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Contamination control in Iranian seedless barberry micropropagation

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Abstract

Iranian seedless barberry is a species with high degree of contamination in *in vitro* culture. The current study was aimed to investigate the effects of mercuric chloride ($HgCl_2$), sodium hypochlorite (NaOCl) + $HgCl_2$, $HgCl_2$ + 8-hydroxyquinoline, cefotaxime + streptomycin, $HgCl_2$ + shoot type, $HgCl_2$ + explant position and $HgCl_2$ + shoot color on the reduction of contamination. The results showed that the combined use of NaOCl and $HgCl_2$ was more effective than their single use, but the contamination rate was relatively high. Interestingly, the results showed that explants sterilized with 100 mg/L $HgCl_2$ and cultured in the medium containing 150 mg/L 8-hydroxyquinoline exhibited the lowest fungal contamination (15%), while those treated with 50 mg/L $HgCl_2$ and cultured in the 8-hydroxyquinoline-free medium showed the highest fungal contamination (96%). Furthermore, treatment of 75 mg/L cefotaxime + 70 mg/L streptomycin exhibited no bacterial contamination compared to the control with 58% bacterial contamination. Explants prepared from suckers and treated with 150 mg/L $HgCl_2$ showed the lowest rates of fungal (16%) and bacterial (13%) contamination. The results also showed that explants collected from upper position had the lowest rates of fungal (20.87%) and bacterial (18.4%) infection. Explants taken from pinkish-colored shoots showed surprisingly the lowest rates of fungal contamination (13.33%), bacterial contamination (10%) and bud mortality (36.47%). In general, choice of explants from upper position of pinkish-colored suckers, sterilization of them with 100 mg/L $HgCl_2$ for 5 min and their culture in medium containing 150 mg/L 8-hydroxyquinoline can be recommended for reducing the contamination.

Keywords: 8-hydroxyquinoline; Bud; Contamination; Iranian seedless barberry; Mercury; Micropropagation.

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Introduction

Iranian seedless barberry (*Berberis vulgaris* L. var. Asperma), belonging to the family of *Berberidaceae*, is widely cultivated in arid and semi-arid areas of Iran due to its high tolerance to drought. The crop has high economic importance in Iran and many people rely on the income obtained from selling its fruits. As most parts of Iran have been recently experiencing severe droughts, cultivation of drought-tolerant crops, such as seedless barberry, is becoming more and more important. There is an increasing trend in the Iran to establish new barberry orchards but difficulty of vegetative propagation is a big problem in the way of extending the cultivation of this crop. Iranian seedless barberry, which has no seeds, is a very recalcitrant species in propagation and use of vegetative methods such as cutting, layering and grafting, all ended in failure (Alemardan *et al*. 2013). Among vegetative methods, sucker is the only way used for propagating this crop, so that all orchards in Iran

are of sucker-established type. Although sucker is the only way of farmers for establishing their new barberry orchards, it has some serious limitations. They should be two or three years old when separated from the mother plants in mid-autumn and also should have a length of 60-70 cm with adequate roots (Kafi *et al*. 2002). Moreover, due to the damages caused during dividing and transporting, a high number of plants are usually failed to grow after planting. In addition, providing large number of high-quality and uniform suckers is difficult. Generally, use of suckers for developing new orchards is very time consuming and expensive. Therefore, there is a great need for the use of new techniques such as tissue culture to improve the propagation of this valuable crop. This technique can be used as an efficient means of propagation of rare, endangered and medicinal plants which are difficult to propagate (Pattnaik and Chand 1996). In tissue culture, microbial contamination is one of the most important limiting factors of culture establishment (Krishna and Singh 2007). Microbial contamination can lead to growth slowdown, necrosis, reduced rates of proliferation and death of cultures (Rai *et al*. 1993). The successful control of pathogens in the initial stage of woody fruits' micropropagation is determined by the type of explant, the sterilization method and the season when explants are collected (Pérez-Tornero and Burgos 2007; Ríoz Leal *et al*. 2007). *In vitro* culture of Iranian seedless barberry, unlike many fruit trees, has for long time been confronted with serious inherent problems of deep seated bacterial and fungal contamination. There are just two studies about the micropropagation of barberry, but both were unsuccessful (Azizi *et al*. 2007; Mohammadi *et al*. 2011). To the best of our knowledge, thus far all the efforts made to eliminate bacterial and fungal contamination problems in *in vitro* culture of this crop failed to materialize. Thus, any attempt to overcome this problem can be a stepping stone toward an improved protocol and can pave the way for doing further research on later steps of micropropagation of this valuable crop. Therefore, the aims of this study were to assess the effects of some chemical compounds as well as several morphological characteristics on the reduction of contamination.

