

EFFECT OF THERMAL HARDENING ON GERMINATION AND SEEDLING VIGOUR OF TOMATO

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Abstract

The study was carried out to investigate the effects of thermal hardening on germination and seedling growth of two tomato cultivars i.e. Nagina and Pakit. Dry heat treatment i.e 50°C and chilling (-19°C), Chilling + Heating, Chilling + Heating + Chilling, Heating + Chilling + Heating treatment each applied for the period of 24h along with a control. The both cultivars responded maximum final germination percentage in case of dry heat treatment applied at 50°C for the period of 24h followed by chilling at -19°C for 24h, while minimum final germination percentage was recorded in case of Heating + Chilling + Heating for 24h. Dry heat treatment at 50°C significantly reduced time taken to 50% germination. Whereas it also gave more GI, root and shoot length, fresh and dry weight, where as minimum EC of seed leachates was recorded in dry heat treatment in Nagina as compared to Pakit.

Keywords: Chilling, Electrical conductivity, Seed dormancy, Thermal hardening.

INTRODUCTION

During development seeds of many species become dormant on the parent plant and this resistance to germination persists after the seeds are dispersed, and this 'primary dormancy' is considered as a key tactic to survive during unfavorable. Primary dormancy may not be always, removed by exposure of dry seeds to high temperatures or of imbibed seed to chilling temperatures [Bewley and Black, 1985]. However, according to Liu *et al.* [1996] dormancy has been reported in freshly harvested tomato seeds. The endosperm rupture is the main limiting factor for germination in tomato seeds, so limited germination, weakening of the micropylar endosperm surrounding the radical tip seems to be required for radical protrusion [Bewley, 1997]. Seed priming increases

respiratory activity of seeds [Halpin-Ingham and Sundstrom, 1992] and when applied to ripened seeds, restores activities of enzymes involved in the cell detoxifying mechanisms such as superoxide dismutase; catalase and glutathione reductase [Bailly *et al.*, 1997].

Tomato is among the crops which are responsive to priming. The purpose of seed priming is to lessen time between seed sowing and emergence so that to protect seeds from biotic and abiotic factors during critical phase of seedling establishment., which helps in uniform crop stand and improved yield. These priming treatments which enhance seed germination include hydropriming [Afzal *et al.* 2002] osmopriming and hormonal priming [Afzal *et al.* 2006]. Dry heat treatment of seeds is used for two purposes, one is to disinfect or sterilize the seeds to control the external and internal seed borne pathogen including fungi, bacteria, virus and nematodes [Nakagawa and Yamagucki, 1989], the other is to break the thermo-dormancy [Zhang, 1990].

Although elevated temperature in dry heat treatment may reduce seed viability and seedling vigour, but optimum temperature for breaking dormancy helps in promotinges seed germination and seedling emergence in cereal crops [Lee *et al.* 2002]. The enhancement in seed germination through dry heat treatments showed a wide intraspecific variation [Herranz *et al.* 1998].

To protect seeds from a-biotic and biotic stresses during critical phase of seedling establishment pre-sowing chilling treatments are being effectively used alone or in combination with other seed vigour enhancement techniques to lysten time between planting and emergence [Basra *et al.* 2002].

Higher vigour of the primed seeds may be due to increased α -amylase activity. During priming with osmotica, ions from potassium nitrate and sodium chloride solutions accumulate within the seeds, reducing water potential and increasing water absorption. It is very important to understand biochemical and physiological changes in tomato induced by thermal hardening. Therefore, the study was carried out to explore the effect of thermal hardening to enhance the germination and seedling vigour of tomato varieties.

MATERIALS AND METHODS

Seeds of two tomato cultivars i.e. Nagina and Pakit, were collected from Ayub Agricultural Research Institute, Faisalabad, Pakistan. The initial seed moisture was 8.15 and 8.12% respectively (dry weight basis). Before use the seeds were surface sterilized by dipping in sodium hypochlorite (5%) solution for 5 min and re-dried on filter paper. These surface sterilized seeds of both tomato varieties Nagina and Pakit were placed in an oven and freezer at varying temperature range for the period of 24 h. Seeds were subjected to heating at 50°C in an oven (EYLA Forced Air oven, WFO-600 ND, Rikakikai Co. Ltd. Tokyo, Japan). Then

the seeds were incubated in glass jars tightly covered with lids to avoid evaporation losses. While for chilling Seeds of both varieties Nagina and Pakit were sealed in polythene bags and placed in a refrigerator at $-19\pm 2^{\circ}\text{C}$ for 24h. The study consisted of following treatment combinations: (T₀: Control, T₁: Chilling, T₂: Heating, T₃: Chilling + Heating, T₄: Chilling + Heating + Chilling T₅: Heating + Chilling +Heating).

