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Study on Thermotherapy Treatment Effects on Seed Germination and Vigor of Tomato Cultivars

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MD and MS designed the study, wrote the protocol, and managed the experimental process. Author MD wrote the first draft of the manuscript and literature searches. Author AK managed the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Hot water treatment presents great potential on the alternative control of seed pathogens. This research aimed to study the relation of hot water treatment to germination and vigor of tomato seeds of 3 varieties, to identify maximum combinations of temperature and exposure time of seeds with no detrimental effect on germination and vigor. The experiment was conducted as factorial based on complete randomized design in 3 replications. The treatments were cultivar at 3 levels (Super Star, Super Urbana and Queen), temperature in 5 levels (48, 52, 56, 60°C and control) and time of treatment in 4 levels (10, 20, 30 and 40 minutes). The measured traits were germination percent, germination rate, and some seed vigor aspects including seedling radicle length and shoot length and seedling dry weight. The analysis of variance showed seeds treatment in temperatures of 56°C and 60°C for 30 and 40 min had detrimental effect on germination and vigor. There was a significant difference among cultivars in germination percent and germination rate, especially at higher temperatures. Queen cultivar showed better germination ability and Super Star was the most sensitive to high temperatures (56 and 60°C) and had the lowest germination ability and vigor.

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1. INTRODUCTION

Heat, in the form of hot water, was first used as a disinfestations treatment of seed potato for late blight in 1882. Heat treatment has been used to control fungal, bacterial and viral diseases, plant-parasitic nematodes, and insect pests. Heat treatments (as vapor or hot water) of seeds and propagating organs not only control pests and diseases, but have benefit to plants by increasing rooting, budding, and vase life. Hot water treatment at 45 to 49°C for 10 to 15 min disinfests flowers, foliage, potted plants and propagation cuttings [1].

The use of hot water treatment is proposed to prevent bacterial diseases of vegetable crops, such as bacterial canker of tomatoes (*Clavibacter michiganensis pv. michiganensis*) and black rot on cole crops (*Xanthomonas campestris pv. capestris*) which can cause serious losses. They reported many companies use chemical treatments such as sodium hypochlorite, which sterilize the surface of the seed and do not reach infections within the seed; research has shown that hot-water treatment can penetrate the seed sufficiently to eradicate bacterial infections inside the seed, but there is a risk that germination will be reduced if the water gets too hot [2].

The most bacterial diseases of annual plants are seed-borne. Only a relatively small amount of infested seeds is sufficient to promote serious disease outbreaks. Hot water and hot air treatments are used to disinfect seeds. Satisfactory control has been obtained for several bacterial diseases (on tomato, tobacco, rice, barley, cucumber, Crucifers, pumpkin and cotton). However, the method requires studies to determine the optimal combination of time and temperature of exposure to use for the best efficiency with the least damage to the host [3].

Thermotherapy (e.g., hot water treatment) is an option that has been explored for seed disinfestations. This approach includes exposing seeds to temperatures of 57 to 60°C for short periods (e.g., 10 min). The elevated temperatures will kill seed borne bacteria; however, the negative impact of hot water treatments on seed germination and sprout vigor is of great concern and there is a narrow optimum range for the temperature and exposure time [4].

Fungicidal seed treatments are not an option for organic growers; however, there are some seed treatments such as hot water treatment that can be used by organic farmers to eradicate some pathogens from seed. Hot water seed treatment at 50°C for 25 minutes is recommended to control early blight on tomato seed [5].

It was recommended hot water treatment to destroy seed-borne fungi by soaking seed at 52°C for 30 minutes. Freshly harvested seed withstands heat treatment better than one or two-year-old seed [6].

Some lots of seed can be vulnerable to heat treatment. Seed treatments can also provide disease management [7].

Tomato seeds of cultivar UC-82 with hot water were treated at 52, 53, 54, 55 and 60°C for 30 min and 60 min and evaluated physiological potential of seeds [8].

