

Original Research Article

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Unclogging Seed Borne Pathogens to Prevent Diseases in Capsicum

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ABSTRACT

The present investigations was carried out to study the effect of hot water seed treatment comprised of different ranges of temperature (47-49, 50-52 and 53-55 °C) and discrete duration of time (30, 45 and 60 min.). The seeds of bell pepper and their most important seed-borne pathogens (*Alternaria* spp., *Curvuleria* spp., *Penicillium* spp., *Fusarium* spp. *Colletotrichum* spp.) have been investigated in laboratory. These numbers of infected seeds were observed and recorded daily and per cent incidence was evaluated. Furthermore, per cent ungerminated seeds and incidence of various diseases like, damping-off, anthracnose, wilt, cercospora leaf spot and virus attack was recorded in nursery as well as in field under protected cultivation. Under *in vitro* conditions, the hot water treated seed with temperature 50-52°C for 30 min. showed significantly lower seed microflora (%) as compared to untreated seed (control). In nursery condition, the hot water treated seed, same temperature 50-52°C for 30 minutes showed lower damping-off and virus incidence as compared to control with 22.75 per cent post emergence damping-off and 5.56 per cent respectively. Though, the incidence of damping off and virus attack is minimum in case of the hot water treated seed same temperature 53-55°C for 60 min. i.e. 5.47 per cent and 1.11 per cent respectively but this high temperature for such long time have strongly affected germination. That's why ungerminated seed percentage in case of is hot water treated with 53-55°C for 60 min. is 41.11 which is significantly very high compared to the hot water treated with 50-52°C for 30 minutes i.e. 12.22 per cent. Under protected condition the hot water treated with 50-52°C for 30 min. showed lower incidence of diseases like anthracnose, cercospora leaf spot, wilt and virus as compared to control. It may be concluded that hot water seed treatment at 50-52°C for 30 min. proved effective in reducing the incidence of diseases like damping-off, anthracnose, cercospora leaf spot and viruses in bell pepper cultivar Solan Bharpur without any ill effect on the germination of seeds.

Keywords

Hot water seed treatment, Bell pepper, Seed microflora, Disease incidence and *Capsicum annuum*

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Introduction

Bell pepper (*Capsicum annuum* L.), also known as sweet pepper, capsicum or Shimla

mirch, belongs to family solanaceae. It is a high value vegetable and an important cash crop grown throughout the world. It has attained a status of high value crop in recent

years because of its delicacy and pleasant flavour coupled with rich content of ascorbic acid and other vitamins and minerals (Agarwal *et al.*, 2007). Bell pepper is mainly cultivated as summer and rainy season crop. Because of high humidity and soil moisture coupled with moderate temperature, the incidence of various diseases is high. Some diseases are seed borne in nature viz., anthracnose (*Colletotrichum capsici*), Cercospora leaf spot (*C. capsici*), bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*), Bacterial wilt (*Pseudomonas solanacearum*), Bacterial canker (*Clavibacter michiganensis*) and viruses like Tomato spotted wilt virus (TSWV). To avoid the occurrence of such diseases, seed treatment with various chemicals has been recommended from time to time (Gupta and Thind, 2006). But in present day agriculture, use of chemicals for crop production is discouraged. Seed borne diseases of bell pepper are considered as alarming problem in organic farming systems because of the non-ecofriendly chemical control methods. Hence, other alternative treatments for disease control have been developed and hot water treatment is one of them.

Hot water soaking is a very old practice but has revived in this era of organic farming to control many seed-borne diseases by using temperatures hot enough to kill the organism but not quite hot enough to kill the seed and it is still being used as a very effective alternative (Floyd, 2005; Muniz, 2001). Hot water seed treatment is thermo physical method of plant protection. At the end of the 19th century, for control of control loose smut (*Ustilago nuda*) the hot water seed treatment was applied to in cereals (Jensen, 1888). During storage of oak seed, hot water seed treatment has been recommended against the fungus *Ciboria batschiana* (Natzke, 1997). Further examples for application of the method are shown by Baker (1962),

Gabrielson (1983), Hoffmann *et al.*, (1994) and Jahn *et al.*, (2000). Hot water treatment is of more importance for organic farming (Trueman and Wick, 1996). It could also become an alternative method for conventional farming especially in case of failure of chemicals permitted for seed treatment. The present investigation was designed to study the effect of different temperature and time combinations of hot water seed treatment on incidence of diseases and seed microflora in bell pepper.