Materials and Methods

The present study was conducted in the Tissue Culture Laboratory of Horticultural Sciences Department, Ferdowsi University of Mashhad, Iran, in 2013-2017. Explants were prepared from barberry shrubs cultivated in research orchard of the Faculty of Agriculture. The initial sterilization was performed based on the common method reported in papers (Tiwari *et al*. 2000; Mukherjee *et al*. 2010; Sivanesan *et al*. 2011).

The first part of the research program tried to find the best sterilizing agent for eradicating microbial infections. Thus, four separate experiments were designed for this purpose. In the first experiment, $HgCl_2$ was applied at concentrations of 50, 100 and 150 mg/L for 5, 10 and 15 min. Due to the low efficiency of $HgCl_2$, combined use of NaOCl and $HgCl_2$ in sequential manner was investigated. To do this, explants were first presoaked in 25 and 50% NaOCl solutions for 10 min and then sterilized with

$HgCl_2$ at concentrations of 50 and 100 mg/L for 4, 8 and 12 min under laminar flow hood. The third experiment included sterilization of explants with $HgCl_2$ (50 and 100 mg/L for 5 min) and their culture in media containing 0, 75 and 150 mg/L 8-hydroxyquinoline. The fourth experiment was designed for elimination of bacterial contamination. Thus, two antibiotics, cefotaxime (0, 25, 50 and 75 mg/L) and streptomycin (0, 35 and 70 mg/L), were added to the media after autoclaving.

The second part of this study evaluated the effect of conditions related to explants, coupled with the sterilizing agent, selected from the previous part, on the contamination rate. This part consisted of three separate experiments. Effects of shoot type, including sucker, current-, one- and two-year old shoots in the first experiment, explant position on sucker including lower, middle and upper in the second experiment, and shoot color including green, dark brown, light brown and pinkish in the third experiment were investigated. In all three experiments, explants were sterilized with $HgCl_2$ at concentrations of 50, 100 and 150 mg/L for 5 min.

All experiments were carried out as factorial based on completely randomized design with five replications. Several variables including fungal contamination rate, bacterial contamination rate and bud mortality rate were measured after one month. In the experiment related to antibiotics, variables measured were the bacterial rate and days to bud break.

In all experiments, the Murashige and Skoog (MS) basal medium containing 30 g/L sucrose and 8 g/L agar was used, and pH of the media was adjusted to 5.8 before autoclaving. All cultures were maintained at 24±2 °C under 8/16 h photoperiod and PPFD of 40 μmol m^{-2} s^{-1} provided by cool white fluorescent light.

Data were subjected to the analysis of variance by JAMP software and means were compared by the LSD method at 5% probability level.

