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Effect of seed disinfectants on *in vitro* seed germination and seedling development in eggplant

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ABSTRACT

The present investigation reports effects of disinfectants on culture contamination, seed germination and seedling growth in eggplant. Mercuric chloride hampers seed germination when seeds are treated with 0.1% solution above 2 min duration and lesser durations are not helpful in controlling *in vitro* contamination. The highest seed germination (89.97%) was recorded with 50% commercial bleach for 20 min in the genotype BL-3, followed by BR-16 (88.53%), BR-14 (86.19%), BL-5 (86.16%) and BSR-23 (85.57%). However, least number of seeds germinated in BR-18 (10.94%), BSR-25 (13.27%) and BL-7 (18.62%) when disinfected with 75% commercial bleach for 25min. Overall results revealed that 50% commercial bleach concentration (73.76%) was better than 75% (36.85%), and 20 min duration (60.82%) was better than 25 min (49.80%) for seed disinfection. Among the varieties, BL-3 was at the top (68.85%) and BR-18 at the lowest (34.16%) edge for per cent seed germination. Seedling growth (cm) with use of commercial bleach was quite satisfactory compared to disinfection with $HgCl_2$, where, 50% commercial bleach favored a good stand of plant lets even after 10 days, showing a healthy root (3cm), hypocotyl (5cm) and cotyledons (1.5cm).

Key words: Seed germination, seedling growth, culture contamination, bleach, mercuric chloride, sodium hypochlorite

INTRODUCTION

Source of the explant plays an important role in successful plant regeneration. Explants collected from the field are a major source of contamination of cultures, and results in a reduction in the number of clean cultures. Various factors like temperature, moisture, light, etc. collectively influence germination. *In vitro* seed germination is the best alternative if a crop is sexually propagated. Seed-borne infections may be an important source of the primary inoculum and the seed should be treated before sowing to obtain good germination and a healthy crop (Habib *et al*, 2007). Mercuric chloride-$HgCl_2$ (Sarker *et al*, 2006) is a widely used chemical for effective control of *in vitro* bacterial and fungal infections in eggplant effectively. Most of the seeds have a hard seed coat, and are easy to disinfect, generally escaping the adverse effects of the chemical. But $HgCl_2$ is found to have a lethal effect on initiation of cultures in solanaceous crops. A number of other chemicals like sodium hypochlorite and hydrogen peroxide are used as disinfectants for *in vitro* seed germination. Rick and Borgnino (1989) advocated the use of 2.7% sodium hypochlorite (half-strength standard household bleach) for 30 min for improvement in seed germination. Commercial bleach can thus used for disinfection. Bleach is readily available and can be diluted to desired concentrations. Chemically, it contains Sodium hypochlorite (4%), Sodium hydroxide (1%), and Amine oxide (1%) that are used as disinfectants for *in vitro* cultures. Sodium hypochlorite (NaOCl) is effective as a disinfecting and sterilizing agent against a broad range of bacteria, viruses and fungi (Aref and Baki, 1974). Germination of both mature and immature seeds was rather stimulated by soaking seeds in 3.5% sodium hypochlorite solution for 15 min (Ho *et al*, 1995). Sodium hypochlorite accelerated germination and improved germination rate in other plant species and vigour index in all the cases studied (Pernezny *et al*, 2002). Objectives of the present study were to examine effects of various chemicals as seed treatments, including a range of concentrations and duration of exposure, on *in vitro* seed contamination, subsequent seed-germination and seedling growth. Emphasis was placed on evaluation of mercuric chloride and the commercial bleach.

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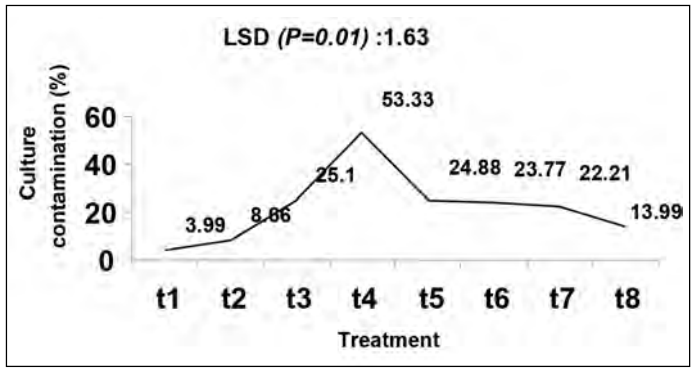
MATERIAL AND METHODS

