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EFFECT OF SALINE WATER ON GROWTH, CHEMICAL COMPOSITION AND MICROBIOLOGICAL SAFETY EVALUATION OF RADISH ETIOLATED SPROUTS

[57]

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ABSTRACT

Egyptian radish sterilized seeds were sprouted for 3 days using tap and saline water NaCl 2000 ppm to study sprout characters, chemical composition, protein fraction and safety aspects such as microbiological examinations and biogenic amines were investigated at 3 days old. The results indicated that sterilized seeds increased sprout growth compared with non-sterilized. Seeds sprouting increased in protein content, moister, ash and fibers while carbohydrate, lipid content and energy decrease in etiolated sprouts. Using saline water and seeds sterilization for sprouting increased albumin, globulin and glutenin fractionation but decreased prolamin compared with sprouts produced with tap water and without seed sterilization. Using sterilization by 2% calcium hypochlorite of seeds before sprouting led to decreasing in total bacterial count compared with non-sterile seeds sprouts, the same was in total coliform, total yeast and total fungi counts. The sprouts product which washed with saline water was contain total bacterial count less than which washed by tap water. All sprouts under investigation were free from feacal coliform and all examined pathogenic microorganism under investigation like *Staph. aureus*, *B. cereus* and *Salmonella* spp. Use sterilized seeds for sprouting caused big decrement on biogenic amines content of radish sprout. Radish sprout contain biogenic amines but it is lower than previous ranges, sprouts can be considered a safe food and germination of seeds either use tap or saline water.

Key words: sprouted seeds, growth, Chemical composition, Protein fraction, food safety, pathogens, saline water, Biogenic amines.

INTRODUCTION

It is well known that the consumption of plant based diet, mainly vegetables and whole grains, is recommended as one of the ways to lower the risk of human diseases **(Cadenas and Packer, 2002)**. Sprouts is one valuable but still under-appreciated dietary supplement which may be considered to be a functional food and to meet consumer demands, as well a popular health food both in China and worldwide **(Li et al 2017).** Eating the fresh sprouts is the best way of gaining all of the health benefits claimed for cruciferous sprouts because only minor losses in health-promoting components are likely to occur **(Maetinez-Villauenga et al 2008)**. Radish (*Raphanus sativus*) is an edible vegetable, was used as medicinal foods for variety ailments in Egyptian folk-medicine long before the pyramids were built and was famous in ancient Egyptian, India; China and Rome as well **(Abdallah, 2008)**.Salinization is increasing on a global scale, decreasing average yields for most major crop plants. So, salt stress is considered as the major limiting factors to seed germination and seedling growth in many places especially in arid and semi-arid regions **(Zivkovic et al 2007)**. Few studies have been carried out to investigate the effect of different concentration of NaCl on sprout germination and Microbiological quality of seed germination. To minimize microbial hazards in sprouts, the Food and Drug Administration **(FDA, 1999)** asked the National Advisory Committee on Microbiologi-

cal Criteria for Foods **(NACMCF,1999)** to review the science behind sprout outbreaks and suggest recommendations to enhance sprout safety **Ding *et al*. (2013)**. As an example, FDA cited 20000 ppm calcium hypochlorite for seed disinfection treatment. It is important to examine the effectiveness of the different seed disinfection treatments as one of the control strategies disinfection **(FDA, 1999).**

The aims of the present research were to evaluate Effect of NaCl 2000 ppm (as one of the abiotic stresses) on Radish seed germination of 3 days old using tap and saline water.

Effect of using tap water and NaCl solution for 3 days on proximate analysis, of seeds sprouting.

Effect of sterilized and non sterilized radish seeds sprouting using tap water and NaCl solution for 3 days on the protein fraction.

microbiological quality of seeds germination using tap water and Nacl solution for 3 days with sanitized and non sanitized seeds.

Effect of sprouting using saline water and sterilized seeds on the content of biogenic amines of 3 days old.

