochlorite for 20 min. +Washing with NaCl then, soaking in 2000 ppm NaCl, 12 hr. / 3 days), T2 (Dipping in Calcium hypochlorite for 20 min. then, washing tap water, 12 hr. / 3 days), T3 (Dipping in tap water + Washing by NaCl, 12 hr. / 3 days) and T4 (Dipping in tap water + washing by tap water, 12 hr. / 3 days).

Trail 2, treated clover seeds in T1 and T4 were low and highly contaminated with microbial load. Clover sprouts were exposed to irradiation doses at 1, 2 and 3 kGy, to study the effect of gamma radiation on quality. Main trail, T4 was modified to dipping seeds in sterilized tap water and washing by sterilized tap water, for 12 hr. /3 days. Sprouts were exposed to irradiation doses and stored at 9°C and quality parameters were evaluated during storage.

Clover sprouts can be treated with gamma radiation, a no thermal food process, to reduce microbial load and forborne pathogens and to increase shelf life. After irradiation at dose of 2 kGy, the total bacterial count decreased from 5.0×10^7 to 6.0×10^3 cfu/g, and the total coliform counts decreased from >1100 to <3 cfu/g. *E. coli* count from 2.9×10^2 to <3 and *Staphylococcus aureus* count went down from 4.5×10^4 to <100 cfu/g.

These results showed clover seeds germinated in sterilized tap water and irradiated at 2 kGy improved microbial safety of clover sprouts without affecting germination, chemical and quality during storage was extended to 15 day. Irradiated sprouts had similar overall acceptability quality as the non-irradiated one.

Keywords: Clover sprouts, Germination, Gamma radiation, Quality, Storability

INTRODUCTION

Sprouts are a rich source of nutrients and beneficial compounds and are often consumed raw, thus since cooking is not used, certain methods must be used to prevent contamination of pathogens. High microbial counts on clover seeds and its sprouts are the main reason of a low shelf life of products and potentially present pathogens may cause human disease outbreak.

Alfalfa (*Medicago sativa*) sprouts are commonly consumed as valuable dietary supplement. It's considered a natural healthy food by consumers in many parts of the world (Donaldson, 2004 & Bari et al 2011), which make them a high-risk commodity (Bari et al 2011 & Scallan et al 2011). Although many sources can contaminate alfalfa sprouts, contaminated seeds are recognized to be the main reason of pathogens in sprouts. Data from the Center for disease control and prevention (CDC) shows that there were 11 sprout related foodborne illness outbreaks between 2009 and 2016. The varieties of sprouts implicated in these outbreaks were alfalfa (6 cases of foodborne illness outbreaks), red clover (2 cases), mung bean (1 case), and soy bean (1 case). The pathogens

associated with these outbreaks included *Salmonella*, *E. coli* and *Listeria monocytogenes* (CDC, 2016).

As a result, consumption of sprouts various outbreaks caused by different pathogens (i.e., *E. coli* and *Salmonella*) due to the consuming of these products have been state the past years (FDA, 1998, Erdozain et al 2013, Gensheimer and Gubernot, 2016 and FDA, 2017). Gensheimer and Gubernot (2016) cited alfalfa, clover, and mung bean as the main public food source for the sprout-related disease, with *Salmonella* spp., *E. coli* and *Listeria* spp. as the more common sprout-concerning pathogens. A comprehensive schedule of all of the sprout-related foodborne illnesses from 1973 to 2016 has been reported in Food Safety News (NACMCF, 1999, Lisa et al 2004 & Food Safety News, 2016).

Due to their high nutritive value and anticarcinogenic properties, raw seed sprouts have become progressively more common as a healthy food (Neetoo and Chen, 2010), and a public food item in markets and restaurants. However, this has led to increase in the happening of disease related to the exhaustion of raw seed sprouts (Golberg et al 2011). Sprouts prepare a distinct food safety challenge approach to other fresh produce, as the sprouting operation facilitates maximum condition for the growth and reproduction of pathogenic bacteria (Csordas et al 2008).

Irradiation of food products is an effective and safe method for food preservation, as it reduces spoilage, enhances food hygiene, and shelf life. In 2000, the U.S. Food and Drug Administration (FDA) confirmed the use of irradiation at doses up to 8 kGy to control microbial pathogens in sprout seeds (CFR 2000). Codex Alimentarius Commission (CAC), World Health Organisation (WHO), the International Atomic Energy Agency (IAEA), and the Food and Agriculture Organization (FAO) assisted the use of irradiation as a food safety technique throughout the world. So far, more than 50 countries have approved irradiation as a sanitary and phytosanitary technique for over 60 foods and food products (IFSAT, 2013).