The present investigation was carried out in Dr G.S. Khush Laboratories of School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, during 2005-2008. Ten genotypes of eggplant, viz., BL-3, BL-5, BR-14, BSR-21, BSR-27, BR-16, BL-7, BSR-23, BR-18 and BSR-25 were used in the investigation. Seeds of each variety were first washed with Teepol™ (Labolene) to remove dirt and light-weight seeds. Only bold seeds were selected and treated with 0.1% $HgCl_2$ and the commercial bleach (75% and 50%) for different lengths of time (2, 4, 6, 8 min for $HgCl_2$, and 20, 25 min for the commercial bleach), respectively. After treatment with $HgCl_2$, seeds were washed thrice, under a Laminar Air Flow Cabinet, with autoclaved MilliQ water to remove traces of $HgCl_2$ on the surface of the seeds. Commercial bleach treated seeds, however, were washed till formation of the foam stopped. Disinfected seeds were shifted to clean and autoclaved petri-dishes. The seeds were then cultured on half-strength MS medium (Murashige and Skoog, 1962) solidified with 0.8% agar for germination, and incubated at 25±2°C in the dark for 20 days. Each jam-jar was inoculated with 10-14 seeds. Observations on culture contamination, seed germination and seedling growth were made. Seed germination (%) was calculated using the number of seeds germinated from among seeds cultured in clean cultures. Statistical analysis was done in CRD factorial design, using CPCS-1 software package (Cheema and Singh, 1990). Results were compared at 1 per cent level of least square differences (LSD), and interpreted.

RESULTS AND DISCUSSION

Culture contamination (%)

Mercuric chloride was found to be more effective of the two in controlling culture contamination. Culture contamination was less (3.99%, 8.66% and 25.10%, respectively), when seeds were treated with 0.1% $HgCl_2$ for 8, 6 and 4 min; however, treatment for 2 min showed highest (53.33%) per cent contaminated cultures even before actual germination started (Fig. 1). Some cultures, although clean, did not show the good seedling growth required for culture initiation. Hypocotyl and the cotyledon remained mostly inside the seed coat. Exposure of seeds to 0.1% $HgCl_2$ for over 2 min further hampered seed germination and seedling growth. Seeds turned brown at first, and then black, as days passed, but did not germinate. Growth of the seedlings may have been hindered due to excessive exposure to the harsh chemical. Seed coat of eggplant is thin compared to that in woody plant species that escape this effect. Thus, the chemical may enter the seed through the thin seed coat, and may affect germination of the seed and growth of seedlings.



t1 0.1% $HgCl_2$ for 8min, t2 0.1% $HgCl_2$ for 6min, t3 0.1% $HgCl_2$ for 4min, t4 0.1% $HgCl_2$ for 2min, t5 50% Commercial bleach for 20min, t6 50% Commercial bleach for 25min, t7 75% Commercial bleach for 20min, t8 75% Commercial bleach for 25min.

Fig 1. Effect of various disinfectants on culture contamination in eggplant

Commercial bleach also reduced culture contamination (24.88%, 23.77%, 22.21% and 13.99, respectively, with 50% conc. for 20 min, 50% conc. for 25 min, 75% conc. for 20 min and 75% conc. for 25 min.) exhibiting good seedling growth while having satisfactory roots, hypocotyl and cotyledons (Fig.1). Increased concentration of the bleach, while reducing the number of contaminated cultures did not hamper seedling growth. Seed germination was delayed by 2-3 days with use of higher concentration (75%) of bleach than with 50% concentration. Chemically, it contains sodium hypochlorite (4%), sodium hydroxide (1%), and amine oxide (1%) that are used as disinfectants in *in vitro* culture. Sodium hypochlorite (NaOCl) is effective as a disinfecting and sterilizing agent against a broad range of bacteria, viruses and fungi **(**Mustafa, 2002**)**. This is also found to increase seed germination in field conditions.

Seed germination (%)

$HgCl_2$ was highly toxic and inhibited seed germination. No germination was seen, when seeds of different genotypes were treated with 0.1% $HgCl_2$ for 8, 6 and 4min time duration; however, 2 min treatment showed 9.27% germination, with poor seedling growth (Fig. 2a). Genotypic differences were also observed, where BR-18 and BSR-25 showed the poorest response to $HgCl_2$ treatment. Mercuric chloride resulted in delayed germination and restricted growth in eggplant seedlings. As per Sarker *et al* (2006),

seeds treated with 0.1% (w/v) mercuric chloride for 5-6 min showed germination in eggplant. However, increased concentration of, and longer exposure to, mercuric chloride while further reducing bacterial contamination, caused a modest reduction in seed germination and growth too, as reported by Ling *et al* (2010).

