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EFFECT OF SEED TREATMENT WITH SODIUM HYPOCHLORITE AND HOT WATER ON SEED-BORNE FUNGI AND GERMINATION OF OKRA SEED

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Abstract

Effect of seed treatment with sodium hypochlorite (NaOCl) and hot water were investigated on the incidence of seed-borne fungi and germination of okra seeds. Seeds were treated with NaOCl solution maintaining the following treatments: 0.1% NaOCl solution for 3 min, 0.1% NaOCl solution for 5 min, 0.5% NaOCl solution for 3 min, 0.5% NaOCl solution for 5 min, 1% NaOCl solution for 3 min and 1% NaOCl solution for 5 min. In case of hot water, the treatments were 48°C for 10 min, 48°C for 12 min, 48°C for 15 min, 50°C for 10 min, 50°C for 12 min, 50°C for 15 min, 52°C for 10 min, 52°C for 12 min at 52°C for 15 min and control. Seed treatment with NaOCl and hot water reduced the seed-borne infection of *Aspergillus* spp., *Fusarium* spp., *Macrophomina phaseolina*, *Colletotrichum dematium* and *Chaetomium* spp. All the three concentrations of NaOCl decreased the prevalence of seed-borne fungi and increased seed germination. Higher concentration (1.0%) with longer period treatment (5 min) was the most effective for reducing fungal infection and increasing seed germination of okra. Hot water treatment at 48, 50 and 52°C for 10-15 min were effective to control the seed-borne infection of okra seed. However, higher temperature (52°C) with longer period (12-15 min) were more effective in reducing seed-borne infection. But seed treatment at 50°C for 12-15 min and at 52°C for 10-15 min negatively affected seed germination. Among the hot water treatments, seed soaking at 48°C for 12 min gave maximum germination (63%) compared to control (57%). Thus seed treatment with 1% NaOCl for 5 min or soaking seeds in hot water at 48°C for 12 min or 50°C for 10 min could be used for minimizing the incidence of seed-borne fungi and also hasten the germination of okra seeds.

Keywords: Seed soaking, seed-borne fungi, control, blotter method.

Introduction

Okra (*Abelmoschus esculentus* L.) is a familiar nutritious vegetable, belongs to the family Malvaceae which is grown annually in tropical and sub-tropical parts of the world. Having many factors behind the low production of okra, among them seed-borne fungal diseases are important. Different seed-borne fungal pathogens such as *Aspergillus* spp., *Fusarium* spp., *Macrophomina phaseolina*, *Colletotrichum dematium* etc. causing diseases like seedling blight, stem rot, anthracnose and die-back of okra (Fakir, 2000). Fungi that affect okra seeds include*; Alternaria alternata, Aspergillus niger, Curvularia lunata, Fusarium oxysporum, F. solani, F. moniliforme, Macrophomina phaseolina, Rhizoctonia solani, Stemphylium botryosum, Penicillium digitatum, Pythium aphanidermatum* (Al-Kassim and Monawar,

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2000; Odofin, 2010). Control of these seed-borne fungi is essential to increase the production of okra successfully.

Sodium hypochlorite (NaOCl) is widely used in laboratories to prevent contamination. It is a potential disinfecting agent and widely used for seed surface disinfection (Kumlachew, 2014; Kaur *et al.,* 2014). NaOCl releases oxygen gas as a by-product which is toxic to all kinds of bacteria, fungi and viruses, by oxidizing biological molecules such as proteins and nucleic acids (Bloomfield and Arthur, 1991). It is also known to favour seed germination or to overcome seed dormancy of different species (Igbinnosa and Okonkowo, 1992; Varasteh *et al*., 2015). Higher concentrations of NaOCl may have negative effects both on the germination and seedling growth in some plant species (Hsiao and Quick, 1984; Sen *et al.,* 2013). It was suggested that weak acids such as hypochlorous (HClO), overcome dormancy by lowering the pH and promoting oxygen uptake. This compound not only protects germinating seeds from all kinds of pathogens but also facilitates the leaching of toxic compounds and scarification of seed coat to improve the permeability for water and oxygen (Bohm, 2003).