GERMINATION TEST

Twenty five seeds with each replicate per treatment were germinated in an incubator at 25°C under continuous fluorescent light (photosynthetic active photon flux density of $330\text{ m mol m}^{-2}\text{ S}^{-1}$) in a growth chamber (Vindon, England) in 9 cm Petri dishes on two layers of Whatman No.1 filter paper and moistened with 4 ml distilled water for seven days. Time to 50% germination (T₅₀) was calculated according to the formulae of Coolbear *et al.* [1984]. Mean germination time (MGT) was calculated according to Ellis and Roberts [1981]. Germination index (GI) was calculated as described by the Association of Official Seed Analysts [1983]. Energy of germination was recorded on the 4th day after planting. It is the percentage of germinating seeds on the 4th day after planting relative to the total number of seeds tested.

EMERGENCE TEST

The haloprimered and unprimered seeds were sown in plastic trays (25 in each) having moist sand, replicated thrice, placed in growth chamber (Vindon, England) maintained at 25°C under continuous fluorescent light for seven days. Emergence data was recorded daily according to the seedling evaluation of the Handbook of Association of Official Seed Analysts [1983]. Seedlings were harvested after two weeks and washed with deionized water after harvest. Afterwards they were separated into root and shoot for the determination of their fresh and dry weight. Dry weight was determined after oven drying the samples at 65°C for 48 h in oven.

ELECTRICAL CONDUCTIVITY OF SEED LEACHATES

All priming treatments were helpful in reducing the electrical conductivity of seed leachates. In general, the electrolyte leakage increased with increasing imbibition period including all treatments and control. After a longer period of imbibition from 1 to 24 h, all the priming treatments lowered down the electrolyte leakage in the seeds of both cultivars. After washing in distilled water, five seeds were weighed and soaked in 10 ml of distilled water at 25°C . Electrical conductivity of seed leachates was measured 0, 3, 6, 12 and 24 h after soaking using a conductivity meter expressed as $\mu\text{S cm}^{-1}\text{g}^{-1}$.

RESULTS

GERMINATION

Dry heat treatments significantly ($P \leq 0.05$) affected the germination and vigour of both the tomato varieties i.e. Nagina and Pakit gave similar results by thermal treatments. Dry heat treatment at 50°C for 24h resulted in lower T_{50} and MGT values and higher FGP, GI, GE, fresh and dry weight followed by chilling at -19°C for 24h compared with other thermal hardening techniques and untreated seeds.

In both cultivars maximum final germination percentage was recorded in dry heat treatment at 50°C for 24 h followed by chilling at -19°C for 24 h, while minimum final germination percentage was recorded in case of Heating + Chilling + Heating for 24h (Fig. 1). Maximum germination index was recorded in dry heat treatment at 50°C for 24h followed by the untreated seeds. Lowest mean germination time was noted in the seeds treated with dry heat treatment at 50°C for 24h followed by chilling at -19°C for 24h, while minimum MGT was recorded in Heating + Chilling + Heating for 24h (Fig. 2).

Time taken to 50% germination was minimum in seeds subjected to dry heating at 50°C for 24h. Chilling + Heating for 24h, followed by Chilling + Heating + Chilling for 24h (Fig. 3). Maximum FGP, GI, GE, fresh and dry weight was noted in seeds treated at 50°C, followed by that of seeds subjected to chilling at -19°C for 24h.

EMERGENCE

Dry heat treatments significantly ($P \leq 0.05$) influenced the seedling vigour of the two tomato varieties tested. Maximum invigoration (dormancy breakdown) as indicated by higher values of FEP was noted in seeds exposed to dry heat treatment 50°C for 24h, followed by chilling at -19°C for a period of 24h (Table 1). Minimum FEP was recorded in Heating + Chilling + Heating for 24h and control and they were statistically at par.

The heat treatment showed a significant effect on mean emergence time (MET); it took lesser time to emerge as compared to the control followed by chilling. Maximum MET was recorded in Heating + Chilling + Heating for 24h.

