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Effects of Field Treatments on Barley Seed Sensitivity to Organic Seed Sanitation (Hot Water)

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ABSTRACT

Seed-borne diseases are one of the most important problems in organic farming systems because of limitation in sanitation methods. Hot water treatment is one of the nonchemical seed sanitation methods that has been predominantly used in cereals. Environmental conditions like water and nutrients availability during grain development can affect on subsequent seed sensitivity to hot water. The aim of this research was to study the effect of various organic and chemical fertilizers and water deficit systems on susceptibility to hot water as an organic seed sanitation method. The field experiment was set up in two successive growing seasons of 2007 and 2008. Seeds produced under different combinations of water deficit (during grain filling) and fertilizing systems were sterilized by hot water (50°C) and then the germination and deep freeze blotter tests were conducted on sterile and none sterile seeds. Results showed that germination rate increased by hot water treatment. Although, hot water treatment significantly decreased germination percentage of seeds produced under well-water (NS) condition but significantly increased the germination percent of seeds which were produced under water stress during generative stage (MS and SS). Application of organic fertilizers including vermicompost and biofertilizer (containing phosphorus and nitrogenous fixing bacteria) increased germination of barley seed after hot water sanitation, however, fertilization with chemical or integrated (chemical+biological) fertilizers decreased germination under the same treatment. Three detected fungi viz., *Fusarium* spp., *Alternaria* spp. and *Penicillium* spp. were detected on different seed lots which were controlled 50-90% with hot water. Hot water is able to control most of the pathogenic fungi with little negative effect on organically produced seeds and induction of water stress during grain filling can improve seed germination after hot water treatment.

Key words: Barely, hot water, organic fertilizer, water stress

INTRODUCTION

In organic farming there is a need to use healthy and vigorous seed to have a unique and good establishment preventing weed invasion to ensure reasonable healthy farm and enough yield. Seed-borne diseases are important in organic agriculture because of limitations in prevention and direct control methods.

Most important pathogenic fungal genera in barley are *Fusarium* spp., *Alternaria* spp., *Ustilago* spp. and *Drechslera* spp. which cause problems in barley production (Nielsen, 2003). Barley infection by *Fusarium* sp. decreased seed germination and caused production of mycotoxin and reduction of malt quality, lower seed quality (due to reduction in storage protein), cellulose and amylase (Argyris *et al*., 2003). Since, there are limitations in organic farming to use chemicals against pathogens and seed-borne diseases, some nonchemical seed treatments have been developed for seed sanitation besides using resistant varieties. The nonchemical seed treatments are divided to biological (Hokeberg *et al*., 1997; Gerhardson, 2002; Jensen *et al*., 2006; Neogi *et al*., 2008; Nor Afifah *et al*., 2010; Islam *et al*., 2001; Bhyan *et al*., 2007), biochemical (Kolasinska, 2008) and physical methods. Physical seed treatments includes washing fungi spore (Maude, 1996), brushing (Borgen, 2002; Borgen, 2005), electron radiation (Jahn and Puls, 1998), radioactive radiation (Cuero *et al*., 1986; Bagegni *et al*., 1990; Kobori *et al*., 2010), laser radiation (Belskii and Mazulenko. 1984), solarization (Luthra and Sattar, 1934), dry hot air (Evans *et al*., 1983) and hot or warm water (Tapke, 1924).

Thermal treatments were established for cereals by Jensen (1888). Among thermal treatments, hot or warm water treatment is an old, simple and easy to use method. There are evidences that combinations of water temperature and time of treatment have been studied on barley to control *Fusarium* (Kottapalli *et al*., 2003) and *Ustilago* (Koehler, 1935) but in some cases despite pathogens control, reduction in seed germination have occurred. It is important to find the best combination of water temperature and duration to control seed-borne diseases in barley without reducing germination percentage.