Results and Discussion

Effect of $HgCl_2$ and time

Results obtained from the analysis of variance (ANOVA) showed that main effects of both $HgCl_2$ and time on the fungal contamination rate, bacterial contamination rate and bud mortality rate were significant at 1% probability level, but their interaction was significant only for the bud mortality rate (table not shown). Fungal contamination rate in explants treated with higher concentrations of $HgCl_2$ was lower than those treated with lower $HgCl_2$ concentrations, but the contamination was generally high (Table 1). For bacterial contamination, results displayed that 50 mg/L $HgCl_2$ showed higher amount of bacterial contamination (78.3%) than 100 and 150 mg/L $HgCl_2$ with 61.6 and 35.5% contamination rates, respectively. Furthermore, longer sterilization time of 15 min exhibited lower bacterial contamination than the two other times of 10 and 5 min. Regarding bud mortality rate, the results showed that there were significant differences among the treatments. On average, 100% bud mortality was recorded when the explants were sterilized with 150 mg/L $HgCl_2$ for 15 min, and no bud mortality was observed in the treatment of 50 mg/L $HgCl_2$ for 5 min. In addition to NaOCl,

$HgCl_2$ is another chemical compound which has been frequently used in surface sterilization of many plants (Dhaka and Kothari 2005; Tao *et al*. 2007; Chen 2009; Mukherjee *et al*. 2010; Swart *et al*. 2012). Singh *et al*. (2013) and Desai *et al*. (2018) in separate studies on *Punica granatum* reported 75.75 and 75% sterilized explants by using 0.1% $HgCl_2$, respectively, showing high efficiency of this compound in sterilizing of explants. Contrary to these results, the present study showed that use of $HgCl_2$ for controlling fungal and bacterial contamination of barberry was not effective.

Table 1. Effects of different levels and times of $HgCl_2$ treatments on contamination rate of Iranian seedless barberry.

$HgCl_2$ (mg/L)	Fungal contamination rate (%)	Bacterial contamination rate (%)	Treatments ($HgCl_2$*Time)	Bud mortality rate (%)
50	92.33±2.92+	78.33±4.13	50,5	0±0
100	78.33±4.21	61.67±3.33	100,5	34.2±2.44
150	55.67±2.71	35.53±3.19	50,10	54.0±4.84
LSD (0.05)	8.094	9.401	100,10	64.0±4.84
Time (min)			150,5	79.0±6.40
5	85.0±4.90	66.67±6.29	50,15	81.0±2.78
10	73.0±5.36	58.33±5.27	100,15	88.0±8.00
15	68.3±4.38	50.53±5.30	150,10	91.0±0
LSD (0.05)	8.09	9.401	150,15	100±5.56
			LSD (0.05)	13.41

+Standard error.

Effect of NaOCl, $HgCl_2$ and time

Results of ANOVA showed that the main effects of NaOCl, $HgCl_2$ and time on fungal and bacterial contamination rate and bud mortality rate were significant at 1% probability level. Moreover, the interaction of NaOCl with $HgCl_2$ for the three variables, as well as the interaction of NaOCl with time for bud mortality rate were significant. Other treatment combinations did not show significant effects on the variables under study (table not shown). Explants presoaked in 50% NaOCl for 10 min and then treated with 100 mg/L $HgCl_2$ showed the lowest fungal (29.33%) and bacterial (26.33%) contamination rates and highest bud mortality rate (64%), while those pretreated with 25% NaOCl and 50 mg/L $HgCl_2$ exhibited the highest rates of fungal (39.67%) and bacterial (59.67%) contamination and lowest rate of bud mortality (35.33%) (Table 2). Also, the lowest fungal (26%) and bacterial (30.25%) contamination rate were observed at 4-min time, averaged over other two factors. Furthermore, the results revealed that explants treated with 25% NaOCl for 4 min, showed the lowest bud mortality rate (36.5%), whereas those sterilized with 50% NaOCl for 8 and 12 min, averaged over $HgCl_2$ levels, displayed the highest bud mortality rates of 66.5 and 66%, respectively (Table 2). Application of NaOCl and $HgCl_2$ in two steps has been successfully reported by Chowdhury *et al*. (2004) in *Dendrocalamus strictus* Nees, Zacchini and De Agazio (2004) in a local olive, Chen (2009) in *Tripterygium wilfordii* and Hossain *et al*. (2010) in *Perilla frutescens*. The findings of these workers were not in concordance with the results of our study that showed the two-step use

Table 2. Effects of NaOCl, $HgCl_2$ and time on contamination rate and bud mortality rate of Iranian seedless barberry.