The effect of commercial bleach concentrations on seed germination in different genotypes of eggplant is given in Fig. 2b. Highest seed germination with 50% commercial bleach was observed in the genotype BL-3 (85.80%), followed by BR-16 (84.25%), BL-5 (82.23%), BR-14 (82.15%), and BSR-23 (82.13%), however, corresponding germination values with 75% commercial bleach were: 51.91, 45.19, 44.44, 43.00 and 44.29%, respectively. The length of treatment with commercial bleach significantly affected seed germination in different genotypes (Fig. 2c). It was highest in BL-3 (74.58%), followed by BR-16 (70.89%), BL-5 (69.17%), BR-14 (68.94%) and BSR-23 (68.93%) with 20min duration. However, increase in duration to 25min lowered germination rate to 63.13, 58.55, 57.50, 56.20 and 57.49 % in the respective genotypes. Interaction effect of concentrations and durations of commercial bleach treatment on seed germination was significant too (Fig 2d). Highest seed germination of 77.30% was seen with 50% commercial bleach for 20min duration in all the genotypes. However, it was lowest (29.38%) with 75% commercial bleach for 25min treatment. Similar increase in germination was also reported in conifer seeds by Wenny and Dumroese (1987).

Interaction of genotype, and concentration and duration of exposure to commercial bleach in Table 1 revealed that seed germination (89.97%) was highest with 50% commercial bleach for 20min in BL-3, followed by BR-16 (88.53%), BR-14 (86.19%), BL-5 (86.16%), and BSR-23 (85.57%). However, least number of seeds germinated in BR-18 (10.94%), BSR-25 (13.27%) and BL-7 (18.62%) when disinfected with 75% commercial bleach for 25min. Overall, the results revealed that 50% commercial bleach concentration (73.76%) was better than 75%

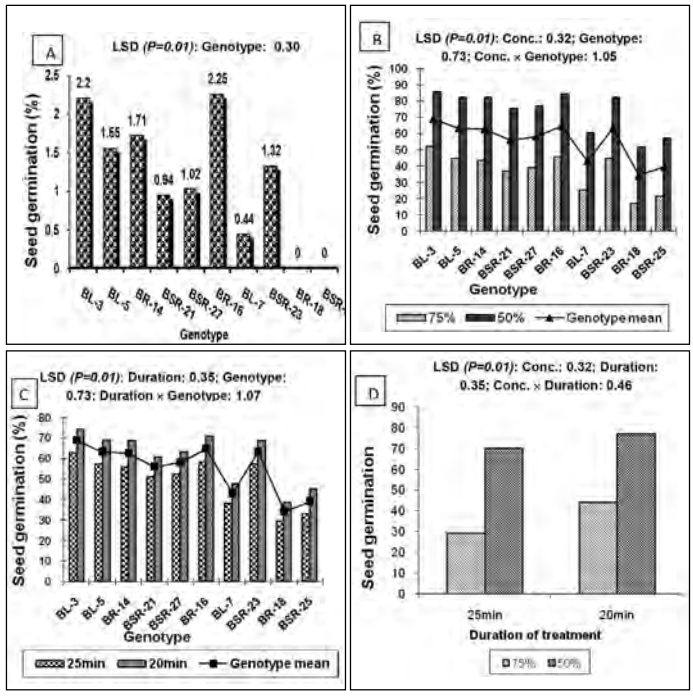


Fig 2. A) Effect of $HgCl_2$ (0.1%) on seed germination (%) in various eggplant genotypes B) Effect of commercial bleach concentrations on seed germination (%) C) Effect of duration of commercial bleach treatment on seed germination (%) D) Effect of commercial bleach concentration and duration of treatment on seed germination (%)

Table 1. Effect of commercial bleach concentrations duration and genotype on seed germination (%) in eggplant

Genotype	Commercial Bleach				Genotype Mean
	75%		50%		
	25min	20min	25min	20min	
BL-3	44.63 (41.90)	59.19 (50.27)	81.64 (64.60)	89.97 (71.52)	68.85 (57.07)
BL-5	36.70 (37.26)	52.18 (46.23)	78.29 (62.21)	86.16 (68.14)	63.33 (53.46)
BR-14	34.30 (35.83)	51.70 (45.95)	78.11 (62.07)	86.19 (68.15)	62.57 (53.00)
BSR-21	29.89 (33.12)	43.66 (41.34)	72.27 (58.20)	78.06 (62.04)	55.97 (48.68)
BSR-27	32.02 (34.44)	46.07 (42.73)	73.46 (58.97)	80.80 (63.99)	58.08 (50.03)
BR-16	37.14 (37.53)	53.24 (46.84)	79.96 (63.38)	88.53 (70.18)	64.72 (54.48)
BL-7	18.62 (25.54)	32.35 (34.65)	58.23 (49.71)	62.85 (52.43)	43.01 (40.58)
BSR-23	36.28 (37.02)	52.29 (46.29)	78.70 (62.49)	85.57 (67.66)	63.21 (53.36)
BR-18	10.94 (19.30)	23.27 (28.82)	48.47 (44.10)	53.96 (47.25)	34.16 (34.87)
BSR-25	13.27 (21.31)	29.36 (32.79)	53.16 (46.79)	60.63 (51.29)	39.18 (38.05)
Conc. Mean	36.85 (36.96)		73.76 (59.76)		
Duration Mean	25min: 49.80 (44.79)		20min: 60.80 (51.93)		

LSD *(P=0.01)* Conc: 0.25 Duration: 0.27 Genotype: 0.56 Conc x Duration: 0.35 Conc x Genotype: 0.80 Duration x Genotype: 0.82 Conc x Duration x Genotype: 1.13

(36.85%) and 20min duration (60.80%) was better than 25min (49.80%) for seed disinfection. Among the genotypes, BL-3 was at the top (68.85%) and BR-18 at the lowest (34.16%) edge for per cent seed germination.