The effect of hot water treatment temperatures (48°C, 50°C, 52°C and control) on germination and the incidence of seed-borne fungi of three varieties of maize consisting Barnali, Khai Bhutta and Mohor was tested [9].

The hot water treatment on wheat seed germination at 54°C for various times of 0 to 20min was assessed [10].

The hot water treatments on seeds of two cotton cultivars of Daytona and Cobalt were conducted to evaluate seed germination and vigor. Eleven treatment times were evaluated: 45 to 180s, in intervals of 15s, and a no treated control [11].

The thermotherapy method (dry-heat and hot water) in the germination and control of microorganisms associated to castor bean seeds was estimated. Using three different temperatures (42°C, 46°C and 50°C) for 15 and 30 minutes in hot water [13]. The hot water treatments to reduce the incidence of *Fusarium circinatum* on *Pinus radiata* seeds at 50°C to 53°C for 30 min and 45 min was evaluated [12].

The thermotherapy as hot water treatment at 60°C for 5, 10 and 20 minutes and chemical treatment in the control of fungi associated with the mulungu seeds was tested. The presence of fungi in seed germination can reduce, causing the death of seedlings or transmit diseases to adult plants. The physiological quality of seeds was evaluated by germination, first-germination counting and dry mass [13]. The effectiveness of thermal treatments of 50 and 55°C for 0.5,1 and 2 hours on Pep MV using commercial hybrid tomato seed lot was evaluated [14].

In total, hot water treatment presents great potential on control of seed pathogens. However, more studies are needed to identify its effect on the quality aspects of seeds, especially germination and vigor, especially vegetable seeds. This research aimed to study the relation of hot water treatment to germination and vigor of tomato seeds of 3 varieties, to identify maximum combinations of temperature and exposure time with no detrimental effect on germination and vigor.

2. MATERIALS AND METHODS

The experiment was conducted as factorial based on completely randomized design in 3 replications in Seed and Plant Certification and Registration institute of Karaj city, in 2013. The treatments were cultivar at 3 levels (Super Star, Super Urbana and Queen), temperature in 5 levels (48, 52, 56, 60°C and control) and time of treatment in 4 levels (10, 20, 30, 40 minutes) in 3 replications.

To measure seed germination percent and vigor, standard germination test and some vigor assessments were performed. The measured traits were germination percent, germination rate, and seedling radicle length, shoot length and seedling dry weight. The analysis of data was done by SAS v.9 and mean comparisons were made by LSD [15].

2.1 Hot Water Seed Treatment

First the seeds were placed in plastic bag which was no tightly closed 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 the seeds were placed at room temperature for 2 days to assure be dry perfectly.

2.2 The Standard Germination Test

The seed germination percent of three cultivars was estimated before and after hot water treatment according to International Seed Testing Association (ISTA) rules. First 4 replications of 100 seeds(totally 400 seeds) of each treatment were planted in moisturized sowing papers by tap water, as between paper method (BP), then planted seeds were placed at alternating temperature of 20-30°C(16 hours at 20°C/8hours at 30°C and 8 hours light/16 hours dark) in a germinator. The number of normal seedlings was counted at 14th day and was considered as germination percent [16].

2.2.1 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 radicle and shoot was measured by ruler [16].

2.2.2 Seedling dry weight

10 seedlings were placed in oven at 75°C for 48 hours till reached constant weight and then the seedlings were weighted by precise scale [16].

2.3 Germination Rate

The germinated seeds were counted every day and germination rate (speed of germination) of seeds calculated by this formula [17].

$$GR=\frac{(n1\times t1)+(n2\times t2)+(n3\times t3)+(ni\times ti)}{T}$$

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

3. RESULTS AND DISCUSSION

According to variance analysis of results most of the measured traits were significant (Table 1).