Therefore, objectives of the present study were to investigate the effect of treating-clover seeds with disinfectants (calcium hypochlorite) and soaking seeds in salt water during germination on quality of sprouts, and to evaluate the effectiveness of the treatment of cloversprouts with gamma radiation (1,2 and 3 kGy) as a potential means of the eliminating microbial load during sprouting and packaging and to extent shelf life of sprouts.

MATERIALS AND METHODS

1- Seeds material

Dry seeds of clover (*Medicago sativa* cv. Masqawy) were purchased from Agricultural Research Center. All the chemical used were of analytical grade and were procured from Sigma.

2. Sprouting process

Clover seed samples (10 g each) were placed into glass jars and covered with a mesh to germinate and produce sprouts as described by Abdallah (2008). After placing the mesh, the jars were divided to four groups for different treatments. **Treatment 1 (T₁):** Sterilized clover seeds (ST) by dipping in 20g/L Calcium hypochlorite for 20 min. and then drained. Seeds were left to soak for 12 h. in 2000 ppm sodium chloride (NaCl) and then drained. This rinse-and-drain procedure was repeated for three days until the sprouts were ready for harvesting. **Treatment 2 (T₂),** (ST) dipping in calcium hypochlorite for 20 min. and washing with tap water, 12 hr. / 3 days, as described above. **Treatment 3 (T₃),** Non-sterilized (NST) dipping in tap water and washing with NaCl, 12 hr. / 3 days. **Treatment 4 (T₄),** control (CTL) dipping in tap water and washing with tap water, 12 hr. / 3 days. All sprouts from each treatment were harvested using tongs, which were sterilized by dipping in ethanol and flaming and were placed into sterile plastic plate, then weighed it. Three samples of 50 g sprouts were collected from each jar. Samples were then analyzed for microbiological and germination parameters.

Yield ratio and sprout length: A known amount of seeds were germinated in a sterile petri dish with filter paper and distilled water. The seeds were germinated for 3 d at 25°C. Yield ratio is the equivalent weight of the sprouts divided by the weight of the seeds. At the end of each yield ratio determination, 100 sprouts were measured for sprout length using a digimatic caliper (Mitutoyo Corp., Japan). All experiments were conducted in triplicate.

3. Irradiation treatment

Gamma irradiation was carried out in ⁶⁰Co Indian Gamma Cell (GC) at National Center for Radiation Research and Technology, NCRRT, Cairo, Egypt. Samples were irradiated at a dose of 0 to 3

kGy. The GC was calibrated against transfer standard alanine dosimeters of NPL (traceability to NPL) and the dose rate at the center of GC chamber was 1kGy/50 min during experimental setup.

Packaged samples were irradiated at a dose of 1, 2, 3 kGy at 4 °C. Following irradiation, the samples were stored at 9 °C for 15 days. During the storage, samples were analyzed for their germination parameter and microbiological quality after 5 days interval.

4. Microbial analyses

Microbial counts of sprouts from untreated seeds were used as initial values for calculating reductions in microbial counts. Clover sprouts were mixed and stomached separately with buffered peptone water (BPW) (Oxoid, Unipath, Ltd., Basingstoke, UK) at a ratio of 1:9 product to medium. Then, 1 ml of each suspension was inoculated in triplicate on different media for the counting of the following microorganisms: Total aerobic mesophilic bacteria on tryptone soya agar (TSA), incubated at 30°C for 72 h. Moulds and yeast on Sabouraud chloramphenicol agar, incubated at 25°C for 3-5 day.

Total coliform was counted on MacConkey broth by the most probable number (MPN) technique using three test tubes with Durham's tubes according to WHO (1993). For *Escherichia coli* counting, positive test tubes from MPN were used to inoculate another tubes of MacConkey broth and incubated at 44 °C for 24-48h. Confirmation of *E. coli* was carried out by IMViC according to APHA (1992).

Staphylococcus aureus: Spread plate technique was used to determine staphylococci count on Baird Parker agar (BPA). Typical black colonies were counted after 48 h of incubation at 35°C. Confirmation of coagulase was done by examining the clot formation in coagulase salt mannitol broth supported by 12% plasma. Analysis for *Salmonella* spp. was done according to the method of Harri-gan, (1998).

