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Assessing Hot Water Treatment Effect on Germination and Vigor of Melon Seeds

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Abstract: Regarding fast development and expansion of organic farming the alternative methods instead of chemical application in seed treatment should be seriously considered. Hot water treatment of seeds can be used to rid seeds of some seed borne pathogens while remain the seed viable. In order to estimate hot water treatment on seed germination and vigor an experiment was conducted as factorial based on complete randomized design in 3 replications on melon seeds of variety Samsuri in Seed and Plant Certification and Registration institute of karaj in 2013. The treatments were temperature in 4 levels(45,50,55˚C and control) and time of treatment in 4 levels(10,20,30 minutes and control).The measured traits were germination percent, germination rate, radicle length and shoot length of seedlings, seedling dry weight, seedling length vigor index. The results showed there was a significant difference in all traits among temperatures except of shoot length, and a significant difference was observed at interaction of time and temperature in germination percent and length vigor index.

Keywords: hot water, temperature, time, seed vigor, germination

INTRODUCTION

Hot water treatment gains more and more importance for organic farming and for the production of spices and medical plants[1]. It could also become an alternative method for conventional farming especially in case of failure of chemicals permitted for seed treatment. It is necessary to determine the optimal parameters of hot water treatment and to develop a technology practicable for vegetable seed.

Effective treatment temperature and duration have to be found out for every vegetable crop and the relevant pathogens[2].

Fungicidal seed treatments are not an option for organic growers; however, there are some seed treatments—such as hot water that can be used by organic farmers to eradicate some pathogens from seed[3]. Hot water treatment of seed, acid treatments or other methods, continue to be a standard method of pathogen elimination in seed. These methods are more ecofriendly and effective compared to chemical treatments (particularly hot water) and also effective; however, they can cause the loss of seed viability[4]. Babadoost[5] declared the hot water treatment is a simple, reliable, safe and cost-effective method. This method is safe to people and the environment because no chemical is used for seed and plant material treatment. It is a reliable method because it controls pathogens in the seeds and sets, has no adverse effect on seeds germination or vigor at the effective temperature-time for control of the pathogens. The method is cost-effective because the costs for implementation of the suggested hot water treatment are ≈$100 per hectare, which is less than 1.5% farm-gate value of the crop. Hot water treatment of seeds has been used for more than 80 years[5]. Hot water treatment can be used to rid seeds of certain seed borne pathogens while leaving the seed viable. For example, the fungi that cause black leg, downy mildew, and anthracnose of cabbage can be eradicated by soaking seed at 122°F for 25 minutes. This treatment will also eliminate the bacteria that cause black rot.. Procedures must be carefully followed. If the water is too cool, the seed borne pathogens will not be killed. If the water is too warm, the seed may be injured or killed[6].

Tuttle[7] declared seed can be treated by growers with hot water or Clorox® bleach (sodium hypochlorite) to kill the pathogen. Hot-water treatment is more thorough than Clorox because it affects bacteria inside the seed; however, high temperatures can adversely affect germination if proper precautions are not taken and bacteria deep inside seed may survive after treatment. Seed that is coated or fungicide-treated should not be treated with hot water or Clorox. Also old seeds should not be treated. Precise control of conditions is essential for successfully hot-water treating seed yourself. There is a small margin between the temperature and length of exposure needed to kill pathogens and the treatment conditions that will kill seeds, and that the highest temperature seed can tolerate varies among crops[7]. Grondeau and Samson[8] suggested hot water treatment is another option that has been used for seed disinfestations and has been employed extensively for seed borne phytopathogens with varying levels of success[8]. Elevated temperatures will kill seed borne bacteria; however, the negative effect of hot water treatments on seed germination and vigor is of great concern. There is a narrow optimum range for the temperature and submerging time that makes hot water treatments risky and difficult to implement commercially[9]. Lee, Ji-Hyun *et al.*[10] reported hot water treatment is the most appropriate seed disinfection method for organic vegetable farming. They evaluated hot water treatment effect on various vegetables seeds[10]. The hot water treatment approach includes exposing seeds to temperatures of 57 to 60°C for short periods (e.g., 10 min)[11]

Aveling et al[12] treated onion seeds with 50˚C hot water for 20 minutes to control *Alternaria porri* and *Stemphylium vesicarium.* Panna *et al.*[13] treated wheat seeds with hot water at 52° for 5, 10, 15 and 20 minutes. Evaluation of hot water (52°c) against *Bipolaris sorokiniana* of wheat Farmer's saved and apparently healthy wheat seeds differed significantly in terms of seed germination treated with hot water (52°C) for different time range. Muniz[14] found that hot water treatment at 50°C for 30 minutes eradicated fungi associated with tomato seed[14]. Bennett and Colyer[15] declared wide adoption of seed thermotherapy by the cotton industry will require not only that the treatments be effective, but also that those treatments do not reduce seed germination or vigor[15]. They investigated hot water treatment on elimination of fusarium from cotton seed and its effect on germination. Eleven treatment times were evaluated: 45 to 180 s, in intervals of 15 s, and a no treated control.