The heat treatment showed significant effect on shoot and root length in both tomato varieties. Maximum shoot length was recorded in unprimed seed, while maximum root length was depicted in case of heating at 50 °C for 24h Heating + Chilling + Heating for 24h. The time to 50% emergence is a useful parameter in determining the vigour and uniformity of emergence as the seeds which took lesser time to complete 50% emergence, are considered healthy which in turn resulted in better crop stand establishment. The heat treatment for 24h at 50°C reduced the time taken to 50% emergence; maximum time taken to 50% emergence was depicted by seeds treated with Heating + Chilling + Heating for 24h tomato seeds (Table 1).

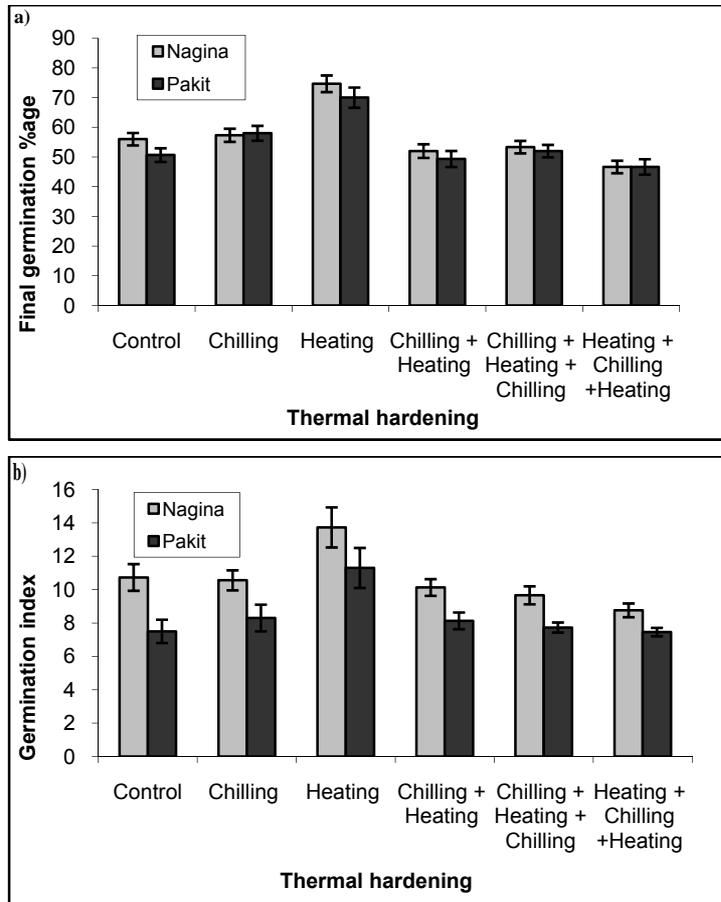


Fig. 1: Effect of different thermal hardening seed treatments on a) final germination % age and b) germination index of two tomato varieties, Nagina and Pakit.

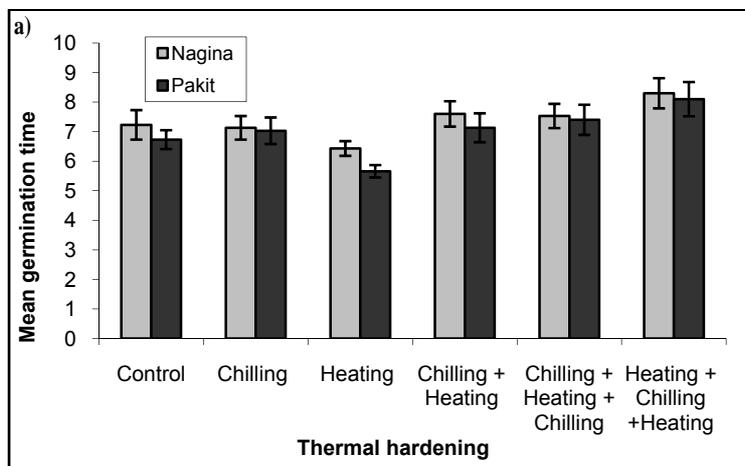


Fig. 2a: Effect of different thermal hardening seed treatments on mean germination time (days).