MATERIALS AND METHODS

This study was carried out in Horticulture Department, Faculty of Agriculture, Ain Shams University and the Regional Center for Food and Feed **(RCFF)**, Agriculture Research Center **(ARC),** Egypt in the period 2016-2018.

1- Seed sprout production

Radish (*Raphanus sativus*), Was obtained from the crops Research Institute Agriculture Research center. Calcium hypochlorite 2% and NaCl were obtained from El- Gomhoria chemical company, cairo, Egypt.

Radish, seeds was cleaned to remove broken, damaged and off-colour grains. Egyptian radish seed sprout was produced using glass jar method and collect it after three days from seed soaking, (for both germinated in tap water and 2000 ppm NaCl solution, washed and hulled before dried according to **Abdallah (2008).** Radish seed and seed sprouts Samples of harvest tap and saline water was oven dried at 60°C for 48 h. and ground in laboratory wiley mill to pass through a 40 mesh sieve. The ground sample was stored at 5°C until chemical analysis while fresh sprouts were used for microbiological examination.

2- Chemical examination of seeds and sprouts

a- Proximate analysis

Proximate analysis moisture, fat, ash, protein and fiber content of seeds and sprouts are determined according to **AOAC (2012)** using subtracting to determine total carbohydrate. The energy value was calculated using the method [(9 x fat) + (4 x carbohydrate) + (4 x protein)] as prescribed by **Chima and Jgyor (2007).**

b- Protein fractionation

Protein fractionation of albumin, globulin, glutelin and prolamin were analyzed in etiolated radish sprout at 3 days old with and without seed sterilized in tap and saline water 2000 ppm Nacl according to **Lookhart and Bean (1995).**

3- Effect of 2000 ppm Nacl concentration on radish seeds sprouts production

Radish sprout was produced using glass jar method and collect it at 3 days from seed soaking for both germinated in tap water and in 2000 ppm NaCl solution and sprout were produced without seed sanitizers.

4- Effect of seeds sanitizing by calcium hypochloride 2% on sprouts production

Radish seeds was soaked in calcium hypchloride solution 2% for 20-30 min as seed sanitizing treatment the seeds was drained and rinsed several times with tap water to remove sanitizer residual, then seeds soaking in both tap water and 2000 ppm NaCl solution for germination.

5- Microbiological examinations

Microbiological examinations were carried out in Regional Center for Food and Feed, Agriculture Research Center (ARC). Radish seeds sprout samples examined for its microbiological quality and safety in, non- sterilized, and sterilized radish seeds sprout, which washed with tap and saline water.

Appropriate dilutions prepared from each sample were used for inoculating different nutrient and

selective media. The microbial detection applied were:

5-1. Total bacterial counts

Bacterial counts were estimated on glucose yeast extract nutrient agar medium (**Difco, 1989**) using pouring plate technique. Suitable plates were counted after incubation at 37°C for 48 hours.

5-2. Coliform and faecal coliform counts

Coliform and faecal coliform counts were estimated on MacConkey agar (**Difco 1989**) using pouring plate technique. Suitable plates were counted after 24 hours at 37ºC and 44.5ºC for total coliform and faecal coliform counts, respectively.

5-3. *Staphylococcus aureus*

The numbers of *Staph. aureus* was carried out according to **Gouda (2002).** The isolation of *Staph aureus* based on appears as black, convex, shiny colonies surrounded by a yellow zone on Vojel Johnson agar medium. The plates were incubated at 37ºC for 48 hours.

5-4. *Bacillus cereus*

The numbers of *B. cereus* were estimated on egg-yolk polymyxin agar medium according to **Kim and Goepfert (1971).** On this medium, presumptive *B. cereus* appears as large pink colonies.

6-5.*Salmonella* spp.

Twenty-five g of each sample were added to 225 ml of peptone water as pre-enrichment medium and incubated at 37ºC for 24 hours. Twenty five ml from pre-enrichment culture were added to 225 ml of tetrathionate broth (**Difco, 1989)** as enrichment medium with incubation at 37ºC for 24 hours. After incubation, the culture was streaked on Difcobrilliant green agar plates and examined after 18-25 hours (**Georgela and Boothroyd, 1965 and Khan and McCaskey, 1973)**.On this medium, presumptive *Salmonell*a appears as pink colonies surrounded by bright red medium.