Freshly harvested as well as stored okra seeds sometimes fail to germinate because of impermeable (hard) seed coat like other species of Malvaceae. Such seeds show staggered germination resulting in uneven plant stand. Germination of such seeds can be enhanced by making their seed coats permeable to water through various physical treatments. Among them hot water treatment is the cheapest and quite effective method. Hot water treatment is probably the most commonly used physical treatment to control seed-borne fungi and bacteria without killing the seed. It kills most of the disease causing organisms on or within the seeds. Hot water treatment was found to be effective due to its higher penetrative ability (Maude, 1996). Seed treatment with hot water, garlic tablet, neem leaf extract, BAU- Biofungicide and Provax-200 significantly reduced the total seed-borne fungal infections as well as the population of individual six target pathogenic fungi *Alternaria tenuis*, *Bipolaris sorghicola*, *Botrytis cinerea*, *Curvularia lunata*, *Fusarium moniliforme* (Masum *et al*., 2009). The fungi that cause black leg, downy mildew, and anthracnose of cabbage can be eradicated by soaking seed at 122°F for 25 minutes (Paulsrud *et al.,* 2001). Babadoost (2006) 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. Hot water treatment of seeds has been used for more than 80 years. This method can be used to rid seeds of certain seed-borne pathogens while leaving the seed viable. The temperature of hot water and treatment of time duration also very influential factors. 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.

Therefore, the investigation was undertaken to optimizing (i) the concentration and duration of treatment with sodium hypochlorite and (ii) temperature and duration of the treatment with hot water on the prevalence of seed-borne fungi and germination of okra seeds.

Materials and Methods

Two independent experiments were conducted to find out the effect of seed treatment with sodium hypochlorite and hot water on seed-

borne fungi and germination of okra seeds. Seeds of okra variety BARI Dherosh-1 were used in both experiment. In first experiment, seeds were treated with NaOCl solution at different concentrations for different periods maintaining the following treatments: 0.1% NaOCl solution for 3 min, 0.1% NaOCl solution for 5 min, 0.5% NaOCl solution for 3 min, 0.5% NaOCl solution for 5 min, 1% NaOCl solution for 3 min, 1% NaOCl solution for 5 min. Fifty gram of seeds were soaked in each of the above treatmnets. After soaking in NaOCl solution it was poured off immediately and the seeds were rinsed twice with sterilized distilled water and subsequently air dried for 1 h.

In the second experiment okra seeds were soaked in hot water maintaining the following treatments: at 48 °C for 10 min, at 48 °C for 12 min, at 48 °C for 15 min, at 50 °C for 10 min, 50 °C for 12 min, at 50 °C for 15 min, at 52 °C for 10 min, at 52 °C for 12 min, at 52 °C for 15 min and control.

Fifty gram of okra seeds were used for each treatment. Before treating with hot water seeds were soaked with normal water for 2 h in a wrapped thin filament of cotton, then placed in a water bath maintaining a constant level of required temperature for desired time at every treatment. After immersion in hot water the seeds were opened from the thin cotton filament and immediately immersed in distilled water at ambient temperature (26°C) for another 30 min for cooling. Seeds soaked in normal water for a period of 2 h at ambient temperature were used as a control for both the experiments. The seeds were air dried for 2 h.

Prevalence of seed-borne fungi in each treatment was determined following standard blotter method (ISTA, 1999). Briefly, 25 seeds were placed to 3 ply moist sterilized blotter paper in 9 cm sterilized petri dish maintaining uniform distances. The plates were incubated at 27°C temperature for 7 days in 12/12 h alternating cycle of NUV (Near Ultra Violet) light and darkness. After incubation, data on the prevalence of seed-borne fungi and seed germination were recorded. Fungi were identified under stereomicroscope based on their growth characters on seed. When identification of fungi was not possible by observing the growth characteristics under stereomicroscope, temporary mounts were prepared and identified under a compound microscope using appropriate morphological characters (Mathur and Kongsdal, 2003).

Data recorded during the study were analyzed statistically using MSTAT-C software after necessary transformations except those for germination capacity (%) which were not transformed and the means were separated following Duncan's Multiple Range Test (DMRT) at 5% level of significance (Gomez and Gomez, 1984).