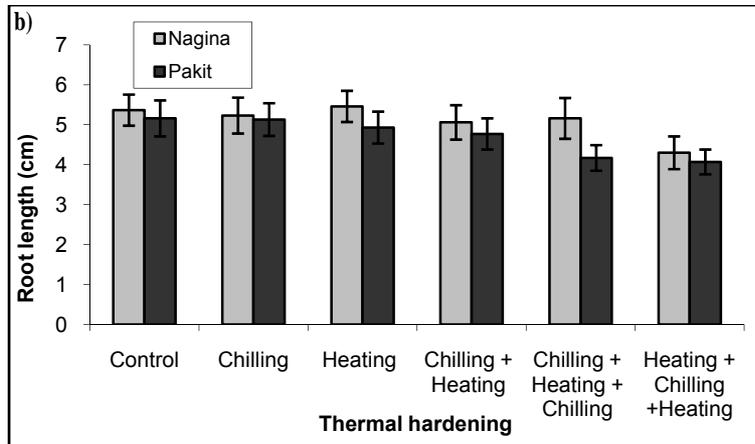


Fig. 2b: Effect of different thermal hardening seed treatments on root length of two tomato varieties, Nagina and Pakit.

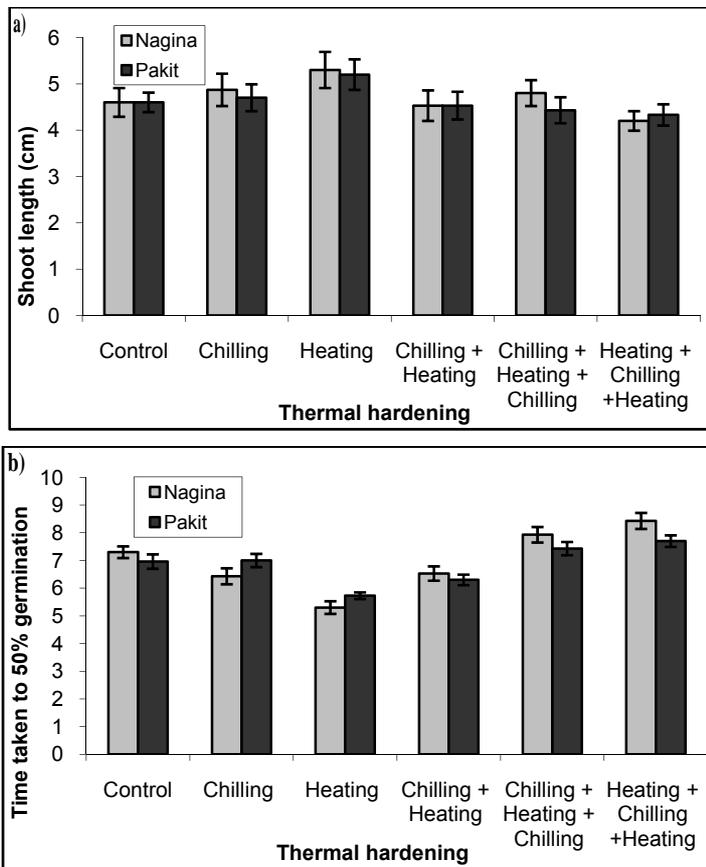


Fig. 3: Effect of different thermal hardening seed treatments on a) shoot length (cm) and b) time taken to 50% germination of two tomato varieties, Nagina and Pakit.

Table 1: Effect of thermal hardening on germination and seedling vigour of tomato.

variety	Priming	FEP	EI	MET (days)	T ₅₀ (days)	Root length (cm)	Shoot length (cm)	Fresh wt. (mg)	Dry wt. (mg)
<u>Nagina</u>	Control	48.00 b	15.93 b	8.30 c	7.70 ab	1.83 a	4.43 a	13.33 b	4.30 b
	Chilling	44.00 bc	14.83 bc	7.60 d	7.50 b	1.70 ab	3.93 ab	12.20 c	4.00 bc
	Heating	65.33 a	21.00 a	6.20 e	6.33 d	1.86 a	3.63 bc	16.03 a	5.80 a
	C + H	40.00 c	13.33 c	9.36 a	7.06 c	1.70 ab	3.33 cd	12.26 c	4.10 b
	C + H + C	41.33 c	13.33 c	9.26 ab	7.50 b	1.33 ab	3.23 cd	10.66 d	4.03 bc
	H + C + H	43.33 bc	13.80 c	9.00 b	7.86 a	1.23 b	2.96 d	9.80 e	3.70 c
	LSD at 0.05	5.2372	1.6976	0.2781	0.2619	0.5451	0.5237	0.7900	0.3583
<u>Pakit</u>	Control	45.66 b	13.56 bc	8.30 b	7.70 a	1.66 b	3.83 ab	13.33 b	4.63 b
	Chilling	46.00 b	15.16 b	7.60 c	7.50 a	1.60 b	3.93 ab	12.20 bc	4.43 b
	Heating	68.00 a	22.00 a	6.23 d	6.20 b	2.06 a	4.70 a	21.26 a	8.50 a
	C + H	37.33 c	11.73 c	8.83 a	7.83 a	1.60 b	3.23 b	11.26 c	4.10 b
	C + H + C	43.33 b	13.00 bc	9.13 a	7.83 a	1.36 bc	3.46 b	11.03 cd	4.00 b
	H + C + H	41.33 bc	12.26 c	9.03 a	7.86 a	1.20 c	3.20 b	9.93 d	3.96 b
	LSD at 0.05	4.8358	2.3905	0.5237	0.7263	0.3558	0.8993	1.2780	0.8470