5-6. Total yeast and total fungi count

Twenty five gram of the sample were transferred to a sterile flask and mixed well with 225 ml of sterile peptone water (0.1%) solution. From which a serial dilutions were done, 1.0 ml was transferred with sterile pipettes from each dilution to a duplicate petri dishes with Rose Bengal chloramphenicol agar. Spread on the surface using a bent glass rod. The plates were placed in well ventilated plastic bags and incubated in an upright position at 25°C for 5-7 days **(NMKL, 1995)**.

6- Biogenic amines determination

Six biogenic amines included histamine, Tyramine, Cadaverine, Spermine, Putresine, Spermidine were extracted and determinates according to (**Frias et al 2007**).

7. Statistical analysis

Statistical analysis was employed for each measured trait by analysis of variance (ANOVA) using completely randomized design and the mean were differentiated by LSD 0.05 **(Snedcor and Cochran, 1980).**

RESULTS AND DISCUSSION

1- Effect of seed sanitizer and non-sanitizers on sprout characters

Data in **Table (1)** showed the effect of both seed sanitizers soaked in calcium hypo chloride 2% for 20-30 min and non-sanitizers on sprouting using saline water 2000 ppm and tap water solution on 3 days old etiolated radish sprouts characters, sprout length, fresh and dry weight and radical hypocotyl length.

Data revealed that all different seed sanitizers were increased the fresh and dry weight in radish compared with non-sanitizers seeds. These results are in agreement by **Rajkowski and Thayer 2001.**

Table 1. Effect of NaCl concentration in sprouting solution on 3 days old etiolated radish sterilized and non-sterilized seeds characters (average of three experiments).

Sterilization (Ste)	NaCl con-centration	Sprout radi-cal length (cm)	Sprout hypocotyl length (cm)	Sprout length (cm)	10 sprout fresh weight (mg)	10 sprout dry weight (mg)
		Radish sprout				
Sterilized seeds	Tap water	5.56a	2.54a	8.13a	954.33a	96.4a
NaCl, ppm	2000 ppm	5.26a	2.36ab	7.60b	946.33b	95.0b
mean		5.41A	2.43A	7.865A	945.00A	95.70A
Non- sterilized seeds	Tap water	5.26a	2.23ab	7.8b	946.33c	95.50ab
NaCl, ppm	2000 ppm	4.66b	2.16b	6.83c	935.0d	95.26b
mean		5.01B	2.20B	7.21B	944.65B	95.38B
Average	Tap water	5.41A	2.36A	7.866A	950.33A	95.95A
NaCl , ppm	2000 ppm	4.96B	2.26A	7.216B	939.33B	95.13B
LSD (0.05)	Ste	0.2607	0.2105	0.2549	2.1051	0.7149
	NaCl	0.2607	0.2105	0.2549	2.1051	0.7149
	Ste× NaCl	0.3686	0.2977	0.3605	2.977	1.011

Means of each column followed by the same letter are not significantly different at the 5% level ($p \leq 0.05$)

2- Effect of sprouting on proximate composition.

The proximate composition of sprouts and dry seeds radish sprouts using tap and saline water are presented in **Table (2).** Data showed using tap and saline water increased moister and protein content as compared with dry radish etiolated sprouts, could be due to a decreased in dry matter through respiration of young sprouts, due to here were no nitrogen source added externally to the water and saline solution used for irrigation during sprouts. This protein percent increase was therefore not a likely true increase (**Chavan and Kadam 1989, Abdallah 2008 and Dung et al 2010)** the increment of moister content in sprouts in a dry weight basis as compared with dry seeds state. The original dry weight of the seeds decreased during sprouting process. The decrement in dry weight may be due to leaching of materials and oxidations of substances from the seeds during sprouting as reported by (**Chavan and Kadam 1989)**. Carbohydrate values by difference recorded opposite results with protein and showed clear eased volute in sprouts compared with dry seeds speedily when using tap and saline water for sprouting. Moister, fiber and ash value increased in radish sprouts than dry seeds, while energy value decreased in radish sprouts using tap and saline water as compared with dry seeds. This observation may be due to decreased in lipid and carbohydrate content.