Materials and Methods

The present experiment was carried out at Experimental Farm and Laboratory of Department of Seed Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Solan. The experimental farm of Department of Seed Science and Technology is located at an altitude of 1183 meters above mean sea level with latitude of 30.51°N and longitude of 77.09°E the mid- hill zone of Himachal Pradesh, India observed with GARMIN'S GPS 12 Personal Navigator. The soil texture of polyhouse was loam to clay loam having pH ranging from 6.85-7.05. The healthy, disease free, bold and uniform seeds of bell pepper cv. Solan Bharpur, were obtained from Department of Seed Science and Technology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.). The obtained seeds were treated with hot water in automatically controlled hot water bath tub at different temperature range for discrete time period (Table 1). The *in vitro* experiment was laid in Completely Randomized Block Design with four replications taking 50 seeds per replication. The experiments in nursery condition and protected condition were both laid in Randomized Block Design with ten treatments replicated three times taking 30 seedlings and 6 plants per replication respectively.

Hot water bath works automatically controlling temperature with time. Firstly seeds were soaked in normal water for 15 min. wrapped in muslin cloth. Then, poured about 3 l. water in the device and it was connected with electricity. With the help of heater coil, the device was heated, with the time and thermostat bulb the device was regulated to the desired temperatures such as (47- 49), (50-52) and (53-55) °C was maintained. With the thermometer the desired temperature was denoted. Fixing temperature and time Thermostat bulb was regulated to fix the desired temperature. At the end of the treatment, seeds were taken out of the hot water bath and spread on blotter paper. After that the blotter paper with seeds was placed in shade for drying of seeds. Then, the seeds were used for test. Seed microflora (%) was observed by following standard Petri plate method as per the ISTA. The seeds of bell pepper were kept in Petri plates (50 seeds per replication). These plates with seeds incubated at 25°C temperatures for 14 days. Numbers of infected seed were observed and per cent incidence was observed by following the method:-

Seed microflora % = (No. of infected seed / Total no. of seed) x 100

Ungerminated seed (%) was also recorded in nursery by following formula:

Ungerminated seed (%) = (Ungerminated seeds / Total number of seeds planted) x 100

Incidence of damping-off, virus attack and disease incidence was recorded in nursery and field under protected condition by using the following formula:

Disease incidence (%) = (Number of diseased seedlings per plot / Total number of seedlings per plot) x 100

The statistical analysis of the data generated was done as per design of the experiment as suggested by Gomez and Gomez (1984).

Results and Discussion

Seed contamination with microflora (*Alternaria spp.*, *Curvuleria spp.*, *Penicilium spp.*, *Fusarium spp.*, *Colletotrichum spp.*) and total microflora was predominant in control group (10.00, 3.00, 12.00, 11.00, 7.00 and 43.00 %, respectively), whereas the least predominant microflora per cent (1.00, 1.00, 3.00, 2.00, 0.00 and 7.00, respectively) were recorded in seeds soaked at 53-55 °C for 60 min (T_{3t3}). Seeds soaked at 50-52°C (T_{2t1}/ T_{2t2}/ T_{2t2}) showed intermediate values between the two extremes.

The temperatures and durations of hot water treatment resulted in significant reduction in the mean incidence of various fungi as the fungi did not tolerate the higher temperature range. Reported 50-52°C temperature for 15-30 min. most suitable against important seed microflora in solanaceous crops like brinjal. Similarly Nega *et al.*, (2003) reported that heating carrot seed to 54°C in water for 20 minutes completely eradicated *A. dauci* found that hot water treatment at 53°C for 10 to 30 min of carrot, cabbage, celery, parsley, and lamb's lettuce seed controlled *Alternaria dauci*, *A. radicina*, *A. alternata*, and *A. brassicicola*.

Hermansen *et al.*, (1999) reported that heating carrot seed to 54°C in water for 20 min completely eradicated *A. dauci*.

Temple *et al.*, (2013) found hot water treatment at 50°C for 20 min. can significantly reduce incidence of fungus (*Cladosporium spp.*, *Fusarium spp.*, and *Alternaria spp.*) (Table 2 and 3).