3.1 Germination Percent

The variance analysis results showed there was a significant difference in germination percent at main effect of cultivars, temperature and time and their interactions (Table 1). There was a significant difference among 56°C and 60°C at 10 and 20 minutes hot water treatment, also these temperatures had significant difference with 48°C, 52°C and control, which a is the highest rate and b is more than c (Table 3, Fig. 1). At 30 and 40 minutes seeds treatments in hot water, temperature of 52°C and 56°C and 60°C s had significant difference with each other and either with control and 48°C, but there was no significant

difference between control and 48°C (Table 4, Fig. 1). The 3 way interaction of cultivar×temperature×time was significant as shown in Table 5.

Table 1. The variance analysis of the measured traits

Source Of Variation	df	Germination percent	Germination rate	Radicle length	Shoot length	Seedling dry weight
cultivar	2	762.0877**	34.79594**	1.17442$^{n.s}$	1.27658$^{n.s}$	0.000009$^{n.s}$
temperature	4	19730.1195**	697.0584**	37.7535**	7.27118**	0.000246*
Time	3	4289.4822**	128.315**	13.34329**	4.1201**	0.00007*
cultivar* temperature	8	79.9142**	2.08408**	0.5655$^{n.s}$	0.27235$^{n.s}$	0.000003$^{n.s}$
cultivar* time	6	85.7591**	2.39491**	0.8120$^{n.s}$	0.43364$^{n.s}$	0.000005n.s
Temperature* time	12	2786.6381**	60.5889**	6.5271**	4.4161**	0.000046**
Cultivar* temperature* time	24	50.4133**	1.92270**	0.475173$^{n.s}$	0.50808$^{n.s}$	0.000003$^{n.s}$
error	120	11.4851	0.397468	0.7221	0.61483	0.0000055
cv		4.568728	5.403289	11.454	14.0225	15.5157

*ns: no significant, *: significant at 5% level of error probability,**: significant at 1% level of error probability*

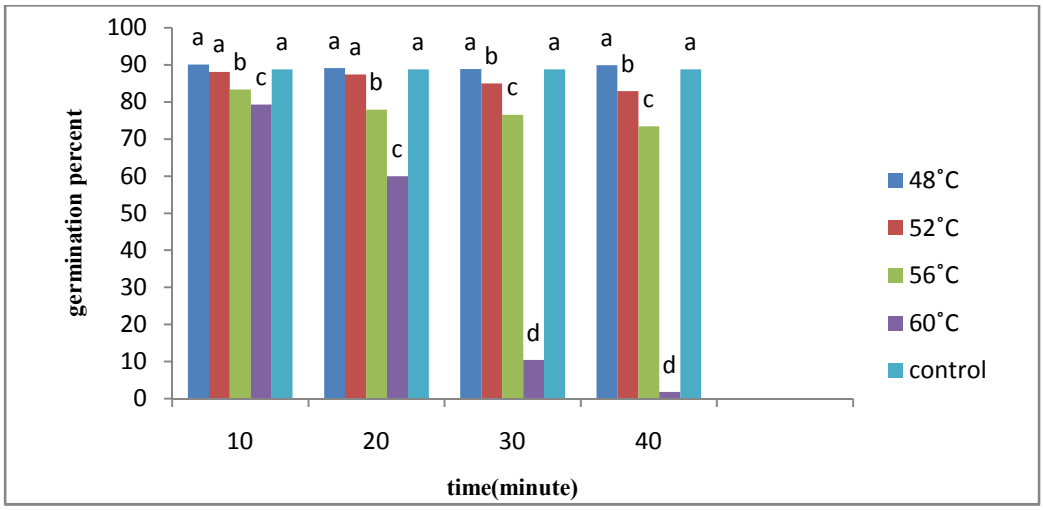

Fig. 1. The interaction of temperature and time on germination percent

This result is somewhat concord with several research results in which it was found that treatment of horseradish sets in hot water at 47°C for 20 min resulted in higher percentages of set germination [18]. But some other researchers reported different results to this experiment, found no reduction in germination after 10 min treatment of wheat seeds at 54°C [19]; also it was reported the hot water treatment up to 50°C up to 30 min did not affect the seed germination percentage of castor bean [19].