Treatments (NaOCl*$HgCl_2$)	Fungal contamination rate (%)	Bacterial contamination rate (%)	Bud mortality rate (%)
25,50	39.67±2.82+	59.67±2.73	35.33±2.54
25,100	30.00±1.88	33.67±1.72	49.67±2.76
50,50	32.67±2.84	28.33±2.42	60.00±2.64
50,100	29.33±1.75	26.33±1.79	64.00±2.31
LSD (0.05)	3.922	4.775	5.191
(NaOCl*Time)			
50,8			66.5±2.89
50,12			66.0±2.70
50,4			53.5±3.43
25,12			53.0±2.36
25,8			38.0±2.24
25,4			36.5±3.39
LSD (0.05)			6.36
Time (min)			
4	43.00±1.60	43.75±3.55	
8	29.75±1.60	37.00±3.52	
12	26.00±1.29	30.25±3.02	
LSD (0.05)	3.397	4.135	

+Standard error; NaOCl: Sodium hypochlorite at 25 and 50% levels; $HgCl_2$: Mercury at concentrations of 50 and 100 mg/L.

of these compounds met with little success. One possible reason for low efficiency of NaOCl and $HgCl_2$ treatments, alone or together, might be the systemic nature of these contaminants (Poonawala *et al*. 1999), as shown in three *Phragmites* species that enhancing the level and time of Savalon liquid bleach treatment from 0.05% to 0.1% and from 8 min to 10 min, as well as $HgCl_2$ from 0.08% to 0.1% and from 8 min to 10 min, couldn't reduce the contamination. Other possible explanation for high fungal contamination of this species can be related to the structure of its bud and bark. Bud of barberry is covered by several layers of tightened scales, which, on the one hand, may provide a good place for fungi establishment and, on the other hand, prevent sterilizing compounds from penetration into layers to kill fungi. Bark of barberry has also many pores and cracks, which expose underlying tissue to invading pathogens.

Effect of $HgCl_2$ and 8-hydroxyquinoline

Results of ANOVA for the effect of $HgCl_2$ and 8-hydroxiquinoline on fungal and bacterial contamination rate and bud mortality rate showed that fungal contamination was significantly affected by the main effects of both 8-hydroxyquinoline and $HgCl_2$, bacterial contamination by 8-hydroxyquinoline and bud mortality rate by $HgCl_2$ (table not shown). The means in Table 3 showed that explants sterilized with 100 mg/L $HgCl_2$ and cultured in medium containing 150 mg/L 8-hydroxyquinoline exhibited the lowest rate of fungal contamination (56.47 and 21.9%, respectively) (Figure 1). Although the 8-hydroxyquinoline × $HgCl_2$ interaction was not significant for the fungal contamination rate, but the means obtained from the combination of these two factors showed interesting results. The explants sterilized with 100 mg/L $HgCl_2$ and cultured in medium containing 150 mg/L 8-hydroxyquinoline exhibited the lowest rate of fungal contamination (15%), while those treated with 50 mg/L $HgCl_2$