Table.1 Treatment details

Treatment	Temperature (°C)	Time (min)
T ₁ t ₁	47-49	30
T ₁ t ₂	47-49	45
T ₁ t ₃	47-49	60
T ₂ t ₁	50-52	30
T ₂ t ₂	50-52	45
T ₂ t ₃	50-52	60
T ₃ t ₁	53-55	30
T ₂ t ₂	53-55	45
T ₃ t ₃	53-55	60
T ₀ (control)	Untreated seeds	

Table.2 Effect of hot water seed treatment on seed microflora in bell pepper cv. Solan Bharpur

Treatments	Seed microflora (%)					
	<i>Alternaria</i> spp.	<i>Curvuleri a</i> spp.	<i>Peniciliu m</i> spp.	<i>Fusarium</i> spp.	<i>Colletotrichum</i> spp.	Total Seed microflora
T ₁ t ₁	7.00	2.00	9.00	8.00	4.00	30.00
T ₁ t ₂	7.00	2.00	7.00	6.00	3.00	25.00
T ₁ t ₃	6.00	2.00	5.00	5.00	3.00	21.00
T ₂ t ₁	6.00	2.00	4.00	3.00	1.00	16.00
T ₂ t ₂	4.00	1.00	4.00	4.00	1.00	14.00
T ₂ t ₃	4.00	1.00	4.00	3.00	1.00	13.00
T ₃ t ₁	4.00	1.00	4.00	3.00	0.00	12.00
T ₃ t ₂	2.00	1.00	4.00	2.00	0.00	9.00
T ₃ t ₃	1.00	1.00	3.00	2.00	0.00	7.00
T ₀	10.00	3.00	12.00	11.00	7.00	43.00
CD at 5%	1.07	NS	0.59	0.94	0.65	3.74

Table.3 Effect of hot water seed treatment on disease incidence in bell pepper cv. Solan Bharpur under nursery conditions

Treatments	Characters		
	Ungerminated seeds (%)	Damping-off (post emergence) (%)	Virus (%)
T ₁ t ₁	31.11	54.84	15.55
T ₁ t ₂	25.56	35.77	13.33
T ₁ t ₃	17.78	25.53	10.00
T ₂ t ₁	12.22	22.75	5.56
T ₂ t ₂	23.33	23.16	4.44
T ₂ t ₃	27.78	15.34	3.33
T ₃ t ₁	26.67	18.07	3.33
T ₃ t ₂	35.55	7.034	2.22
T ₃ t ₃	41.11	5.47	1.11
T ₀	30.00	63.59	23.33
CD at 5%	3.65	7.00	0.86

Table.4 Effect of hot water seed treatment on disease incidence in bell pepper cv. Solan Bharpur under protected conditions

Treatments	Disease incidence (%)			
	Anthracnose	Cercospora leaf spot	Wilt	Virus
T ₁ D ₁	23.18	38.89	0.00	27.78
T ₁ D ₂	19.79	27.78	0.00	22.22
T ₁ D ₃	11.27	11.11	0.00	16.67
T ₂ D ₁	9.59	16.67	0.00	11.11
T ₂ D ₂	10.63	11.11	0.00	11.11
T ₂ D ₃	7.59	5.56	0.00	5.56
T ₃ D ₁	7.62	5.56	0.00	5.56
T ₃ D ₂	4.97	0.00	0.00	5.56
T ₃ D ₃	3.16	0.00	0.00	5.56
T ₀	24.23	44.44	5.56	44.44
CD at 5%	0.30	19.55	NS	20.46

Disease incidence and ungerminated seed (%) under nursery conditions

Significantly higher ungerminated seeds (41.11%) were recorded, when seeds were soaked at 53-55 °C for 60 min (T₃t₃) and lowest proportion of ungerminated seeds (12.22%), when soaked at 50-52°C for 30 min (T₂t₁). Seedlings kept as control had intermediate proportion of ungerminated seeds (30.00%). In contrast, the per cent damping off (post-emergence) was highest in control groups (63.59 %) and lowest (5.47%) in seeds were soaked at 53-55°C for 60 min (T₃t₃). Seeds soaked at 50-52°C (T₂t₁/ T₂t₂/ T₂t₂) showed intermediate values between the two extremes. Similarly, the presence of virus (%) was shown to be highest in control (23.33 %) and lowest (1.11 %) in seeds soaked at 53-55°C for 60 min (T₃t₃). In case of T₂D₁ (seeds soaked at 50-52 °C for 30 min) incidence recorded due to post emergence damping-off and virus (%) was 22.75 per cent and 5.56 per cent respectively which was significantly less as compared to control. Hot water seed treatment has been used to control many seed borne diseases by using