Groot *et al*. (2006) showed that seed maturity affect sensitivity to hot water treatment though less mature *Brassica oleracea* seeds were more adversely affected by seed-borne pathogens and were more susceptible to hot water treatment. It is assumed that physical or physiological quality of seed will affect the sensitivity of seed to hot water treatment. Seeds developed at different conditions, perform different qualities (Geetha *et al*., 2011). This experiment was conducted to examine the sensitivity of seeds produced under different fertilizing and water deficit systems, to hot water sanitation method.

MATERIALS AND METHODS

Seed production conditions: Field studies were conducted at the Experimental Farm of the College of Agriculture, University of Tehran, Karaj, Iran (35°56'N and 50°58'E with an altitude of 1312 m) during the 2006-2007 and the 2007-2008 cropping seasons. Seed of the spring cultivated barley Turkman used in this experiment was provided by the Seed and Plant Breeding Research Institute, Karaj, Iran.

The soil was a clay loam with a pH of 8.4 and 1.02 EC. Karaj has an average annual rainfall of 400 mm and it was about 450 mm for first year (2007), however, in second year (2008) the total rainfall was about 680 mm that most of the precipitation was as snow during January and February in 2008 (Table 1).

The experimental design was a split plot arrangement based on a randomized complete block design with four replications. The barley was sown in 2 by 5 m plots with 1 m alleys between replications on March 17th, 2007 and March 1st, 2008 at a rate of 300 seed m^{-2}, respectively. The treatments consisted of three irrigation regimes (main plots) and six fertilizing systems (sub-plots). The irrigation treatments were applied at different phenological stages of barley according to Zadoks (1974) scale and consisted of: (1) non-stressed (NS, full irrigation until the end of

Table 1: Total monthly precipitation, average monthly temperature, average monthly relative humidity and maximum air temperature (T_{max}) for the period 1 October to 31 July in 2007 and 2008, Karaj, Iran

	Precipitation (mm)		Temperature (°C)		Relative humidity (%)		T_{max} (°C)	
Month	2006-2007	2007-2008	2006-2007	2007-2008	2006-2007	2007-2008	2006-2007	2007-2008
October	71.200	5.50	18.9	17.6	51.0	43.0	31.0	30.0
November	16.000	33.20	8.4	11.6	61.0	44.0	22.2	25.0
December	62.900	69.80	1.3	3.6	70.0	64.0	9.6	16.6
January	45.900	475.20	1.9	-5.7	58.0	76.0	14.0	6.4
February	44.000	95.00	5.6	1.5	61.2	65.8	14.6	15.0
March	82.200	3.20	7.3	14.7	60.0	34.0	18.2	37.0
April	100.400	4.10	14.4	17.7	52.0	34.0	23.6	33.0
May	13.100	0.00	20.3	22.0	45.0	34.0	22.4	35.0
June	12.600	0.20	24.3	24.6	38.0	36.0	38.0	37.0
July	6.800	0.10	27.0	27.8	37.0	34.0	38.4	39.8

Table 2: Characteristics of applied fertilizers in 2007 and 2008

Parameter fertilizer	Organic carbon (%)	pH	EC (dS m^{-1})	P (mg kg^{-1})	N (mg kg^{-1})	K (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})
Chemical Fertilizer (CF)				460000.0	460000	500000				
Vermicompost (VC)	22.2	8.4	6.705	547.5	22950	4729	30.562	156.75	74.925	1666.5
Biofertilizer (BF)	Nitrogen and phosphorous biofertilizers were a complex of different free living nitrogen fixing and phosphorus solubilizing bacteria including *Azospirillum* and *Azetobacter* as nitrogen fixing bacteria and *Bacillus lentus* and *Pseudomonas putida* as phosphorus solubilizing bacteria									

physiological maturity), (2) moderate stress (MS, ceased irrigation from the beginning of flowering (Zadoks, 65) to the beginning of the grain filling stage (Zadoks, 70) and (3) severe stress (SS, ceased irrigation from the beginning of flowering stage to the end of physiological maturity). Fertilizing systems consisted of (1) no fertilizer (control) (NF), (2) phosphorous and nitrogen biofertilizers (Biofertilizer is a complex of different free living nitrogen fixing and phosphorus solubilizing bacteria) (BF), (3) 100% chemical fertilizer (NPK) (based on soil chemical analysis) (CF), (4) vermicompost (VC) (applied 5 t ha^{-1}), (5) 50% chemical fertilizer (NPK)+50% vermicompost (2.5 t ha^{-1}) (CV) and finally (6) 50% chemical fertilizer (NPK)+50% biofertilizer (CB), assigned to the sub plots. Fertilizers characteristics are presented in Table 2.