Results and Discussion

Effect of NaOCl on prevalence of seed-borne fungi and germination

Five genera of fungi, namely, *Aspergillus* spp. (*Aspergillus flavus* and *A. niger*) *Fusarium* spp. (*Fusarium oxysporum* and *Fusarium* sp.) *Macrophomina phaseolina*, *Colletotrichum dematium* and *Chaetomium* sp. were recorded in okra seed with the NaOCl treated and untreated (control). Their prevalence varied with concentration of NaOCl and duration of treatment. Prevalence of *Aspergillus* spp. and *M. phaseolina* under different treatments including control ranged 42.50-47.00% and

0.5-1.0%, respectively. The differences among the treatments were not significant. Prevalence of *Fusarium* spp., *C. dematium* varied within the range of 24.00-26.50% and 1.00-2.00%, respectively. Soaking of okra seeds in 1.0% NaOCl solution for 5 min gave significantly lower prevalence of these two fungi compared to control. Prevalence of these two fungi under other treatments was statistically similar to control. Soaking of seeds in 1.0% NaOCl solution for 5 min completely eliminated *Cheatomium* sp. Seed soaking in 0.5% and 1.0% NaOCl solution for 3 or 5 min reduced prevalence of *Cheatomium* sp. significantly compared to control. Prevalence of *Chaetomium* sp. was also decreased when seeds were soaked in 0.1% solution of NaOCl for 3 or 5 min compared to control but not significant. Prevalence of total fungi was significantly lower in seeds treated with 1% NaOCl for 3 min (72.00%) and 5 min (68.00%) compared to control (79.00%) (Table 1). Different treatments with NaOCl reduced prevalence of total fungi by 2.53 to 13.92% over control. The higher reduction in seed-borne fungi was recorded in seed treatment with 1% NaOCl for 5 min followed by 1% NaOCl for 3 min (Fig. 1).

Table 1. Effect of seed soaking in NaOCl solutions on the prevalence of seed-borne fungi and germination of okra seed

Treatment with NaOCl	% seed-borne infection						Germination (%)
	Aspergillus spp.*	*Fusarium* spp.**	*Macrophomina phaseolina*	*Colletotrichum dematium*	*Chaetomium* spp.	Total fungi	
0.1%, 3 min	46.00 a*** (6.77)****	26.00 ab (5.10)	1.00 a (1.18)	2.00 a (1.57)	2.00 ab (1.57)	77.00 a (8.77)	57.00 a
0.1%, 5 min	46.00 a (6.77)	26.00 ab (5.10)	1.00 a (1.18)	2.00 a (1.57)	2.00 ab (1.57)	77.00 a (8.77)	58.00 a
0.5%, 3 min	45.50 a (6.74)	26.00 ab (5.10)	1.00 a (1.18)	2.00 a (1.57)	1.50 bc (1.40)	76.00 ab (8.71)	58.00 a
0.5%, 5 min	45.00 a (6.70)	25.50 ab (5.05)	1.00 a (1.18)	2.00 a (1.57)	1.50 bc (1.40)	75.00 ab (8.66)	59.00 a
1%, 3 min	44.00 a (6.63)	25.00 ab (5.00)	0.50 a (0.96)	1.50 a (1.40)	1.00 c (1.18)	72.00 b (8.48)	60.00 a
1%, 5 min	42.50 a (6.51)	24.00 b (4.89)	0.50 a (0.96)	1.00 b (1.22)	0.00 d (0.71)	68.00 c (8.24)	61.00 a
Control	47.00 a (6.85)	26.50 a (5.14)	1.00 a (1.18)	2.00 a (1.57)	2.50 a (1.73)	79.00 a (8.88)	57.00 a
CV (%)	4.17	2.88	30.43	8.25	12.94	1.82	8.74