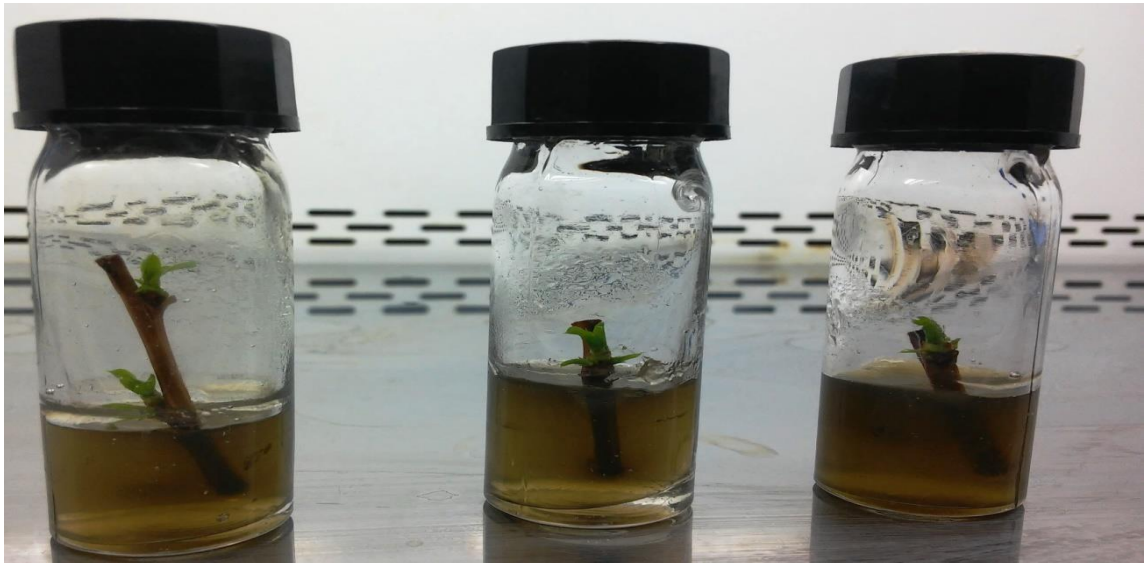

Figure 1. Explants of Iranian seedless barberry cultured in media containing 150 mg/L 8-hydroxyquinoline.

Table 3. Effects of different levels of $HgCl_2$ and 8-hydroxyquinoline on contamination rate and bud mortality rate of Iranian seedless barberry.

Treatments ($HgCl_2$*8-hydroxi	Fungal contamination rate (%)	$HgCl_2$	Fungal contamination rate (%)	Bacterial contamination rate (%)	Bud mortality Rate (%)
50,0	96.0±4.00	50	56.47±7.88		0±0
100,0	88.0±3.48	100	45.73±8.65		27.33±1.63
50,75	44.6±2.69	LSD (0.05)	7.038		3.604
100,75	34.2±4.89	8-hydroxi			
50,150	28.8±2.78	0	92.0±3.27	80±3.80	
100,150	15.0± 6.12	75	39.4±2.73	14±4.39	
LSD (0.05)	8.619	150	21.9± 3.90	0±0	
		LSD (0.05)	8.62	9.21	

[+]Standard error; $HgCl_2$: Mercury at concentrations of 50 and 100 mg/L; 8-hydroxi: 8-hydroxyquinoline at concentration of 0, 75 and 150 mg/L.

and cultured in 8-hydroxyquinoline-free medium showed the highest fungal contamination (96%). Bacterial contamination was the highest in media lacking 8-hydroxyquinoline, while it was the lowest in media containing 150 mg/L 8-hydroxyquinoline. No bud mortality was recorded when explants were treated with 50 mg/L $HgCl_2$, irrespective of 8-hydroxyquinoline concentration. 8-hydroxyquinoline is a chemical compound which has been mainly used in forcing bud break and also as an antimicrobial agent for preservation of cut flowers (van Doorn *et al*. 1989). To the best of our knowledge, there is no report about the addition of 8-hydroxyquinoline to culture medium for reducing contamination, and this is the first study showing the successful application of this compound for control of contamination. Our results showed that addition of this compound to medium at concentration of 150 mg/L could cause a significant reduction in fungal and bacterial contamination rates without any harmful effect on bud break rate. High effectiveness of this compound in elimination of fungal and bacterial contamination as well as in increase of bud break is related to its antimicrobial properties (van Doorn *et al*. 1989) and its ability in breaking bud

as a forcing agent (Yang and Read 1992).