5. Statistical analysis

All data were analyzed by analysis of variance (AVOVA) using statistical analysis system (SAS) program (Completely Randomized design CRD), and the means were compared by detecting the LSD at $P < 0.05$ according to SAS (2006).

RESULTS AND DISCUSSIONS

Preliminary experiment No. 1

Weight of 50 seeds equal 0.14g increased to 0.99g fresh weight within 3 days germination but decreased to 0.11g dry weight in the same period. These results indicated that the fresh yield was about 7- fold while dry weight lost about 21.4% in 3 days old etiolated clover sprouts. Similar results were as obtained by Tahany et al (2015).

From Table (1) it is evident that the total bacterial count (TBC) of seed sterilized with calcium hypochlorite and non sterilized ranged from 5.90 to 6.29 CFU/g. Seeds treated with dipping and washing in tap water had the highest TBC. Fresh sprouts had TBC as high as 10^8 – 10^9 CFU/g

Mould and yeast log counts was 3.29 CFU/g in seeds sterilized and dipped in NaCl. A study by Tournas (2005) found that mung bean sprouts contained approximately 1.7×10^7 cfu/g yeasts and molds and alfalfa sprouts contained approximately 8.1×10^5 cfu/g.

Total coliform count usually has been used as an indicator of hygiene quality. All examined samples contained high levels of coliform bacteria. Count of *E. coli* by MPN ranged from 1.49 to 2.31 cfu/g indicating poor hygienic conditions (may be due to the use of tap water or contaminated seeds) during processing. A study by Rangel-Vargas et al (2015) tested 100 samples of alfalfa sprouts from retail supermarkets and found that all samples contained coliform bacteria ranging from 6.2 to 8.6 cfu/g. Additionally, a study by Kim et al (2012) found that coliforms increased to over 6 log CFU/g during germination even when coliform counts for the seeds were zero before the germination process began.

Staphylococcus aureus was detected in all treatments. It was present in relatively high density, ranging from 3.02 to 5.05 cfu/g. The clover sprouts were negative for *Salmonella* sp.

Preliminary experiment No. 2

The above-mentioned findings show the importance and need for a suitable methods to control various microorganisms contaminating clover sprout in order to extend their shelf-life and to ensure their safety.

Therefore, T1 (ST) and T4 (CTL) were exposed to 1,2 and 3 kGy (Table 2). To evaluate irradiation effect on presence of microorganisms during storage at $9 \pm 1^\circ\text{C}$. Microbiological parameters were

Table 1. Microbiological evaluation of four treatments of clover sprouts germinated in tap water and NaCl (2000 ppm).

Treatments	Total bacteria (log cfu/g)			Total mould (log cfu/g)			Total ciform (log cfu/g)			E. coli (log cfu/g)			Staph. aureus (log cfu/g)			Salmonella	
	NaCl	Tap water	Mean	NaCl	Tap water	Mean	NaCl	Tap water	Mean	NaCl	Tap water	Mean	NaCl	Tap water	Mean	NaCl	Tap water
Sterilized T(T ₁)	5.90	6.61	6.25	3.29	4.01	3.65	3.03	3.04	3.03	1.49	1.96	1.73	3.02	4.71	3.86	ND	ND
	6.29	6.90	6.59	5.30	6.95	6.13	3.04	3.04	3.04	1.87	2.31	2.09	3.48	5.05	4.26	ND	ND
Mean	6.10	6.75		4.30	5.48		3.03	3.04		1.68	2.14		3.25	4.88			
LSD of Mean (5%)	0.04		0.04	0.03		0.03	0.02		0.02	0.43		0.43	0.04		0.04	0.04	
LSD of Interaction	0.057			0.047			0.028			0.617			0.06				

Sterilized by calcium hypochloride (ST), non sterilized (NST), Tap water (TW), NaCl (2000 ppm).