Application of chemical fertilizer was performed based on soil analysis. The amounts of N, P and K applied were 105 kg N ha^{-1}, 32 kg P_2O_5 ha^{-1} and 170 kg K_2O ha^{-1}, respectively. All P (triple superphosphate), K (K_2SO_4) and organic fertilizers were applied to the soil during seedbed preparation (as basal fertilizers), whereas one third of N (urea) was applied during seedbed preparation period (as basal fertilizer) and the rest as topdressing during the tillering and flowering stages, respectively. Full irrigation was performed at weekly intervals whenever soil moisture reached 50% available soil water in the root growth zone. There was no effective rain during post anthesis period (Table 1).

After harvest, the barley ears were threshed by hand and were equilibrated to 7-8% moisture content (by fresh weight) and stored at 4°C until the laboratory seed tests were performed.

Hot water treatment: Laboratory seed tests were performed at Plant Research International, Wageningen, Netherlands on harvested seeds from both experimental years (2007 and 2008). The hot water treatment was performed by incubating the seeds in water bath (Nega *et al*., 2003). Some preliminary tests were performed with different temperature and time to find best combination to control pathogenic organisms with little effect on germination. The combination of 30 min at 50°C was selected. The treated seeds were dried by blowing air at 20°C and 32% RH.

Germination test: Germination test was performed according to methods of the International Seed Testing Association (ISTA, 1999) on none treated and hot water treated seeds. Seeds were considered germinated when radicles emerged at least 2 mm. Seedling quality was evaluated after 7 days in respect to the number of normal and abnormal seedlings (Don, 2006). Parameters related to germination, such as maximum germination (G_{max}) and mean germination time (MGT, calculated by integration of the fitted germination curve) were calculated using the software package Seed Calculator V3.0 (Plant Research International, Wageningen, The Netherlands). The final number of germinated seeds after the germination period (7 days) were used in analysis of variance by transformation by function of:

$$y = \arcsin\sqrt{\frac{p}{100}}$$

where, p is the germination percentage.

Seed health test (deep freeze blotter test): Seeds from none treated and hot water treated batches were placed in a tray on a sterile thick filter paper (T10D) and 60 mL of sterile water was added. The trays were incubated in dark at 20°C for 24 h to hydrate the seeds, then the incubation continued for another 24 h in a freezer at -20°C to kill the embryo. The treated seeds subsequently were kept for 10 days at 20°C under 12 h near-ultraviolet light/12 h dark cycle to stimulate fungal growth (Elwakil *et al*., 2009; Srivastava *et al*., 2011). The barley seeds were then examined under a binocular microscope for the presence of fungi. Fungal occurrence on seed was expressed as percentage of seed where a fungal species was identified.

Statistical analysis: Data were statistically analyzed separately for each production year by analysis of variance (ANOVA) using MSTATC (Michigan State Univ., East Lansing, MS, USA) and SAS (SAS Inst., 1990) programs. Homogeneity of error variances was tested using Bartlett's Chi-square. Since the χ^2 was not significant, a combined analysis of the data was performed for two years.

RESULTS AND DISCUSSION

Seed quality tests: Hot water treatment

Mean germination time (MGT): There was a significant interaction between irrigation regime and fertilizing system across hot water treatment on MGT. Drought stress in field significantly decreased mean germination time of seeds produced under all fertilizing systems over two years (Fig. 1). MGT decreased significantly under severe water stress (SS) in all seed types. The shorter MGT corresponds to the effect of water stress in field on seed size (Maleki Farahani *et al*., 2010). Though smaller seeds produced under water stress conditions need less water for germination.