Effect of cefotaxime and streptomycin

Results of ANOVA showed that combined use of cefotaxime and streptomycin antibiotics had significant effect on bacterial rate but not on day to bud break. Significant differences were observed among different treatments (Table 4). Treatment containing 75 mg/L cefotaxime + 70 mg/L streptomycin showed no bacterial contamination, and when the media were devoid of antibiotics, 58% of the explants showed the contamination. Bacterial contamination is considered as a major obstacle in plant tissue culture, seriously hampering the successful establishment of the explants. Addition of different antibiotics solely or in combination to media has been found as an efficient method for eliminating this problem (Leifert *et al*. 1992; Dangi *et al*. 2009; Misra *et al*. 2010; Nadha *et al*. 2012). However, the combined uses of antibiotics for removing bacterial contaminants have been more successful considering other factors such as toxicity (Bohra *et al*. 2014). Zacchini and De Agazio (2004) in micropropagation of a local olive cultivar reported a significant improvement in decontaminated explants from 25% by applying 0.1% $HgCl_2$ + 15% NaOCl to 86% by adding antibiotics to culture media. In line with the results of the above mentioned reports, our investigation indicated greater success rate of antibiotics in controlling bacterial infection when used in combination, which can be owing to their synergistic effect (Leifert *et al*. 1992). Moreover, not much difference was found in day to bud break among antibacterial combinations and control, suggesting that antibiotics were not harmful to bud sprouting of the explants. In line with our results, positive effects of cefotaxime antibiotic on promoting shoot regeneration have been reported in several plants (Shi and Cheng 1994; Yepes and Aldwinckle 1994; Rao *et al*. 1995), showing that this antibiotic not only does not have adverse effect on the explant growth in several plant species, but also has beneficial influence on shoot growth and development.

Table 4. Combined effects of antibiotics on bacterial contamination rate of Iranian seedless barberry.

Treatments (Cefotaxime * Streptomycin)	Bacterial contamination rate (%)
0,0	58±3.74
25,0	50±2.44
0,35	44±1.87
75,0	39±3.16
50,0	39±2.46
25,35	31.6±1.22
50,35	31±1.87
0,70	21±1.87
25,70	18±3.67
50,70	14±1.87
75,35	7±3.00
75,70	0±0
LSD (0.05)	7.06

[+]Standard error; Cefotaxime: Concentrations of 0, 25, 50 and 75 mg/L; Streptomycin: Concentrations of 0, 35 and 70 mg/L.

Effect of $HgCl_2$ and shoot type

According to ANOVA results, there was significant interaction of $HgCl_2$ with shoot type for fungal and bacterial contamination rates but not for bud mortality rate. Furthermore, bud mortality rate was significantly influenced by the main effects of HgCl2 and shoot type. Comparison of means in Table 5 showed that explants prepared from the two-year-old shoots and treated with 50 mg/L $HgCl_2$ for 5 min had the highest amounts of fungal (100%) and bacterial contamination (90%), while those prepared from sucker and treated with 150 mg/L $HgCl_2$ showed the lowest rates of fungal (16%) and bacterial (13%) contamination. In terms of bud mortality rate, explants treated with 150 mg/L $HgCl_2$ exhibited the highest rate of bud mortality (49.15%), but those treated with 50 mg/L $HgCl_2$ showed the lowest bud mortality rate (32.65%). Explants taken from the two-year-old shoots exhibited the lowest rate of bud mortality (32.27%), and those taken from suckers displayed the highest rate of bud mortality (47%); however, they did not show significant differences from the explants prepared from the current- and one-year old shoots. In addition to suitable sterilizing agents with optimum concentrations, other factors including age of the shoots from which explants are taken, bud position, method of micropropagation and genotype have been reported to be crucial to the success of establishment phase (Chang *et al*. 2001; Wu *et al*. 2009; Bonga *et al*. 2010; Cardoso and da Silva 2013). Studying the micropropagation of *Taxus mairei* from mature trees, Chang *et al*. (2001) showed that explants collected from the one-year-old shoots showed lower rate of contamination compared to those taken from old trees. Wu *et al*. (2009) in a study on raspberry and blackberry reported that the 2-year-old canes showed higher contamination than shoots. In line with these reports, we also showed the lower contamination rate of suckers compared to other types of shoots, which can be attributed to the presence of recalcitrant microbes in the old shoots that affect surface sterilization and subsequent culture process (Shekhawat *et al*. 2014).