temperature hot enough to kill organisms but not quite hot to kill the seed (Miller,2005). It is believed that hot water treatment may activate pathogenesis-related (PR) proteins. PR proteins coded by host plant genes are induced by pathogen infection or related situations, and are thought to play a major role in plant defence responses against a wide variety of pathogens (Van Loon and Van Strien, 1999). Among the PR proteins, the most characterized enzymes are those of group 2 that have β -1, 3-glucanase activity (Kauffmann *et al.*, 1987) and group 3 that have chitinase activity and both hydrolyze polymers of fungal cell walls and are, therefore, thought to be involved in the plant defence mechanism against fungal infection (Schlumbaum *et al.*, 1986, Collinge *et al.*, 1993). These enzymes were capable of inducing plant resistance against pathogen infection in transgenic plants which over express chitinase and β -1, 3-glucanase genes (Zhu *et al.*, 1994; Jach *et al.*, 1995). Hot water temperature at 50-53 °C has found as the optimum ranged for reducing various seed borne diseases including fungi, bacteria and viruses of vegetable crops and other crops by

earlier workers (Nega *et al.*, 2003; Nandini and Kulkarni, 2015). The use of hot water treatments to control seed-borne diseases is regarded as very efficient in destroying pathogens borne both, outside the testa, such as TSWV (Edmund and Pottorff, 2009) and inside the seed testa (Miller and Ivey, 2004;). Zitter *et al.*, (1989) have reported that hot water seed treatment at 50-55 °C for 25-30 min. controlled viruses affecting tomato, such as the *Tomato mosaic virus* (TMV), *Pepino mosaic virus* (PMV), *Tobacco mosaic virus* (YMV) and *Tomato spotted wilt virus* (TSWV). Winter *et al.*, (1997) compared hot water treatments (52 °C for 10 min.) of cereal seeds were with seed fungicides and found that it was equally effective in controlling damping-off disease. The mode of action of hot water seed treatment could be direct killing of seed borne inoculum in and on the seed.

Disease incidence under protected conditions

Hot water treatment of seeds has significant effect on disease incidence under protected condition (Table 4). Incidence (%) of antracnose, cercospora leaf spot, wilt and presence of virus was highest in control (24.23, 44.44, 5.56, and 44.44, respectively) and lowest in T3t3 (4.97, 0.00, 0.00 and 5.56, respectively). However, in the remaining treatment-time combinations, the values remained intermediate between two extremes. The probable reasons behind the reduced incidence of various diseases after hot water seed treatment were found to be the killing of seed borne inoculum of these pathogens due to increased temperature. Although, elimination of seed microflora was effective, seed quality i.e. percent ungerminated seeds deteriorated with higher temperature-time combinations. Muniz (2001) reported Hot water treatment as potent practice to neutralise the seed borne organisms but not

quite enough to kill the seed. Similarly, Miller and Ivey (2004) and Miller and Ivey (2005) reported reduction in the occurrence of anthracnose, bacterial canker, bacterial spot, bacterial wilt and bacterial speck after treatment of seeds of tomato and bell pepper with hot water at 50 to 55°C for 25 to 30 minutes. Treatments of bell pepper seed with hot water at 45°C for 15 min or 53°C for 4 min prior to storage at 8°C reduced the incidence of fungal infections (Aguilar *et al.*, 1998). This method is more eco-friendly and effective compared to chemical treatment, however, they can cause the loss of seed viability (Meah, 2004). Seed of okra (*Abelmoschus esculentus*) when treated with hot water at 52°C for 30 min. resulted in the improvement of crop, both in greenhouse and field conditions (Begum and Lokesh, 2012). Enotomo *et al.*, (2002) described hot-water treatment as an alternative method to hypochlorite treatments for disinfecting pathogenic bacteria in seeds for alfalfa.

It may be concluded that hot water seed treatment at 53-55°C for 60 min. can be effectively utilized in reducing the incidence of seed microflora and various seed borne diseases, but this temperature-time combination has subsequently increased ungerminated seed (%) so hot water seed treatment at 50-52°C for 30 min. could be considered as an holistic approach as it has reduced seed microflora and seed borne disease with least ungerminated seed (%).

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