Nega *et al*.[2] examined efficacy and applicability of hot water treatment to five important vegetable crops including carrot, cabbage, celery, parsley and lettuce and developed hot water treatments effective against *Alternaria, Phoma,* and *Septoria* spp. infecting carrot, cabbage, celery, and parsley seeds[2].

Zinnen and Sinclair[16] found that the seed coat of soybean seeds sloughed off in water but remained intact when heat-treated[16,] Piorr[17] evaluated the effect of hot water treatment on Tilletia tritici spores and wheat seed germination[17]. Jaquette *et al*.[18] studied the treatment of alfalfa seeds in hot water for elimination of *S. Stanley.* Inactivation of *S. stanley* on seeds as affected by dipping in hot water for 5 or 10 min at 21, 54, 57, 60, 63, 66, or 71˚C was determined[18]. Du Toit and Hernandez-Perez[19] evaluated spinach seed treatments in hot water (40, 45, 50, 55, and 60ºC) for 10 to 40 min, for eradication of *C. variabile* and *V. dahliae* fungi from seed[19].

MATERIALS AND METHODS

The experiment was conducted as factorial based on complete randomized design in 3 replications on melon seeds of variety Samsuri in Seed and Plant Certification and Registration institute of karaj in 2013. The treatments were temperature in 4 levels (45, 50, 55˚C and control) and time of treatment in 4 levels (10, 20,30 minutes and control). The measured traits were germination percent, germination rate, seedling radicle length and shoot length, seedling dry weight, seedling length vigor index. The analysis of data was done by SAS software.

Seed treatment: first the seeds were placed in plastic bag and were placed in water bath at 37˚C for 10 minutes for pre warming. Then the seeds were treated with determined temperatures and durations in water bath. After treatment with hot water the bags containing seeds were placed in cool water for 5 minutes and finally he seeds were dried in room temperature for 2 days.

The standard germination test: 4 replications of 100 seeds were cultured as between paper method and were placed at 20-30˚C in germinator. The number of normal seedlings at 8th day was considered as germination percent[20].

Radicle and shoot length of seedling: For assessing seed vigor 10 seedlings were selected randomly from each replication at 14th day and the length of radical and shoot was measured by ruler.

Seedling length vigor index: This vigor index was obtained by germination percent × seedling length.

Germination rate: The germinated seeds were counted every day and germination rate(velocity) of seeds calculated by this formula[21]:

$$GR = \frac{(n1 \times t1) + (n2 \times t2) + (ni \times ti)}{T}$$

GR=Germination Rate

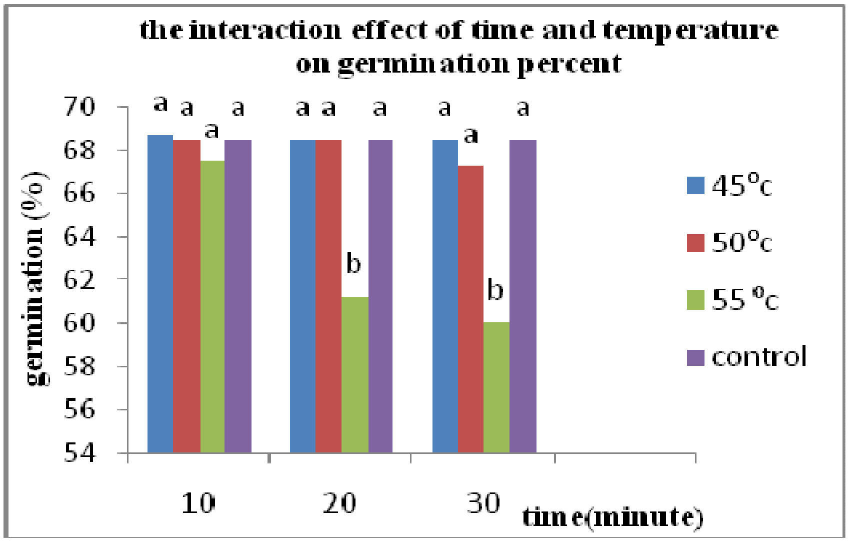
n= Number of days for each counting of germinated seeds

t= Number of germinated seeds in each counting day

T= Total number of germinated seeds

RESULTS AND DISCUSSION

Germination percent: The variance analysis results showed there was a significant difference in germination percent in main effect of time and temperature **(Table 1).** Also the interaction of time and temperature was significant, in 30 min treatment, the temperature of 55˚C showed the least germination percent(mean of 60%) and had significant difference with 45ºc and 50ºC and control**(Figure 1).** Soaking seeds in hot water at 55ºC for 20 min showed significant difference with 50ºC and 45C and control, in 10 minutes treatment there was no significant difference between treatments**(Figure 1).**



Zobaer *et al.*[22] reported that hot water treatment of wheat seeds at 52°C for 5 minutes increased seed germination up to 74.90%[22].Also the results of Khaleduzzaman[23] study revealed that wheat seed treated at 49°C,52°C, 55°C, for 5-10 minutes gave highest control of *B. sorokiniana* and increased seed germination[23].