The treatment with hot water (55°C for 30 min) is consistent option to control of fungi *Rhyzopus* sp., *Aspergillus* sp. and *Cladosporium* sp., without impairing the physiological potential of tomato seeds. The treatments 52 to 54°C for 30 or 60 min did not damage the physiological potential of tomato seeds. The treatments at 60°C for 30 or 60 min are effective to control fungi, but lethal to tomato seeds [8].There was a significant difference among cultivars germination at different temperatures. The temperature level of 48°C, 52°C

and control resulted in lower germination percent compared to 50ºC except in khai variety [9], also the decrease in germination percentage of treated seed compared to non-treated seeds of *Pinus radiata* was recorded for treatment temperatures <53°C [12], hot water at 60°C for 5, 10 and 20 minutes on mulungu seeds indicated that the heat treatments reduced significantly the germination and first count of the locations studied [13].

Hot water soaking of seeds for 2h at 55°C resulted in a detrimental effect on seed germination [14].

3.2 Seedlings Radicle Length

According to results of variance analysis there was no significant difference among cultivars, but the main effect of time and temperature and also the interaction of temperature and time were significant (Table 1). At 10 and 20 minutes treatments, a significant difference was observed between control and 60°C (Fig. 2). Also a significant difference was recorded between 56°C, 60°C at 30 min treatments and both of them had a significant difference with control and 48°C and 52°C with them (Fig. 2). There was a significant difference among 60°C with control and other treatments at 40 min treatment of hot water, also control had a significant difference with 52°C (Fig. 2).

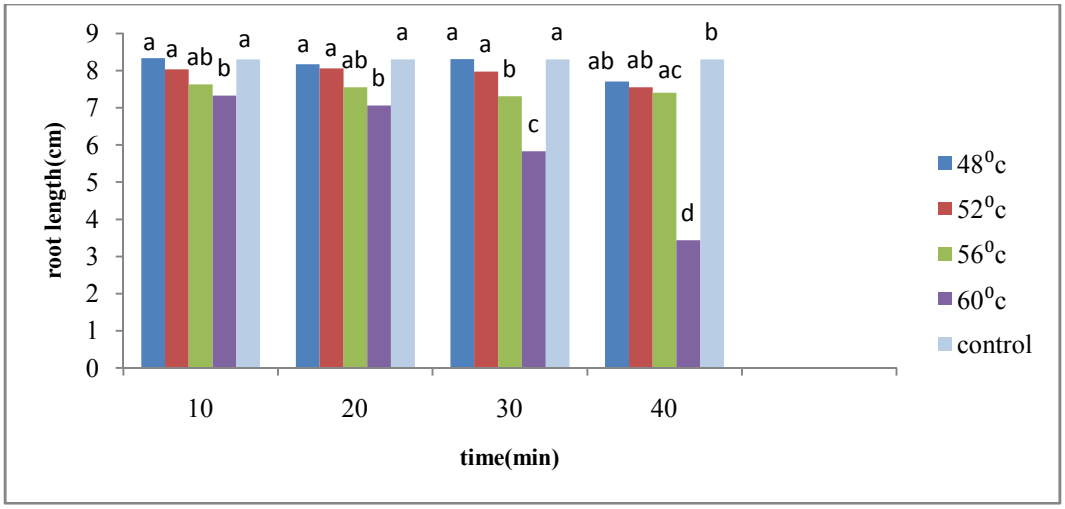

Fig. 2. The interaction of temperature and time on seedling root length

3.3 Seedlings Shoot Length

The results showed that the main effects of variety, temperature and time were significant (Table 1). Queen cultivar had a significant difference with Super Star but there was no significant difference between Super Urbana and Queen, also between super Urbana and Super Star (Table 2). Also a significant difference was recorded at the interaction effect of temperature and time (Table 1). According to Fig. 3 there was a significant difference at 30 minutes treatment between 60°C with 48°C and control. At 40 min 60°C had significant difference with all other treatments.