Table 2. Effect of sprouting on proximate analysis of radish etiolated sprouts using tap water and saline water 2000 ppm dry seeds (g/100g DW).

Nutrient (%)	moisture	Protein	Carbohydrates by different	Total lipid	fiber	ASH	Energy (Kcal/g) *
	Radish						
Dry seeds	4.9	23.14	22.94	32.3	8.82	7.90	475.02
Tap water	6.8	28.22	16.76	28.5	11.32	8.40	436.42
Saline water	8.2	31.30	13.56	26.1	11.94	8.90	414.34

*Energy calculated according to **Chima and Jgyor (2007)** Kal/g.

1- Effect of sterilization and water saline 2000 ppm on protein fractionation in radish sprouts:

Data in **Table (3)** revealed that radish seed sprout with saline water 2000 ppm Nacl recorded significant increase 56.18, 36.23 and 4.26 (mg/ 100mg) sample in albumin, globulin and glutenin fractionation respectively. Whereas significant decrease occurred in prolamin 3.32(mg/ 100mg) sample compared with sprouts produced with tap water with and without seed sterilized. Finally, albumin was the major radish sprout protein fraction

extracted from Nacl 2000 ppm sprout followed by globulin. Therefore, the radish sprout could have excellent applications for future product development due to their nutritional properties. These results may be due to Exposure of salt stress altered the protein profiles and promoted the accumulation of salt-specific proteins was depended on genotypes, salt concentration and salt treatment duration. It was suggested that appearance of synthesized proteins in response to NaCl treatments might be related with the capability of metabolism to adjust or adapt to varying requirements in response to NaCl treatments and may be involved in osmotic adjustment **(Win and Oo 2017).**

Table 3. Effect of sterilized and non-sterilized radish seed sprouting using saline water (Nacl 2000 ppm) on the protein fraction of 3 days etiolated radish sprouts characters (mg/ 100mg) samples.

Sterilization	parameter	Albumin	Globulin	Glutelin	Prolamin
Sterilization	Tap water	53.5 c	33.64 d	3.8 b	9.06 a
Nacl , ppm	2000	55.3 b	35.66 b	4.00 b	5.04 c
Mean		54.40 B	34.65 B	3.90 B	7.05 A
Non sterilization	Tap water	55.00 b	34.70 c	3.90 b	6.40 b
Nacl , ppm	2000	57.06 a	36.80 a	4.53 a	1.61 d
Mean		56.03 A	35.75 A	4.21 A	4.00 B
Average	Tap water	54.25 B	34.17 B	3.85 B	7.37 A
Nacl , ppm	2000	56.18 A	36.23 A	4.26 A	3.32 B
LSD 0.05	Ste	0.330	0.358	0.285	0.356
	Nacl	0.330	0.358	0.285	0.356
	Ste×Nacl	0.467	0.507	0.403	0.504

Means of each column followed by the same letter are not significantly different at the 5% level ($p \leq 0.05$).

Microbiological examination (CFU/g) of radish seeds sprout with tap and saline water 2000 ppm at home conditions