Table 2. Microbiological evaluation of clover sprouts germinated in tap water with both treatments

Treatments	Total bacteria				Total mould				Total cliform						
	Con.	1	2	3	Mean x̄	Con.	1	2	3	Mean x̄	Con.	1	2	3	Mean x̄
T ₁ ST (hypo+NaCl)	6.88	4.32	3.66	2.58	4.36	3.53	2.61	1.65	1.28	2.27	3.04	2.19	1.94	0.48	1.91
T ₄ N-St (Water+water)	6.96	5.86	3.94	2.89	4.91	4.93	3.79	2.19	1.62	3.13	3.04	2.66	2.19	0.48	2.09
Mean K	6.92	5.09	3.80	2.73		4.23	3.20	1.92	1.45		3.04	2.42	2.06	0.48	
LSD of mean (5%)	0.005				0.0071	.027				0.039	0.016				
LSD of interaction	0.1					0.55					I=0.033				
	E. coli					Staph aureus					Salmonella				
	Con.	1	2	3	Mean x̄	Con.	1	2	3	Mean x̄	Con.	1	2	3	
ST T ₁ (hypo+NaCl)	1.91	1.63	1.22	0.48	1.31	2.08	1.71	2.00	2.00	1.94	ND	ND	ND	ND	ND
T ₄ N-St (Water+water)	2.36	2.18	1.38	0.48	1.60	2.61	1.96	2.00	2.00	2.14	ND	ND	ND	ND	ND
Mean K	2.13	1.90	1.30	0.48		2.35	1.83	2.00	2.00		ND	ND	ND	ND	
LSD of mean (5%)	0.042				0.059	0.024				0.036	ND = not detected				
LSD of interaction	0.08					0.05									

evaluated on irradiated and un-irradiated clover sprout samples after irradiation. Total bacteria were considered the predominant spoilage microflora in clover sprout. Mould and yeasts also play a role in spoilage of sprout as well as they have pathogenic, allergic and toxic action.

Results showed that irradiation caused a significant decrease in counts of microbial load and this decrease was proportional with irradiation dose. Irradiation with 3 kGy reduced log of TBC, and mould and yeast (M&Y) count in T₁ to 2.58 and 1.28 cfu/g, respectively. Our results found to be similar to the results of **Kim et al (2009)** who reported that clover sprouts irradiated with UV-C light (1–10 kJ/ m²) decreased the total aerobic bacterial count by 1.03–1.45 log cfu/g depending on the radiation dose.

Irradiation dose at 2 kGy decreased log total coliform and *E. coli* to 1.94 and 1.22 cfu/g in T₁, 2.19 and 1.38 cfu/g in T₄, respectively. Meanwhile, counts were below detectable level < 0.48 at dose 3 kGy in both treatments.

Staphylococcus aureus is the one of the most significant public health pathogens, and thus was tested for ensuring microbial safety of irradiated clover sprout. *Staph. aureus* was found in control clover sprout at level of 1.96 and 1.71 cfu/g at dose 1 kGy in T₁ and T₄, respectively. Irradiation at dose 2 kGy eliminated *Staph. aureus* Less than the detectable level (< 2 cfu/g).

From **Table (3)** treated sprouts with calcium hypochlorite reduced percentage of germination to 78%, while, treatment with water germination was 97%. According to the experience of sprouts producers, germination rate of 95% and above is acceptable. Yield ratio of T₄ (9.55) was higher than that of T₁ (7.74). Sprout length was 3.26 in T₁, which is lower than in T₄. This means treated seeds with hypochlorite and NaCl reduced the values to two folds.

It can be summarized that, treatment of clover seeds with calcium hypochlorite and sodium chloride (T₁) decreased percentage of germination.

Table 3. Percentage germination, yield ratio and length of clover sprouts grown from two treatments washing by tap water

Doses (kGy)	Germination (%)	Yield ratio (g sprout/g seed)	Sprout length (mm)
Treatment 1 (Hypo+NaCl)	78	7.74	3.26
Treatment 4 (Water + Water)	97	9.55	6.46
T test	- 0.04*	- 2.79*	- 1.80

*significant at 0.05.

Main experiment No. 3 (Treatment 4, T₄)

The changes in microbial load of clover sprout as a result of irradiation and subsequent refrigeration storage are shown in **Table (4)**. The average initial TBC, M&Y in unirradiated clover sprout were 7.69 and 6.04 cfu/g, respectively. Irradiation significantly reduced initial log counts TBC and M & Y to 1.47 and < 1.0 cfu/g, at 3 kGy. **Sastry et al (2000)** reported that the inhibition of microorganisms by UV irradiation is fundamentally due to DNA injury by UV-C light, which rise cross-linking between neighboring pyrimidine bases (thymine and cytosine) in DNA strand. Thus, the structure of hydrogen bonds to the purine bases is impaired, create blocking of DNA transcription and replication and final cell death (**Unluturk et al 2008**).