Table 2. Mean comparison of measured traits at different cultivars

Cultivar	Germination (%)	Germination rate	Radicle length(cm)	Shoot length(cm)
Queen	79.020a	12.370a	7.677a	5.863a
Super Urbana	74.549b	12.083b	7.374ab	5.377b
Super Star	69.058c	10.563c	7.209b	5.540b

The same letters show no significant difference at 5% multiple Duncan test. Different letters show significance at 5% level of probability

Table 3. Mean comparison of measured traits at different temperatures

Temperature	Germination (%)	Germination rate	Radicle length(cm)	Shoot length(cm)	Seedling dry weight(g)
48	89.50a	14.717a	8.1306a	5.9094a	0.0170a
52	85.861b	13.9872b	7.9350a	5.6417a	0.0164a
56	77.806c	12.046c	7.4028b	5.7631a	0.0155a
60	38.914d	5.0832d	5.9343c	4.961b	0.0113a
control	88.778a	14.2852b	8.3444a	5.888a	0.0168b

The same letters show no significant difference at 5% multiple Duncan test. Different letters show significance at 5% level of probability

Table 4. Mean comparison of measured traits at different times

Time	Germination (%)	Germination rate	Radicle length(cm)	Shoot length(cm)	Seedling dry weight(g)
10	85.222b	13.8180b	7.811b	5.812a	0.0161a
20	78.611c	12.059c	7.693b	5.762a	0.0163a
30	65.222d	10.119d	7.355b	5.563b	0.0145b
40	63.714d	9.973d	6.560c	5.141b	0.0134b
control	88.778a	14.285a	8.344a	5.888a	0.0168a

The same letters show no significant difference at 5% multiple Duncan test. Different letters show significance at 5% level of probability

Table 5. 3-way effect of tomato cultivar* temperature* time on germination percent

Temperature	Time	Super urbana	Queen	Super star
48	10	97.333a	85.666b	87.333b
	20	92.666a	88.333b	86.33b
	30	88.0000b	94.333a	84.333c
	40	94.6666a	92.333a	82.666b
52	10	86.333b	92.00a	86.00b
	20	84.6666b	95.333a	82.333b
	30	81.666b	93.666a	79.6666b
	40	80.333b	87.666a	80.666b
56	10	84.0000a	85.00a	79.00c
	20	78.00b	83.333a	72.333c
	30	78.0b	83.0a	68.6666c
	40	76.0a	78.333a	66.00b
60	10	80.333a	82.66a	75.000b
	20	62.00b	73.00a	45.000c
	30	9.33b	15.333a	6.666b
	40	1.666a	2.00a	1.6666a
control	control	95a	88.00b	83.333c