**Aspergillus* spp. include *A. flavus* and *A. niger*

***Fusarium* spp. include *F. oxysporum,* and *Fusarium* sp.

***Means followed by the same letter (s) in a column are not significantly different at 5 % level

****Figures in the parentheses are square root transformed values

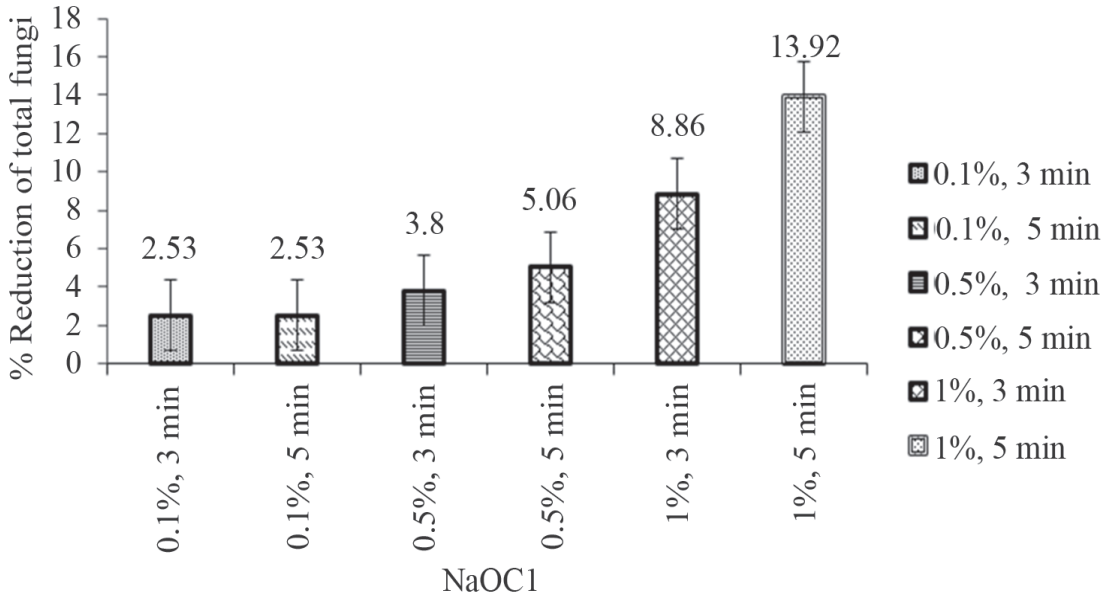

Fig. 1. Effect of seed treatment with NaOCl on % reduction of total seed-borne fungi in okra seed.

Seed germination ranged between 57.00 to 61.00% under different treatments and no significant differences were observed among the treatments. Numerically the lowest germination was (57%) recorded in control and treatment with 0.1% NaOCl for 3 min. On the contrary, the highest germination (61%) was observed in seeds soaked in 1.00% NaOCl for 5 min (Table 1). Soaking seeds in 0.1, 0.5 and 1.0% solution of NaOCl increased germination within the range of 1.75-7.02%. The highest increase was observed in seed treated with 1% NaOCl for 5 min (7.02%) followed by those treated with 1% NaOCl for 3 min (Fig. 2).

The results of the present experiment suggested that treatment of okra seeds with NaOCl at 0.1 and 0.5 % for 3 or 5 min was not satisfactory to reduce seed-borne fungi or to increase germination. However, seed treatment with NaOCl at 1% concentration for 5 min was effective to reduce total seed-borne fungi and to increase okra germination though this increase was not significant. Various researcher reported that NaOCl (bleach) is used as an effective disinfecting agent for seed surface sterilization and promotes seed germination. Kaur *et al.* (2014), Odofin (2010) reported that 2 and 3% of bleach increased germination percentage and vigour of okra seed. Nwangburuka *et al.* (2013) reported that sodium hypochlorite pre-treatment at 4% and 6% concentrations inhibited the population of seed-borne fungi such as *A. niger, F. oxysporum* and *Penicillium* sp. The results of the present study and past reports agreed well with the indication that NaOCl is a useful seed treating agent.