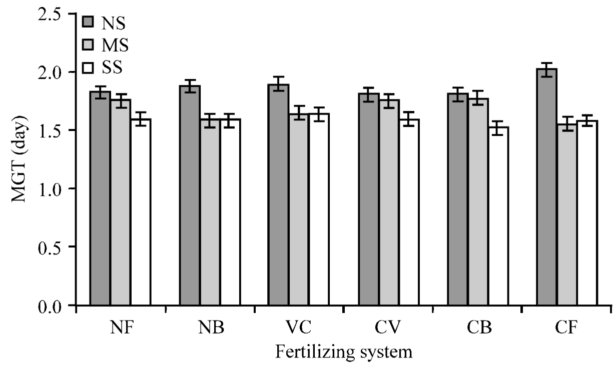

Fig. 1: Effects of irrigation regimes and fertilizing systems on mean germination time of barley seeds. NS: Full irrigation until the end of the plant physiological maturity, MS: Ceased irrigation from the beginning of flowering (Zadoks, 65) to the initiation of seed filling stage (Zadoks, 70), SS: Ceased irrigation from the initiation of flowering stage (Zadoks, 65) to the end of the physiological maturity, NF: No fertilizing, NB: Phosphatic and nitrogenous biofertilizer, VC: Vermicompost, CV: 50% chemical fertilizer including NPK+50% vermicompost, CB: 50% chemical fertilizer including NPK+50% biofertilizer and CF: 100% chemical fertilizer. Bars indicate±SD of means

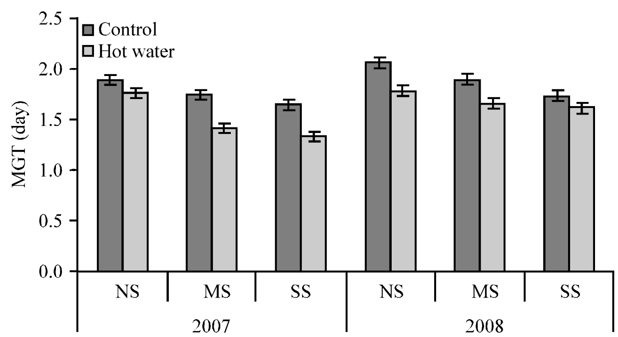

Fig. 2: Effects of year, irrigation regime and hot water on mean germination time of barley seeds. NF: No fertilizing, NB: Phosphatic and nitrogenous biofertilizer, VC: Vermicompost, CV: 50% chemical fertilizer including NPK+50% vermicompost, CB: 50% chemical fertilizer including NPK+50% biofertilizer and CF: 100% chemical fertilizer. Bars indicate±SD of means

There was a significant interaction between year, irrigation regime and hot water treatment on MGT. Hot water treatment decreased MGT compared to control (without hot water) in seeds produced in both years under all water stress conditions. However, no significant reduction in MGT was observed under SS in 2008 (Fig. 2). MGT of water stressed seeds were less than non stressed seeds. Seeds treated with hot water had significantly less MGT (across all fertilizing systems) compared to control (Fig. 3). Seed sanitation with hot water decreased MGT in all fertilizing systems; however, a significant difference was only seen in organic fertilizers (NB and VC) compared to control (none fertilized).

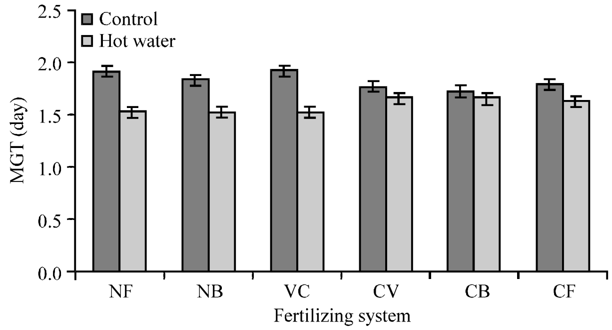

Fig. 3: Effects of hot water and fertilizing systems on mean germination time of barley seeds. NF: No fertilizing, NB: Phosphatic and nitrogenous biofertilizer, VC: Vermicompost, CV: 50% chemical fertilizer including NPK+50% vermicompost, CB: 50% chemical fertilizer including NPK+50% biofertilizer and CF: 100% chemical fertilizer. Bars indicate±SD of means