Different letters in same rows show significance at 5% level of probability

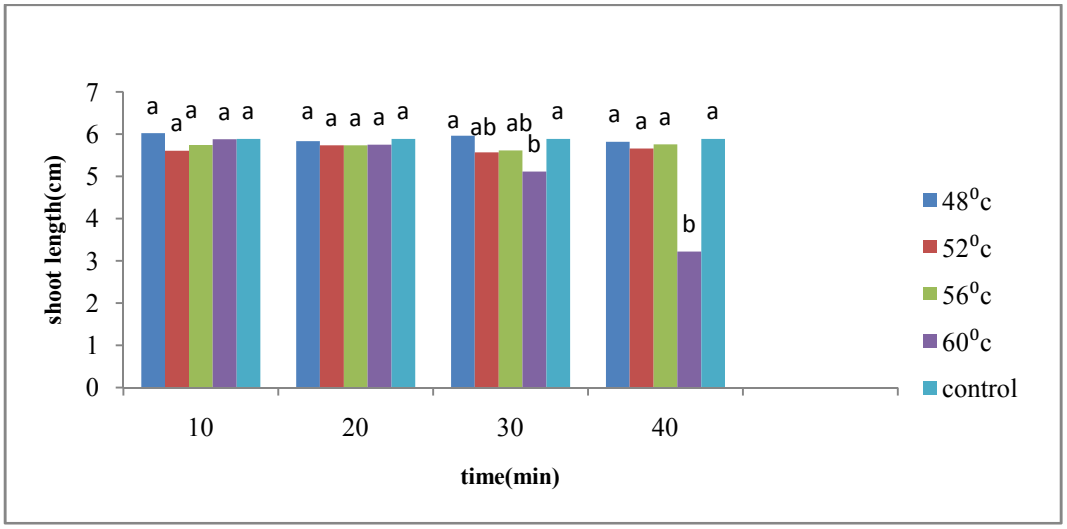

Fig. 3. The interaction of temperature and time on shoot length of seedlings

3.4 Seedling Dry Weight

According to variance analysis results a significant difference was recorded in main effects of temperature and time, but the main effect of cultivar was not significant (Table 1).The interaction of time and temperature was also significant, which in 20, 30 and 40 min of hot water treatments the temperature of 60ºC showed a significant difference with other treatments and control, but there was no significant difference among other treatments (Fig. 4).

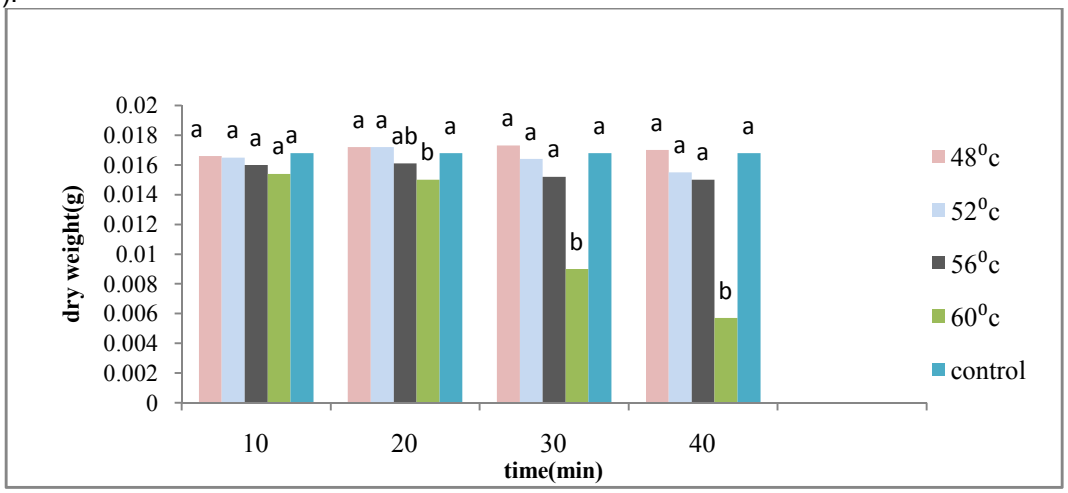

Fig. 4. The interaction of temperature and time on seedling dry weight

The duration of seeds immersion in hot water had a significant effect on both germination and vigor of both cultivars of cotton. They suggested that the cotton seeds could be immersed in 90°C water for up to 105 seconds without significant loss of germination or vigor compared to no treated seed. Seeds of Cobalt exhibited a significant reduction in

germination and vigor after 120 s of immersion in comparison with non treated control. Daytona seed did not demonstrate a reduction in germination or vigor compared to the non treated seeds until seeds were immersed for 150s [11].