Clover sprout control treatment (T₁) samples contained coliform and *E.coli* at average log levels > 3.04 and 1.04 cfu/g, respectively. The irradiation dose of 2 kGy was efficient in reducing both coliform and *E.coli* less than 0.48. **Rajkowski and Thayer (2001)** reported a decrease of 4 log in aerobic plate count and coliform counts in alfalfa sprouts after exposure to 2 kGy. There was no

Table 4. Microbial quality of irradiated clover sprouts during cold storage at 9 °C germinated in sterilized tap water

Microbial quality Parameter log cfu/g	Total bacterial				Molds and yeasts				Total coliform				Escherichia coli				Staphylococcus								
	Control	1	2	3	Mean R	control	1	2	3	Mean R	control	1	2	3	Mean R	control	1	2	3	Mean R					
Doses (kGy)																									
0	7.69	5.07	2.00	1.47	4.90	6.04	3.77	1.90	1.00	3.18	3.04	1.56	0.48	0.48	1.39	1.04	0.96	0.48	0.48	0.74	2.92	2.55	2.00	2.00	2.36
5	7.04	4.93	2.56	1.99	4.13	6.55	3.25	1.99	1.01	3.20	2.18	0.98	0.48	0.48	1.03	1.38	0.48	0.48	0.48	0.70	2.73	2.25	2.00	2.00	2.24
10	6.62	4.70	3.53	2.35	4.30	6.68	3.95	2.08	2.17	3.72	1.94	0.48	0.48	0.48	0.84	2.19	0.48	0.48	0.48	0.90	2.57	2.00	2.00	2.00	2.14
15	7.43	5.59	3.78	2.81	4.90	6.90	3.47	2.11	1.50	3.49	3.04	0.48	0.48	0.48	1.12	2.43	0.48	0.48	0.48	0.96	2.44	2.00	2.00	2.00	2.11
Mean K	7.19	5.07	2.97	2.15	0.017	6.54	3.61	2.02	1.42	0.12	2.55	0.878	0.48	0.48	0.14	1.76	0.60	0.48	0.48	0.018	2.67	2.20	2.00	2.00	0.007
LSD (5%)					0.017					0.12		0.014			0.14					0.018		0.007			0.007
LSD of interaction					0.034					0.24		0.029			0.037					0.014					0.014

significant increase in aerobic plate count, coliforms, yeast and mold count, and staphylococci counts throughout storage up to 15 days at 9 °C.

Staphylococcus aureus was present in unirradiated clover sprout at level 2.92 log cfu/g. Irradiation with 1 kGy was not sufficient in complete elimination of *Staphylococcus aureus*; while dose of 2 kGy was very effective in complete elimination.

During refrigeration storage, total coliform and *E. coli* of unirradiated samples progressively increased. No coliform or *E. coli* growth was recorded in irradiated clover sprout samples at dose 2 kGy till the end of their storage periods.

Staphylococcus aureus in unirradiated samples significantly decreased during storage in control and 1 kGy. On the other hand, clover sprouts irradiated with 2 and 3 kGy were free of *Staph. aureus* throughout their 15 day of storage.

During refrigeration storage at 9± 1°C, clover sprouts TBC and M&Y increased significantly in all samples. However, the rate of increase in the irradiated samples was lower than of unirradiated ones. After 15 day of storage log counts TBC and M&Y of unirradiated samples reached 7.43 and 6.90, respectively which more than the accepted level. Meanwhile, log TBC of clover sprout samples exposed to 1,2 and 3 kGy significantly increased to reach 5.59, 3.78 and 2.81 log cfu/g indicating that the microorganisms injured by irradiation were able to recover somewhat. The same trend was found in M&Y. *Sallmonella* spp. was not detected in all clover sprout samples at zero time and during storage periods. **Saroj et al (2006)** reported that radiation treatment of mung, matki, chana and vana sprouts with 1 and 2 kGy caused reduction of aerobic and coliform counts by 2 and 4 log cfu/g, respectively. Staphylococci, yeast and mold counts decreased by 1 and 2 log cfu/g, respectively. However, during post-irradiation storage at 9 °C, aerobic plate, mold, yeast, coliform, staphylococci counts stay stable during the incubation period.