Effect of $HgCl_2$ and explant position on sucker

Based on ANOVA, main effects of $HgCl_2$ and explant position were significant for fungal and bacterial contamination rates, and bud mortality rate; however, their interaction was not significant for these variables (table not shown). The highest rates of fungal (62.2%) and bacterial (56.53%) contamination, and the lowest bud mortality rate (48.53%) were related to the explants treated with 50 mg/L $HgCl_2$ (Table 6). On the contrary, explants treated with 150 mg/L $HgCl_2$ showed the lowest fungal (36.2%) and bacterial contamination (16.73%) rates, and the highest rate of bud mortality (72%). Explants collected from lower position showed the highest rates of fungal contamination (70.66%), bacterial contamination (53.4%) and bud mortality (76%), but those of upper position had the lowest rates of fungal contamination (20.87%), bacterial contamination (18.4%) and bud mortality (37.6%). Our results are in agreement with the results reported by Meier and Reuther (1994), who used upper buds

in *Fagus sylvatica*, Nadel *et al*. (1991), who used only the upper four to five buds in this plant, and Vieitez *et al*. (1985) and Pardos (1981), who only used terminal buds in *Quercus robur L* and *Q. suber* L, respectively. However, Poonawala *et al*. (1999) by clonal micropropagation of three *Crataeva adansonii* species, reported that the lowest bud break rate and the highest contamination rate were related to the upper buds of the shoot, to this fact that the buds were too weak to resist high levels of sterilizing agents.

Table 5. Combined effects of $HgCl_2$ and shoot type on contamination rate and bud mortality rate of Iranian seedless barberry.

Treatments ($HgCl_2$*Shoot type)	Fungal contamination rate (%)	Bacterial contamination rate (%)	$HgCl_2$	Bud mortality rate (%)
50,4	100±0	90.0±6.12	50	32.65±1.97
100,4	94.0±4.00	70.2±4.24	100	41.35±2.63
50,3	89.0±5.09	75.0±4.18	150	49.15±2.49
150,4	89.0±5.09	53.0±4.35	LSD (0.05)	6.238
100,3	74.0±4.30	58.0±4.63	Shoot type	
150,3	46.6±4.64	40.6±3.14	1	47.07±3.37
50,2	43.6±3.23	37.6±3.50	2	43.27±3.11
50,1	35.6±1.96	39.2±3.12	3	41.60±2.95
100,2	35.2±3.41	29.0± 1.87	4	32.27±2.36
100,1	24.0±1.87	27.6±3.90	LSD (0.05)	7.203
150,2	22.6±3.04	26.6±2.24		
150,1	16.0±1.87	13.0±2.00		
LSD (0.05)	10.062	10.802		

[+]Standard error; $HgCl_2$: Mercury at concentrations of 50, 100 and 150 mg/L; Shoot type: 1: Sucker, 2: Current-year shoot, 3: One-year old shoot, 4: Two-year old shoot.

Table 6. Combined effects of $HgCl_2$ and explant position on contamination rate and bud mortality rate of Iranian seedless barberry.

$HgCl_2$ (mg/L)	Fungal contamination rate (%)	Bacterial contamination rate (%)	Bud mortality rate (%)
50	62.20±6.47	56.53±5.46	48.53±4.85
100	46.33±5.70	39.73±4.19	57.07±5.55
150	36.20±5.59	16.73±3.68	72.00±5.24
LSD (0.05)	6.765	7.006	9.016
Explant position			
Lower	70.67±4.22	53.4±5.51	76.0±3.69
Middle	53.20±3.75	41.2±4.95	64.0±4.08
Upper	20.87±2.68	18.4±4.25	37.6±4.07
LSD (0.05)	6.765	7.01	9.02

[+]Standard error.