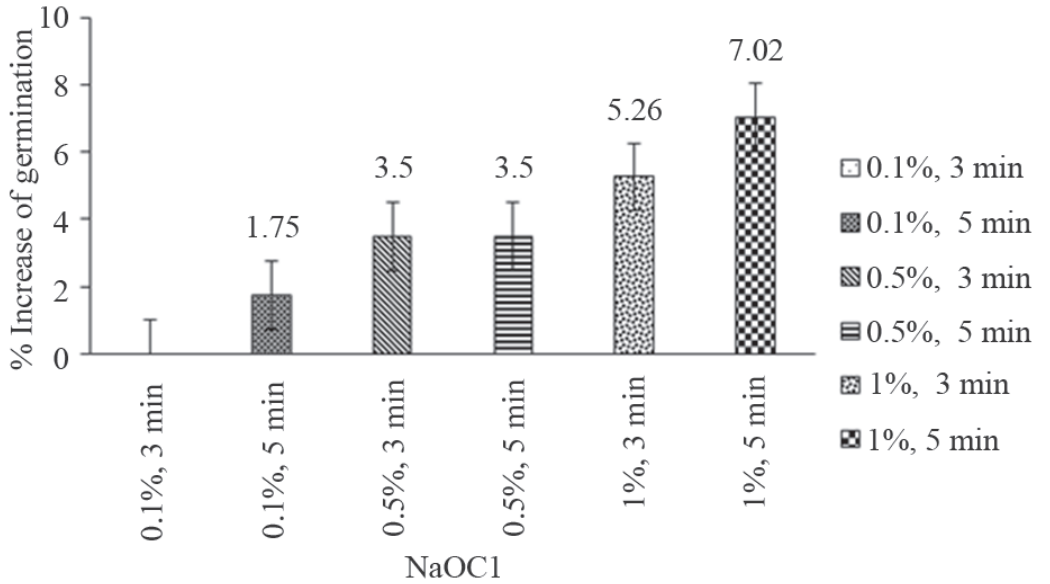

Fig. 2. Effect of seed treatment with NaOCl solution on % increase in seed germination of okra.

Effect of hot water treatment on prevalence of seed-borne fungi and germination

Treatment of okra seeds with hot water at 48-52°C for 10-15 min significantly reduced the prevalence of seed-borne fungi of okra. Prevalence of total seed-borne fungi was significantly reduced in all treatments. The rate of reduction was higher at higher levels temperature and longer exposure time. Effect of hot water treatment of seeds at 48°C for 10 and 12 min on prevalence of *M. phaseolina* was not significant compared to control. Other treatments with hot water completely eliminated *M. phaseolina* from seeds. Complete elimination of seed-borne *C. dematium* was achieved with hot water treatment at 50°C for 15 min, and at 52 °C for 10, 12 and 15 min. Soaking of seeds in hot water at 48°C for 12 and 15 min and at 50°C for 10 min also significantly reduced the prevalence of *C. dematium*. While at 50 and 52°C for 10, 12 and 15 min caused total elimination of seed-borne *Chaetomium* spp. Treatments at 48°C for 10, 12, and 15 min gave significant reduction in prevalence of *Chaetomium* spp. (Table 2). Hot water treatments also reduced prevalence of total fungi within the range of 48.10-72.15%. The highest reduction was achieved at 52°C for 15 min followed by 12 and 10 min at same temperature (Fig. 3).

Incased germination over control was observed in seeds treated with hot water at 48°C for 10, 12 and 15 min and at 50°C for 10 min although the increase was not statistically significant. Treatment of the seeds at 50°C for 12 and 15 min and at 52°C for 10, 12 and 15 min reduced germination (Table 2). The treatments at 48°C for 10, 12, and 15 min and 50°C for 10 min increased seed germination by 5.26 to 10.53% (Fig. 4). The increased germination due to hot water treatment might

Table 2. Effect of hot water seed treatment on the prevalence of seed-borne fungi and on germination of okra seed