Figures not sharing the same letters in a column differ significantly at p=0.05; FEP = Final emergence percentage, EI = Emergence index, MET = Mean emergence time, T₅₀ = Time taken to 50% emergence

Emergence index indicates the power of a seed to emerge; this is a good indicator of seed vigour because higher the value of emergence index more will be the vigour of a seed. Highest emergence index was noted in seeds treated with dry heat at 50°C for 24h followed by chilling at -19°C, while minimum emergence index was recorded in untreated (control) seeds.

Dry heat treatment 50°C for 24h significantly affected ($P \leq 0.05$) fresh weight of seedling (Fig. 4). Maximum seedling fresh weight (16.03 mg) was recorded in seeds treated with dry heat treatment 50°C for 24h followed by untreated seeds (13.33 mg), while minimum seedling fresh weight (9.8 mg) was recorded in seed treated with Heating + Chilling + Heating for 24h. Seed treatments had a significant effect on seedlings dry weight. Maximum seedlings dry weight was recorded in seed priming with dry heat treatment 50°C with (5.8 mg) followed by untreated seeds (4.3 mg), while minimum seedling dry weight (3.7 mg) was achieved in seeds treated with Heating + Chilling + Heating for 24h.

ELECTRICAL CONDUCTIVITY OF SEED LEACHATES

Pre-sowing seed treatments heating for 24h at 50°C was helpful in lessening electrolyte conductivity of seed leachates (Fig. 5). Generally the electrolyte leakage increased with increasing imbibition period including all treatments and the control. After a longer period of imbibition ranging from 1h to 24h all the priming treatments lowered the electrolyte leakage except control. Maximum decrease in electrolyte leakage was induced by heating for 24h at 50°C on all measuring periods. Hormonal priming with 10ppm IAA followed by 25ppm was successful in decreasing electrolyte leakage.

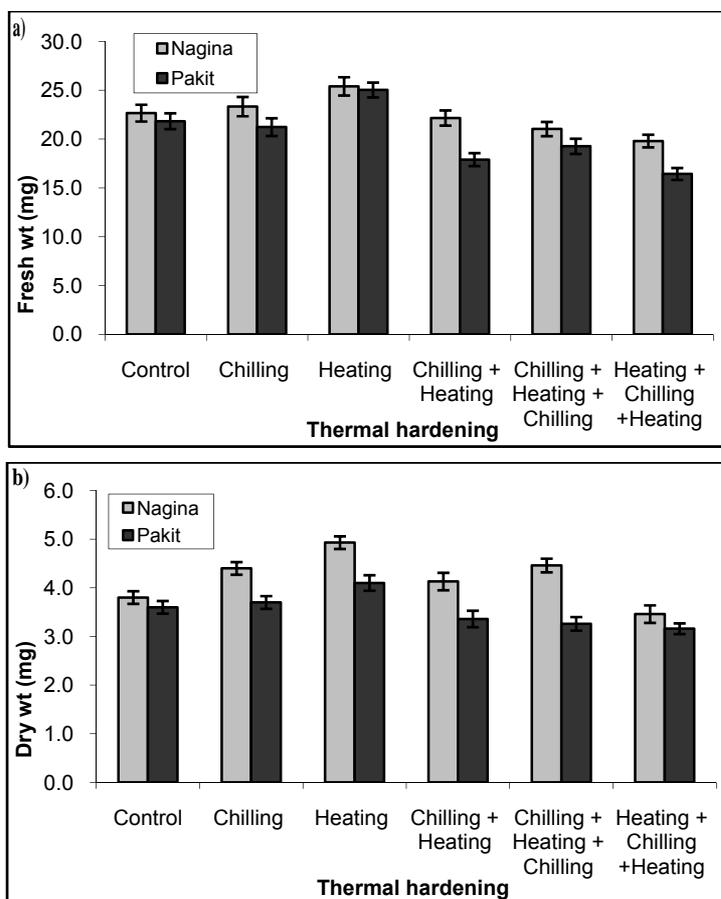


Fig. 4: Effect of different thermal hardening seed treatments on a) fresh weight (mg) and b) dry weight (mg) of two tomato varieties, Nagina and Pakit.