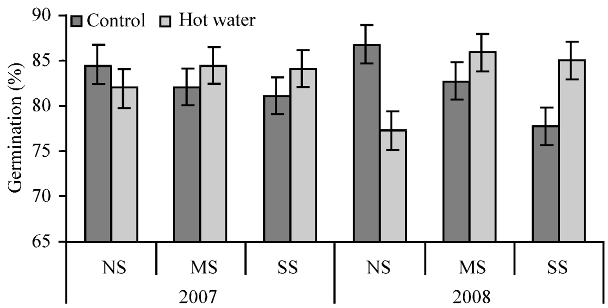

Fig. 4: Effect of year, irrigation regimes and hot water on germination percentage of barley seeds. NS: Full irrigation until the end of the plant physiological maturity, MS: Ceased irrigation from the beginning of flowering (Zadoks, 65) to the initiation of seed filling stage (Zadoks, 70), SS: Ceased irrigation from the initiation of flowering stage (Zadoks, 65) to the end of the physiological maturity. Bars indicate±SD of means

It seems that hot water enhances the activity of seed enzymes that stimulate the commencement of germination. On the other hand, the presence of fungi on none treated seeds might have postponed the germination. Shorter MGT in seeds produced under water stress or organic fertilizers in field after hot water treatment, showed that in areas in which the plants are grown under water and nutrient stress, sanitation of seed with hot water tends to enhance germination which in turn provides with better completion ability for plants to over dominate the weeds to produce a good and unique establishment.

Germination percentage: There was a significant interaction of year, irrigation regime and hot water on germination percentage. The germination percentage of none sterile seeds produced under water stress, decreased significantly over two years, however, water stress in field increased germination of subsequent seeds after hot water treatment (Fig. 4). It seems that drought stress in field induced drought tolerance in developed seed which consequently the stressed seeds showed better germination after hot water treatment. On the other hand seeds produced under SS and MS water stress treatments matured earlier than NS seeds, thus it can be concluded that NS seed was

Table 3: Fungi infection of barley seed before and after hot water treatment

Fungi	Control (without hot water) (%)	30 min in 50°C (%)
Fusarium sp.	96	10
Alternaria sp.	92	8
Penicillium sp.	8	4

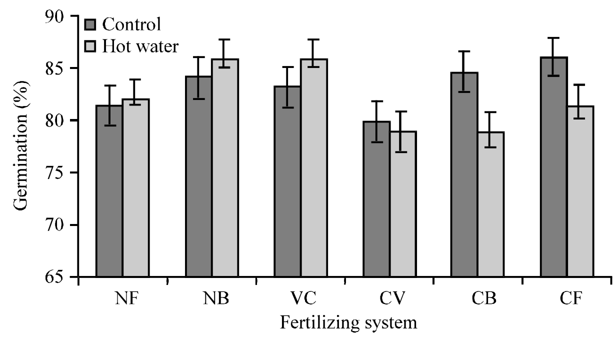

Fig. 5: Effect of fertilizing systems and hot water on germination percentage of barley seeds. NF: No fertilizing, NB: Phosphatic and nitrogenous biofertilizer, VC: Vermicompost, CV: 50% chemical fertilizer including NPK+50% vermicompost, CB: 50% chemical fertilizer including NPK+50% biofertilizer and CF: 100% chemical fertilizer. Bars indicate±SD of means

less mature than SS and MS seeds and herein less mature seeds germinated less than mature seeds. These results are confirmed by Groot *et al.* (2006) who found premature seeds of cabbage and carrot were more susceptible to physical seed sanitation including hot water treatment. Germination of NS seeds after hot water treatment decrease compared to non treated seeds which had more fungi contamination. It is in contrary with results of Konstantinova *et al.* (2002) who found barley seed lot with more fungi contamination had less germination.