The data in **Table (4)** cleared that total bacterial counts in non sterile seeds sprouts was ranged from 9× 10^2 to 12× 10^3CFU/g in seeds sprouts which washed with saline and tap water respectively, while in sterile seed sprouts the total bacterial count was 6× 10 and 5× 10 CFU/g for sprouts washed with tap and saline water. Total coliform group was recorded as 5× 10 CFU/g only in non sterile seed sprouts washed with tap water. All sprouts under investigation were free from coliform group, *Staph. aureas*, *Salmonella* spp. and *B. cereus* was ranged from 10 to 70 CFU/g in non sterile seed sprouts washed with saline and tap water respectively. Total yeast and total fungi counts were counted as 7× 10^2, 3× 10^2 CFU/g and 15× 10, 11× 10 CFU/g in non sterile seed sprouts washed with tap and saline water respectively. Similar results for undetected *Salmonella spp* and faecal coliform were reported by **Prokowich and Blank (2001) and Ibrahim (2010).** On contrary **Ibrahem (2010)** detected *Staphylococcus aureus* in radish seeds sprouts and rocket sprouts. Data in **Table 4** explain the microbiological quality of sterilized radish seeds sprout with calcium hypochlorite and washed with tap and saline water were free from any microbial groups expect total bacterial count.

Table 4. Microbiological examination (CFU/g)* of non-sterile and sterile radish seeds sprout at home conditions.

Species	Non-sterile sprout		Sterile sprout	
	Tap water	Saline water (2000 ppm)	Tap water	Saline water (2000 ppm)
Total bacterial count	12× 10^3	9× 10^2	6× 10	5× 10
Total coliform count	5× 10	ND	ND	ND
Faecal coliform count	ND	ND	ND	ND
Staphylococcus auraus	ND	ND	ND	ND
Bacillus cereus	7× 10	1× 10	ND	ND
Salmonella spp.	ND	ND	ND	ND
Total yeast count	7× 10^2	15× 10	ND	ND
Total fungi count	3× 10^2	11× 10	ND	ND

* Colony Forming unit, ND: not detected

Therefore, these results were in harmony with **Ding et al 2013** reported that Microbial contamination of sprouts by *Salmonella* and *Escherichia coli* cause foodborne diseases, they reduce the risk of illness associated with contaminated sprouts with these microbes by using Food and Drug Administration recommendation using 20000 ppm calcium hypochlorite as treatment for seed treatment and this treatment has been considered the reference standard for seed disinfection treatment. Fur-

thermore, our results showed that sprouts treated with saline water at concentration 2000 ppm was reduced the total microbial count of radish sprouts without using 20000 ppm calcium hypochlorite. As a result, we recommend treat sprouts with saline water at 2000 ppm to disinfestation them from microbial contamination.

Effect of sprouting using saline water and sterilized seeds on the content of biogenic amines of 3 days old etiolated radish sprouts.

The results of biogenic amine contents of radish sprouts and grains are given in **Table (5).** Six biogenic amines were investigated in the experiment spermine, putrescine, cadaverine, histamine, tyramine and spermidine were 11.59,14.62, 14.39, 15.47, 7.37 and 16.68 mg/ Kg respectively non strilized of radish sprouts with tap water. However, all biogenic amines were detected in big amounts of radish sprouts with saline water. Sterilization of radish seeds decrease the amines content in radish sprout of tap and saline water **Table (5).** From the obtained results its clearly to say that all radish sprout samples were free from any biogenic amines except cadaverine and histamine were 0.11 and 0.10 mg/ Kg respectively in radish sprouts with saline water. On the other hand, cadaverine was 0.11 mg/ Kg in radish sprouts with tap water.

Radish dry seeds contain 4.28 mg of spermine, 9.57 mg of putrecine, 11.20 mg of cadaverine, 10.93 mg of histamine, 2.76 mg of tyramine and 8.42 mg of spermidine and most of these amines were slightly increased after sprouting seeds either use tap or saline water. These results were in parallel with **Shalaby (2000)** who showed that cadaverine and putrescine increased during the germination period in bean, lupine and chickpea seeds. Tryptamine can induce blood pressure increase, therefore causes hypertension, however there is no regulation on the maximum amount of tryptamine **Shalaby (1996).** However, **Parente et al (2001) and Nout (1994)** pointed out that the maximum and daily intake of histamine and tyramine should be in the range of 50-100 mg/Kg and 100-800 mg/ Kg respectively over 1080 mg/Kg tyramine becomes toxic.