But Piorr[17] observed hot water treatment at 50°C for 60-90 minutes reduced wheat seed germination to 10-20 percent, also Aveling *et al.*[12] observed hot water treatment at 50°C for 20 minutes in onion seed, reduced germination compared with non treated seed. Broda[24] found the temperature of 55 °C for 20 min for the treatment of carrot seed had negative effect on seed germination. Nega *et al.*[2] concluded Seed-borne pathogens could be reduced without significant losses of germination by hot water treatments at 50 °C for 20 to 30 min up to 53 °C for 10 to 30 min. At higher temperature, however, treatment time must be lowered to avoid reduced germination of sensitive crops.

They observed hot water treatment in the temperature ranges of 50 to 53 °C with treatment times up to 30 min at 50 °C and treatment times of 10 min at 53 °C showed no significant reduction in seed germination for all crops under investigation. Extended treatments especially at higher temperatures could reduce germination rate.

Nega *et al.*[2] found to avoid reduction in germination at temperature of 53 °C, shorter treatment time must be realized, especially on more sensitive crops[2]. The results of du Toit and Hernandez-Perez[19] research showed soaking spinach seed in 50ºC water for ≥30 min, or 55 or 60ºC water for ≥10 min resulted in germination reduction of seeds demonstrating the need to control water temperature precisely during hot water seed treatments to avoid damaging spinach seed[19]. Jaquette *et al.*[18] found treatment alfalfa seeds at >54˚C for 10 min caused a substantial reduction in viability of the seeds.

They found treatment at 57 or 60˚C for 5 min appears to be effective in killing *S. stanley* without substantially decreasing germinability of alfalfa seeds[18]. Bennett and Colyer[15] reported the duration of immersion in hot water also had a significant effect on both germination and vigor .

Nonetheless, seed of both cultivars could be immersed in 90°C water for up to 105 s without significant loss of germination or vigor compared with nontreated seed. The results of Panna *et al.*[13] showed in apparently healthy seeds treatment by hot water, the highest seed germination was recorded in the treatment 10 min followed by 5, 15 and 20. The lowest seed germination was observed under the control[13]. Hermansen *et al.*[25] found submerging carrot seeds for 20 min in water heated to 54°C to eradicate *A. dauci* did not adversely affect germination, seedling emergence, or yield.

Table-1: the analysis of variance of measured traits

S.O.V	Germination percent	Radicle length	Shoot length	Seedling Dry weight	Length vigor index	Germination rate
temperature	85.2972**	0.557104**	0.05664n.s	0.000116*	23613.486**	3.847426**
time	32.3333**	0.05673n.s	0.01434n.s	0.000022ns	7083.9366**	0.23459ns
Temperature*time	17.79166**	0.16756ns	0.0741ns	0.000046ns	6791.5012**	0.060838ns
error	3.24166	0.088729	0.04894	0.000035	6089.32586	0.122537
c.v	2.696313	4.01651	5.19192	4.90992	1.69039	4.6499

n.s= non significant, *: significant at 5%level of error probability,: significant at 1% level of probability**

Seedling radicle and shoot length: There was a significant difference among the temperatures in radicle length but there was no significant difference in shoot length **(Table-1).** The treatment of time was not significant **(Table-1).** According to mean comparison results**(Table-2)** the highest radicle length was for 45 ºC and 50 ºC treatments which had a significant difference with control(7.0875 cm) and 55 ºC (7.1958 cm) , but control and 55 ºC had no significant difference**(Table -).**

The results of Nega *et al.*[2] showed in hot water treatment the seedlings from treated seed were smaller than seedlings of untreated seed. This effect was observed for all crops investigated.

Table- 2: mean comparison of some measured traits at different temperatures

Temperature	Germination percentage	Radicle length	Seedling Dry weight	Length vigor index	Germination rate
Control	68.5000a	7.0875b	0.12000ab	769.820b	6.0597c
45	68.5833a	7.5708a	0.124000a	814.579a	7.9269a
50	68.5000a	7.5917a	0.123333ab	811.222a	7.7736a
55	62.9167b	7.1958b	0.11716b	719.180c	7.3736b

The same letters show no significant difference at 5% multiple Duncan test.