Table 6. 3-way interaction of cultivar*temperature*time on germination rate

Temperature	Time	Super urban	Queen	Super star
48	10	15.877a	15.261a	13.972b
	20	15.205a	14.177ab	14.122b
	30	14.8111a	14.311ab	13.316b
	40	15.166a	15.342a	15.044a
52	10	15.483a	13.588b	13.056b
	20	14.716a	14.4055a	12.672b
	30	14.455a	15.050a	12.411b
	40	13.738b	15.138a	13.128b
56	10	14.538a	14.927a	13.005b
	20	14.155a	13.750a	11.738b
	30	11.622a	12.044a	10.488b
	40	9.533b	11.745a	7.007c
60	10	13.644a	11.527b	10.930b
	20	6.4055b	8.592a	4.773c
	30	0.479b	1.933a	0.505b
	40	0.222a	0.100a	0.222a
control		15.355a	14.311b	13.188b

Different letters show significance at 5% level of probability

Others found that treatment of horseradish sets in hot water at 47°C for 20 min resulted in higher vigor in the greenhouse and fields. Temperatures higher than 48°C negatively affected plant vigor of horseradish. Also treatment in hot water at 47°C for 30 min was effective for control of the set-borne pathogens without significant effect on plant vigor [18].

3.5 Germination Rate

Consider to results of variance analysis, the main effect of cultivars, temperature and time and their interactions indicated a significant difference in germination percent (Table 1). The Fig. 5 shows the interaction of time and temperature in which the treatment 48°C had significant difference with all other treatments at 10 min hot water treatment, also 60°C indicated significant difference with other treatments. At 20 and 30 min treatments, the temperatures of 56°C and 60°C revealed significant difference with each other and also with other treatments. At 40 min treatment a significant difference was observed among all treatments except of control and 48°C and 52°C (Fig. 5). According to Table 6 a significant difference was observed in the 3-way interactions of cultivar×temperature×time.

It was reported that some varieties or seed lots are more vulnerable to heat treatment than others and an increase in germination rates after hot-water treatment is due to that other microbes that attack the seed during germination are killed [11].

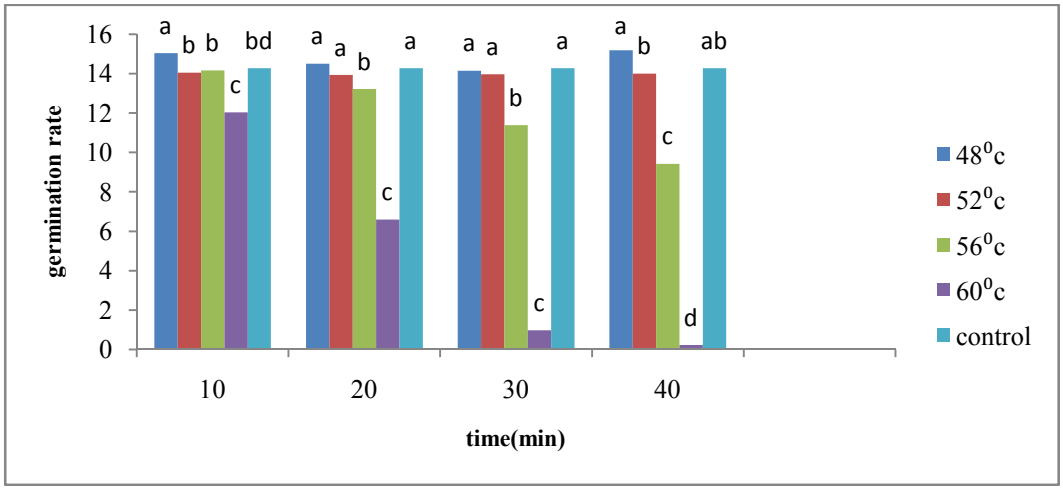

Fig. 5. The interaction of time and temperature on germination rate

4. CONCLUSION

The hot water treatment of seeds is an environmental friendly and safe approach in organic farming, but it should be determined the optimum temperature and time for each vegetable seed to avoid the effect on seed germination and vigor. Based on the results of this study the hot water treatment at 48°C and 52°C can be considered as non detrimental treatment on seed germination and vigor. Also there is an obvious difference in cultivars susceptibility to high temperatures; Queen Cultivar was recorded the best germination ability at higher temperatures than other cultivars. It's proposed to fulfill more research to evaluate the effectiveness of these treatments in eliminating fungi and bacterial diseases from seeds of different tomato cultivars.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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