Treatments with Hot water soaking	% seed-borne infection						Germination (%)
	Aspergillus spp.*	*Fusarium* spp.**	*Macrophomina phaseolina*	*Colletotrichum dematium*	*Chaetomium* spp.	Total fungi	
48º C, 10 min	21.50 b*** (4.63)****	16.00 b (4.00)	0.50 ab (0.96)	2.00 a (1.57)	1.00 b (1.22)	41.00 b (6.40)	60.00 a
48º C, 12 min	19.00 bc (4.35)	15.50 bc (3.93)	0.50 ab (0.96)	1.00 b (1.22)	0.50 c (0.96)	36.50 c (6.03)	63.00 a
48º C, 15 min	20.00 b (4.47)	14.50 bcd (3.80)	0.00 b (0.71)	1.00 b (1.22)	0.50 c (0.96	36.00 c (6.00)	61.00 a
50º C, 10 min	19.50 bc (4.41)	13.00 d (3.60)	0.00 b (0.71)	1.00 b (1.22)	0.00 d (0.71)	31.00 d (5.55)	61.00 a
50º C, 12 min	18.00 bcd (4.23)	14.00 cd (3.74)	0.00 b (0.71)	0.50 c (0.98)	0.00 d (0.71)	32.50 cd (5.70)	56.00 ab
50º C, 15 min	16.50 cde (4.06)	13.00 d (3.60)	0.00 b (0.71)	0.00 d (0.71)	0.00 d (0.71)	29.50 de (5.43)	49.00 bc
52º C, 10 min	15.00 def (3.87)	11.50 e (3.39)	0.00 b (0.71)	0.00 d (0.71)	0.00 d (0.71)	26.50 ef (5.14)	43.00 cd
52º C, 12 min	14.00 cf (3.73)	11.00 e (3.31)	0.00 b (0.71)	0.00 d (0.71)	0.00 d (0.71)	25.00 fg (5.00)	39.00 d
52º C, 15 min	12.50 f (3.52)	9.50 f (3.08)	0.00 b (0.71)	0.00 d (0.71)	0.00 d (0.71)	22.00 g (4.69)	36.00 d
Control	47.00 a (6.85)	26.50 a (5.14)	1.00 a (1.18)	2.00 a (1.57)	2.50 a (1.73)	79.00 a (8.88)	57.00 ab
CV (%)	5.63	3.59	22.31	10.91	14.98	3.90	11.36

**Aspergillus* spp. include *A. flavus* and *A. niger*

***Fusarium* spp. include *F. oxysporum,* and *Fusarium* sp.

***Means followed by the same letter (s) in a column are not significantly different at 5 % level

****Figures in the parentheses are square root transformed values

be due to weakening of seed coat, opening of micropyle and increased water uptake. Seed treatments at 50°C for 12 and 15 min and 52°C for 10 and 15 min reduce germination by 1.75 to 36.84 % (Fig. 4). This suggests that these treatments were injurious to the okra seeds. These results fully supported the results of Maude (1996) and Kaur *et al.* (2014). Raju and Sivaprakasam (1994) found that cabbage seeds treated with hot water recorded higher germination than untreated seeds after six months of storage. Mashooda and Lokesh (2012) found that hot water treatment reduced the incidence of mycoflora in the okra seeds and thereby enhanced the seed germination percentage and vigour index of the seedlings. The results of the present study suggested that seed treatment at higher temperature for longer

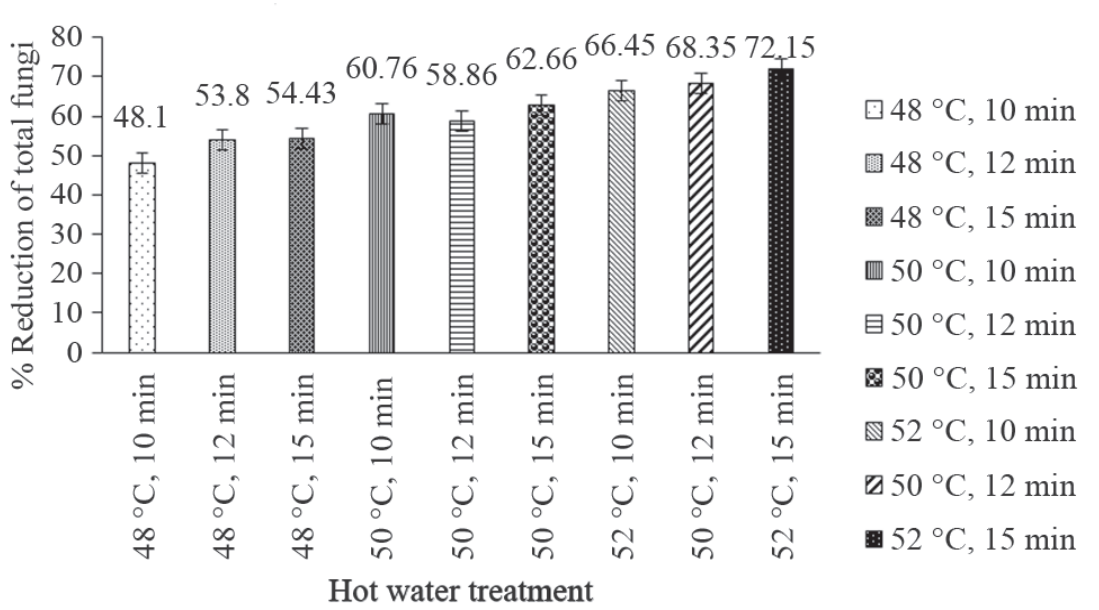

Fig. 3. Effect of seed treatment with hot water on % reduction of total fungi in okra seed.