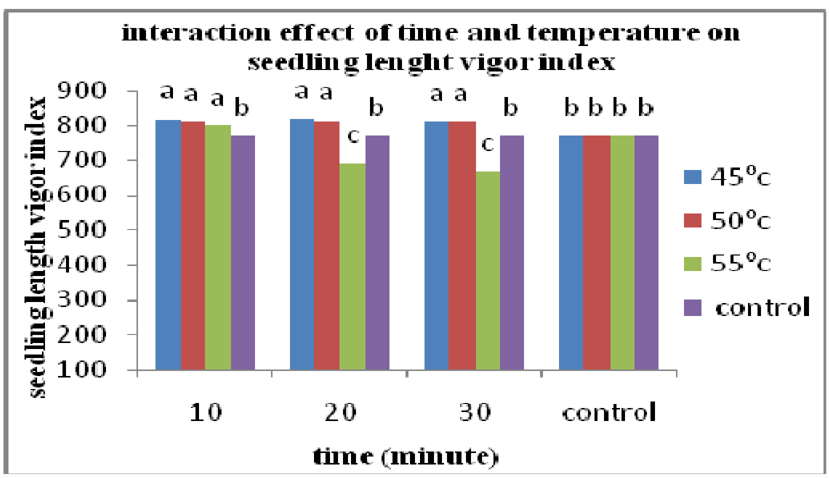
Table- 3: mean comparison of some measured traits at different times

Time	Germination percentage	Length vigor index
Control	68.5000a	769.947b
10	68.2500a	808.947a
20	66.0833b	773.667b
30	65.5000b	762.368b

The same letters show no significant difference at 5% multiple Duncan test

Seedling dry weight: According to analysis of variance results there was a significant difference among temperatures for the trait of seedling dry weight, but there was no significant difference in 3 levels of time **(Table-1).** The hot water treatment of 45ºC (mean of 0.12400g) showed the significant difference with 55ºC, but other treatments had no significant difference **(Table -2).**

Seedling length vigor index: The results indicated the index of seedling length vigor showed the significant difference in hot water treatments of time and temperature **(Table- 1).** Also the interaction of temperature and time was significant which showed at 10 minutes seed treatment in hot water 3 temperatures had no significant difference but they had significant difference with control **(Figure 2).** The temperature of 55 ºC at 20 min with the least seedling length vigor index(mean of 691.1) had significant difference with 50 ºC and 55 ºC and control, also control with mean of 769.8 showed significant difference with 3 temperature, but treatments of 45 ºC and 50 ºC had no significant difference. At 30 min treatment 55 ºC showed the lowest seedling length vigor index(668.4) and had significant difference with other treatments and significant difference was also observed between control and 45 ºC and 50 ºC and 55 ºC**(Figure 2).**



Bennett and Colyer[15] recorded 90 °C hot water treatment of cotton seed for 105 s appeared most effective at removing fusarium from cottonseed with minimal loss of germination and seed vigor and this treatment had no statistically significant loss of seed germination or vigor.

Germination velocity: The variance analysis results indicated there was a significant difference among the temperatures in germination rate (**Table-1**). The lowest germination rate was in control (mean of 6.0597) and the highest germination rate was observed in 45 ºC (mean of 7.9269) and 50 ºC(7.7736).the treatment of 55ºC (mean of 7.3736) also had a significant difference with other treatments**(Table-2).** The main effect of time treatment was not significant **(Table- 1).** The results of

Nega *et al*.[2] showed hot water treatment primarily caused a delay in germination and emergence. Depending on species, cultivar and fastness of germination, the delay in these processes decreased after 3 to 11 days and seedlings grew normally. They concluded it may be due to a loss of nutrients during hot water treatment and drying. The results of Ji-Hyun *et al.*[10] showed lettuce seeds that were the most sensitive to hot water were suitable to treat at 45 for 25 min, while Chinese cabbage and radish seeds were optimally treated at 50 for 25 min. The treatments resulted in similar or higher seed germination rate than non-treated seeds and promoted plant growth. Among the vegetable seeds pumpkin which is a member of Cucurbitaceae like melon was vulnerable to hot water and was suitable to treat at 50 for 15 min, while cucumber and hot pepper seeds and also Chinese cabbage and radish seeds indicated optimum treatment at 50 for 25 min as. The treatment also showed similar or higher seed germination rate and growth than non-treated seeds[10].

CONCLUSION

The hot water treatment of vegetable seeds is a useful method in organic farming, but the optimum temperature and time should be determine for each vegetable seed to avoid of detrimental effect on seed germination and vigor. According to results of this study hot water treatment at 50 ºC for 30 minutes and 55 ºC for 10 minutes had no detrimental effect on seed germination and vigor, but more research is needed to evaluate its effectiveness in eliminating fungi and bacterial diseases from melon seeds.

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