Interaction effect of fertilizing system and hot water was significant on germination. Seeds produced with organic fertilizers (NB and VC) showed significantly higher germination compared to other fertilizing treatments moreover their germination percentage significantly increased after treatment with hot water (Fig. 5). Germination in seed produced under conventional (CF) and integrated (CV and CB) fertilizing systems, significantly decreased after hot water treatment. Nitrogen availability in conventional and integrated systems is more than organic treatments, as Scott (1969) described, higher nitrogen availability postpones crop maturity, thus the seeds produced under CF, CV and CB fertilizing systems had less maturity though it made them more susceptible to hot water. It seems that different fertilizers have adverse effect on seed structure and it's tolerance to hot water.

Effect of hot water on the occurrence of fungi: In seed health analysis three fungal genera of *Fusarium* spp., *Alternaria* spp. and *Penicillium* spp., were identified. on seeds. *Fusarium* spp. and *Alternaria* spp., were the most common fungi observed on 95-100% of the seeds. On average over two years incidence percentage of fungi decrease significantly after treatment with 50°C hot water (Table 3). Kottapalli *et al.* (2003) also found hot water decreased infection level of seed by *Fusarium* spp.

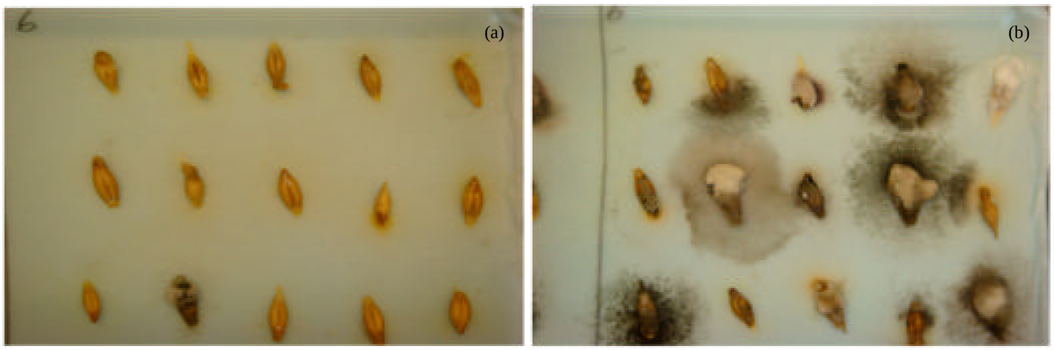

Fig. 6(a-b): Fungi infection of barley seed (a) After and (b) Before hot water

Seed infection by *Fusarium* spp. and *Alternaria* spp., were better controlled by hot water treatment than *Penicillium* spp., *Fusarium* spp., fungi as an important pathogen cause a lot of diseases (Konstantinova *et al.*, 2002) in cereals including ear blight, (head or scab blight), brown foot rot and seedling blight. This pathogen also has the potential to produce mycotoxins which are harmful for livestock, humans and malt industry. Taking these problems into consideration, prohibitions to use chemical fungicides to control *Fusarium* spp. in an organic farming system, as the results of this experiment support the efficiency of hot water treatment to prevent the incidence of diseases caused by *Fusarium* spp. in barley (Fig. 6).

CONCLUSIONS

As data showed, treatment of barley seed with 50°C hot water for 30 min can significantly reduce seed fungi contamination with little negative effect on germination. Hot water treatment increased germination speed regardless to germination percentage, it can help earlier germination than weed that it is an important parameter to establish a unique field in organic farming to use water and nutrient reserves properly. Hot water treatment reduced germination percentage of seeds produced under conventional and integrated fertilizing systems. However, the same treatment increased germination percentage in seeds produced with organic fertilizers or under water stress in field. It can be concluded that to reduce the negative effect of hot water on germination, it is useful to induce water stress in field to promote germination after hot water treatment. In overall it seems that hot water treatment has the ability to apply in organic farming systems as an efficient sanitation method besides its other characteristics like simplicity and low cost.

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