The relationship between yield ratio and sprout length of clover sprouts from irradiation doses presented in **Table (5)**. Significant decrease in the percent germination of sprouts were observed with increases in the radiation dose. Germination was > 90 % for dose levels in control and irradiated samples up to 3 kGy. This significant decrease in germination which resulted in lower yield ratios with the dose increased.

Table 5. Germination parameters of clover sprouts which germinated seeds in sterilized tap water and irradiated packaged sprouts

Doses (kGy)	Quality parameter		
	Germination %	Yield ratio (g sprout /g seed)	Sprout length (mm)
Control	98.06	9.64	5.583
1	97.17	9.64	5.63
2	96.50	9.35	5.55
3	95.34	9.13	5.53
LSD (5%)	0.82	0.27	0.13

Yield ratio was 9.64 (g sprout/g seed). The yield ratio also significant lower with the increase in irradiation dose, which coincide to the decrease in the germination percent. Results reported by **Rajkowski and Thayer (2001)** showed that as irradiation dose raise above 3 kGy, germination percent of alfalfa seeds was not influenced but the yield ratio decreased. The delayed growth of sprouts probably due to the stress response of seeds to irradiation (**Fan et al 2004**).

No significant decrease in clover sprouts length was observed in the control or radiation treated samples. The U.S. FDA advisable irradiation up to 8 kGy for sprout seed sanitation, study appear that an irradiation dose of over 4 kGy minimize germination of broccoli seeds to ≤ 90% accompanied with a essential reduction in the yield ratio and sprout length. **Fan et al (2004)** reported that germination of alfalfa seeds was not significantly influenced at 2 kGy in both irradiation processing but the yield ratio and sprout length was slightly reduced. To compensate for the yield loss, it was proposed that sprouts to be propagated for longer time to obtain the same yield as the non-irradiated seeds.

CONCLUSION

The results showed that germinated clover seeds in sterilized tap water and irradiation of sprouts at 2 kGy may be efficient for microbial safety and quality of clover sprouts during storage for 15 days.

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تأثير بعض المعاملات على بذور البرسيم وجودة النبتة اثناء التخزين بعد معالجتها بالاشعاع

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الموجز

الميكروبي وكذلك الميكروبات الممرضة وزيادة فترة التخزين وذلك بالتشعيع عند جرعة 2 كيلوجراى. حيث انخفضت الأعداد اللوغاريتمية الكلية للميكروبات من 7.69 الى 3.78 خلية/جرام. والأعداد الكلية لميكروبات القولون من <3.04 الى >0.48 خلية/جرام واعداد ميكروبات ايشريشياكولاي من 1.04 الى 0.48 خلية /جرام وستافيلوكوكس أوريوس من 2.92 الى <2.0 خلية/جرام.

أوضحت النتائج أوضحت أن تثبيت بذور البرسيم باستخدام ماء حنفية معقم ثم تشعيع النبتة بجرعة 2 كيلوجراى مفيد فى تحسين الجودة الميكروبية للنبتة دون تأثير يذكر على عملية الانبات مع الاحتفاظ بجودتها أثناء فترة التخزين لمدة 15 يوم.

الكلمات الدالة: نبت البرسيم، الإنبات، أشعة جاما، الجودة، التخزين

فى هذه الدراسة تم انبات بذور البرسيم بعد معالجتها بأربعة معاملات. المعاملة الأولى (تعقيم البذور)النقع فى هيبوكلوريت الكالسيوم 20جم/لتر لمدة 20 ق ثم الغسيل والنقع بصوديوم كلوريد بتركيز 2000 جزء فى المليون لمدة 12 ساعة / شطف 3 أيام). المعاملة الثانية (تعقيم البذور بالنقع فى هيبوكلوريت الكالسيوم 20 جم/لتر لمدة 20 ق ثم الغسيل والنقع بماء حنفية لمدة 12 ساعة / شطف 3 أيام). المعاملة الثالثة (النقع والغسيل فى صوديوم كلوريد 12 ساعة /3 أيام). المعاملة الرابعة (النقع والغسيل فى ماء حنفية 12 ساعة / شطف 3 أيام).

تم تعريض النبت الى التشعيع بجرعات 1 و 2 و 3 كيلوجراى ثم التخزين على 9م° وقيم الجودة أثناء التخزين. أوضحت النتائج أن نبتة بذور البرسيم التى تم معالجتها بالاشعاع ادى الى تقليل وتثبيط الحمل