Table 5. Effect of sprouting using saline water on the biogenic amines content (mg/kg/) of 3 days old etiolated radish sprouts.

Treatment		Spermine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Total
Sterilized	TW	ND	ND	0.11	ND	ND	ND	0.11
	SW 2000	ND	ND	0.11	0.10	ND	ND	0.21
Mean		ND	ND	0.11	0.10	ND	ND	0.16
Non sterilized	TW	11.59	14.62	14.39	15.47	7.37	16.68	80.12
	SW 2000	13.42	17.23	15.65	16.38	8.46	17.18	88.32
Mean		12.51	15.93	15.02	15.93	7.92	16.93	84.22
Average	TW sprout	5.79	7.31	7.25	7.74	3.69	8.34	40.13
	SW sprout	6.71	8.62	7.88	8.24	4.23	8.59	44.27
	Dry seed	4.28	9.57	11.20	10.93	2.76	8.42	47.16

ND= not detected, TW= Tap water, SW= Saline water.

Since, radish sprouts contain biogenic amines but it's lower than previous ranges, sprouts can be considered a safe food and germination of seeds either use tap or saline water did not cause harmful effect on the health of food. Besides, use sterilized seeds for sprouting caused big decrement in the amines content of radish sprout **Table (5).**

Over all, sterilization of seeds plays an important role to decrease the amines content which can be recommended treatment for decline the biogenic amines content of sprouts.

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تاثير الماء المالح على النمو و التركيب الكيماوى والسلامه الميكروبية لنبت بذور الفجل [57]

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تم تقدير النمو و التركيب الكيماوى و تفريدة البروتين والجودة الميكروبية والأمينات الحيوية فى نبت الفجل عمر 3 ايام فى الظلام حيث استخدمت البذور غير المعقمة والبذور المعقمة باستخدام هيبوكلوريت الكالسيوم 2% للتنبيت باستخدام ماء الصنبور و الماء المالح 2000 جزء فى المليون. اظهرت النتائج ان نبت البذور المعقمة ادت الى تحسين صفات النبت الناتج بالمقارنة بنبت البذور غير المعقمة. كما تم تقدير التركيب الكيماوى للبذور النابتة بماء الصنبور و الماء المالح واظهرت النتائج ان البذور النابتة وخاصة فى الماء المالح ادت الى زيادة فى نسبة البروتين والرطوبة والالياف والرماد مع انخفاض فى نسبة الكروهيدرات والدهون والطاقة بالمقارنة بالبذور الكاملة .كما حدث زيادة معنوية فى كل من الالبيومين، الجلوبيولين والجلوتيلين على التوالى في النبت المعامل بالماء المالح، بينما حدث نقص معنوى للبرولامين بالمقارنة بالنبت الناتج من ماء الصنبور. هذة الدراسة تم تقدير كل من العدد الكلى للبكتريا والعدد الكلى للكوليفورم والكوليفورم والسالمونيلا والاستافيلوكوكس والباسيلس والعدد الكلى للخمائر والفطريات للنبت واظهرت النتائج ان البذور المعقمة كانت خالية من جميع الميكروبات السالفة الذكر ما عدا الاعداد الكلية للبكتريا وان البذور غير المعقمة كانت خالية من الميكروبات المرضية والاعداد الميكروبية الموجودة كانت فى الحدود الامنة. تم تقدير الأمينات الحيوية ووجد ان تنبيت بذور الفجل المعقمة بالماء المالح وماء الصنبور ادى الى انخفاض ملحوظ فى محتوى النبت من الأمينات الحيوية بالمقارنة بغير المعقمة ولذلك ادت عملية تعقيم بذور الفجل قبل التنبيت فى كل من الماء المالح وماء الصنبور الى انتاج غذاء امن.

الكلمات الداله: نبت البذور، الفجل، التركيب الكيماوى، تفريدة البروتين، السلامة الميكروبية، البكتريا المرضية، الماء المالح، البيوجينك امين

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