DISCUSSION

Earlier and more synchronized, germination and seedling emergence was observed in seeds treated with dry heat at 50 °C followed by seeds treated with chilling. The lower MET, T_{50} , MGT, and higher GI, FEP, FGP observed is primarily attributed to dormancy breakdown, because fresh seeds were used and postharvest dormancy has been reported in freshly harvested tomato seeds [Liu *et al.* 1996]. The thermal treatments resulted in seed invigoration, as it is generally used to break the dormancy of seeds our results are in line with findings of [Dadlani and Seshu, 1990]. The larger root most likely to be the result of earlier germination and seedling emergence that might be due to membrane integration as indicated by lower rate of seed leachates. Due to dormancy breakdown and invigoration both tomato varieties depicted higher GI and FGP followed by the seed chilling treatment. However other hardening

treatments like Chilling + Heating for 24h, Chilling + Heating + Chilling for 24h and Heating + Chilling + Heating for 24h could not gave better results as compared to untreated seeds. Lee *et al.* [2002] reported similar results in rice and Sacheti and Sacheti [1996] in legumes. Farooq *et al.* [2005] found that chilling in japonica and dry heat treatment in indica rice has resulted in invigoration compared with that of untreated seeds.

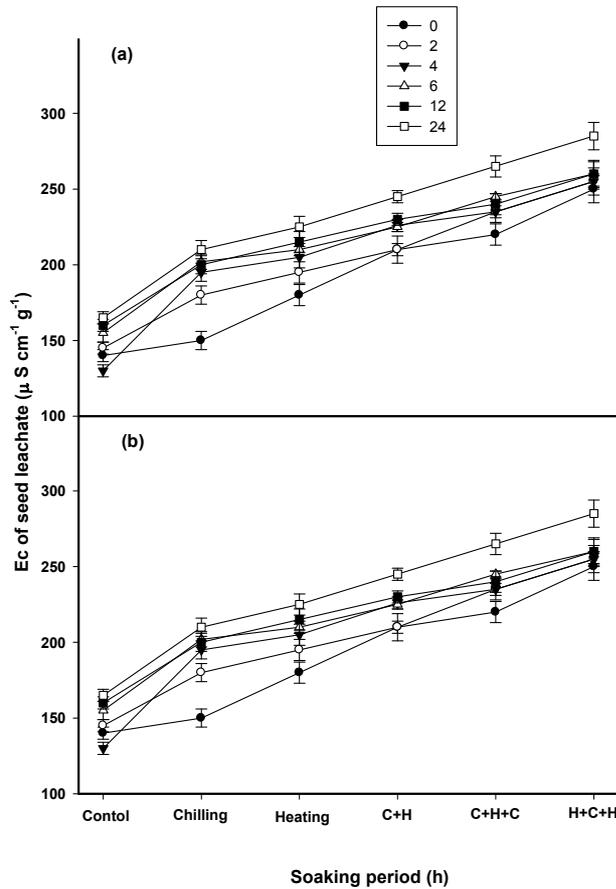


Fig. 5: Effect of thermal hardening treatments on electrical conductivity (EC) of seed leachates in two tomato varieties (a) Nagina and (b) Pakit.

A similar trend was noted in conductivity of seed leachates. The observation that seeds dry heat treated at 50°C for 24h resulted in lowest EC of leachates which may be the result of membrane repair, as earlier discussed by Farooq *et al.* [2005] in rice. This trend is confirmed incorrigible by EC of seed leachates which showed that due to better membrane repair as well as integrity of membrane resulted in lower rate

of seed leachates in chilled japonica rice and dry heat treated seeds in indica rice.

Dry heat treatments significantly ($P \leq 0.05$) influenced the seedling vigour of the two tomato cultivars tested which is shown by more root and shoot length as well as higher fresh and dry weight of the seedlings. Our results are in line with the results of Farooq *et al.* [2008].

It may be concluded that dry heat treatment at 50°C for 24h gave significant results followed by untreated seed as compared with other heating and alternate chilling treatments.

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