Seed germination using the commercial bleach was quite satisfactory compared to that in $HgCl_2$ treatment, exhibiting normal growth and development of seedlings (Plate 1a). Seed germination was observed after 15 days in $HgCl_2$ treatment, while, it was within a week using the commercial bleach. Commercial bleach contains 4% sodium hypochlorite (NaOCl), which acts as a sterilizing agent. Kaur *et al* (2011a & b) also used it for sterilizing eggplant seeds. However, Mir *et al* (2008) used 0.1% $HgCl_2$ for 3 min for disinfecting eggplant seeds. Differential response in seed germination using various disinfectants for different length of time can be due to genotypic differences, method of treatment and cultural condition during the experiment. Seed germination (%) and seedling growth in eggplant were also seen to be negatively affected by increasing concentration of the disinfectant. A delayed germination (by 3-4 days) and reduced seedling growth was observed with increase in concentration and time of exposure in commercial bleach treatment. Mustafa (2002) also reported similar results.

Seedling growth

Seedling growth (cm) with the use of commercial bleach was quite satisfactory compared to disinfection with $HgCl_2$. Seedlings exhibited normal growth and development, as visible in Fig III and Plate1b. Seeds did not germinate when treated with 0.1% $HgCl_2$ for over two minutes.

Two-minute treatment also gave a poor stand of germinated seedlings with restricted growth having only root growth after 15 days of culturing. However, 50% commercial bleach favored good stand even after 10 days with healthy root (3cm), hypocotyl (5cm) and cotyledons

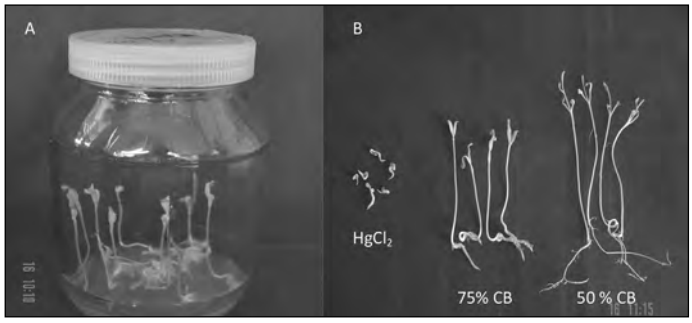


Plate 1. a) Seed-germination enhanced with 50% commercial bleach (CB) b) Effect of $HgCl_2$ (0.1%) and commercial bleach (75% and 50%) on seedling growth (cm) in eggplant after 10 days of culture

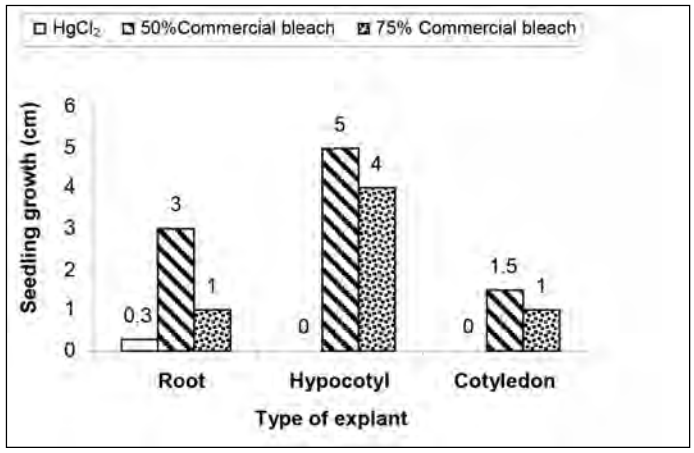


Fig 3. Effect of $HgCl_2$ and Commercial bleach on seedling growth (cm) in eggplant

(1.5cm). Increased concentration of commercial bleach (75%) also produced seedlings with slow growth rate of root (1cm), hypocotyl (4cm) and cotyledons (1cm). Mustafa (2002) also reported similar results in flax.

Type of seed disinfectant in eggplant affects culture contamination, seed germination and seedling growth significantly. Moreover, the concentration, time duration of disinfection and genotype also affect seed germination and seedling growth potential. It is concluded that commercial bleach is better than $HgCl_2$ for initiation of *in vitro* cultures in eggplant.

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