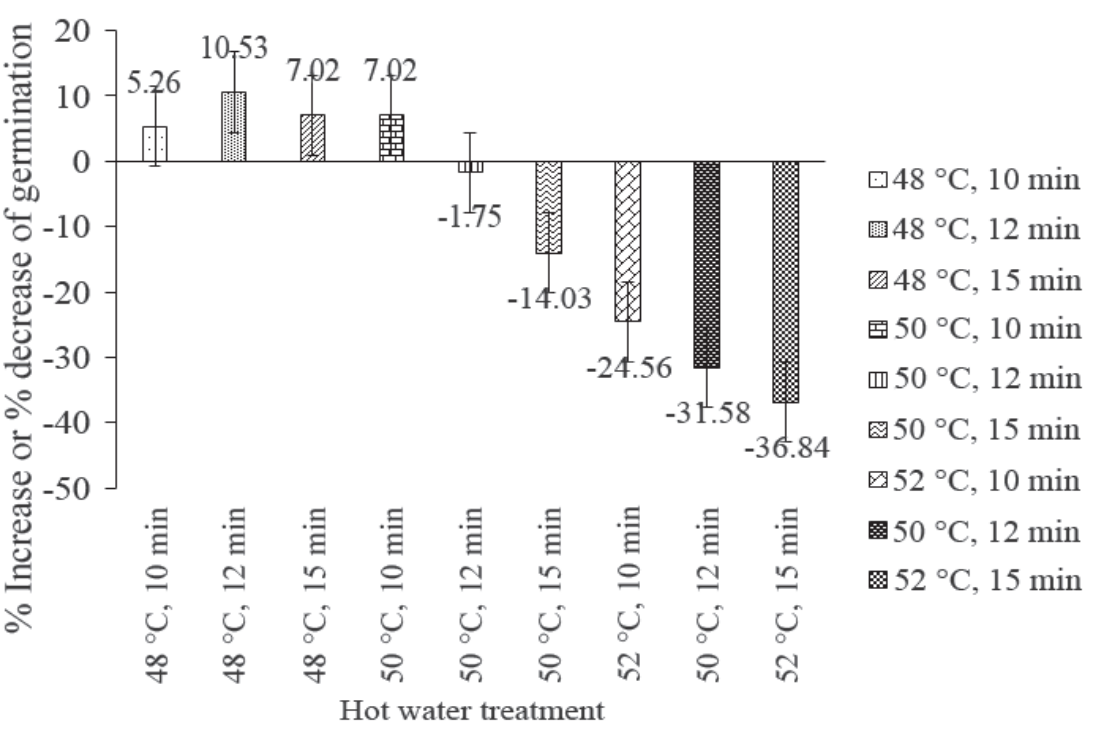

Fig. 4. Effect of seed treatment with hot water on % increase or % decrease of germination of okra seed.

period (at ≥ 52 °C for 10-15 min) although reduced the incidence of seed-borne fungi, but reduced the percent germination of okra seeds. However, Divsalar (2014) found, hot water treatment at 48°C and 52°C as non-detrimental on seed germination and vigor of melon seeds. In the present study, considering both fungal incidence and germination percentages of the seeds 48°C for 12 min and 50°C for 10 min were effective for controlling seed-borne mycoflora of okra. These results closely agreed with the report of (Meah, 2004; Rahman *et al.,* 2008; Farahani and Chaichi, 2012).

Conclusion

The present study revealed that NaOCl @ 1% for 5 min and soaking seeds in hot water at 48°C for 12 min and 50°C for 10 min could be used as the alternative to chemical for minimizing the incidence of seed-borne fungi and also hasten the germination of okra seeds.

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