Effect of $HgCl_2$ and shoot color

As ANOVA results showed, main effects of $HgCl_2$ and shoot color on fungal and bacterial contamination rates, and bud mortality rate were significant, while the interaction of shoot color with $HgCl_2$ concentration was not significant. Comparison of means in Table 7 showed that explants sterilized with 50 mg/L $HgCl_2$ had the highest fungal (46.25%) and bacterial (33.3%) contamination rates, and the lowest bud mortality rate (33.4%). On the contrary, the treatment containing 150 mg/L $HgCl_2$ showed the lowest rates of fungal (18.75%) and bacterial (11.5%) contamination, and the highest rate of bud

Table 7. Combined effects of $HgCl_2$ and shoot color on contamination rate and mortality rate of Iranian seedless barberry.

$HgCl_2$ (mg/L)	Fungal contamination rate (%)	Bacterial contamination rate (%)	Mortality rate (%)
50	46.25±4.52	33.3±2.65	33.40±1.68
100	31.40±.3.34	22.1±2.30	41.10±2.06
150	18.75±3.78	11.5±2.78	52.55±2.91
LSD (0.05)	4.525	4.08	6.278
Shoot color			
1	23.33±3.26	16.67±3.66	47.60±3.75
2	55.00±4.30	33.33±3.02	41.60±2.87
3	36.87±3.30	29.13±2.33	43.73±3.51
4	13.33±.3.07	10.00±2.43	36.47±2.56
LSD (0.05)	5.225	4.715	7.249

[+]Standard error; Shoot color: 1: Green, 2: Dark brown, 3: Light brown, 4: Pinkish.

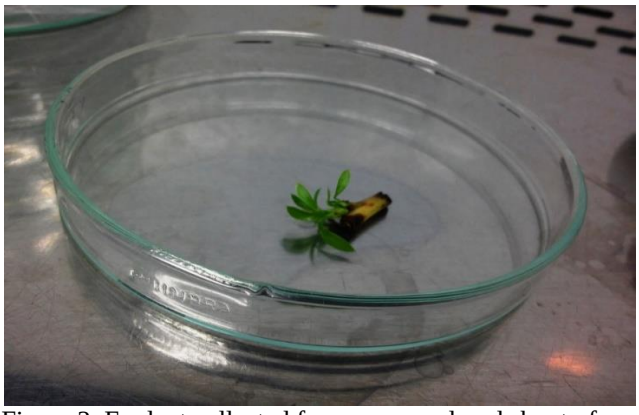

Figure 2. Explant collected from green-colored shoot of Iranian seedless barberry.

mortality (52.55%). Explants taken from dark brown-colored shoots had the highest fungal (55%) and bacterial (33.33%) contamination rates, while those taken from pinkish shoots showed the lowest fungal (13.33%) and bacterial (10%) infection rates. In addition, the highest bud mortality rate was observed in the explants of green-colored shoots (47.6%) (Figure 2), while the lowest rate was found in the explants of pinkish-colored shoots (36.47%). Surprisingly, we found that shoot color is an important factor which can be taken into consideration when selecting explant from the barberry plant. Generally, there are shoots with different colors of dark brown, light brown, green and pinkish in barberry that can be seen at any time of the year. Shoots with pinkish green colors contained bigger buds in size, which, were more resistant to sterilizing compounds, and less prone to the attack of microbial agents. Diametrically opposite, explants collected from shoots with dark and light brown colors showed higher contamination and mortality rates. This can be associated to smaller-sized buds that, besides not being strong enough to withstand higher concentrations of sterilizing agents, were more susceptible to microbial contamination. Showing newer and higher growth, pinkish- and green-colored shoots had also no cracks that are suitable place for the penetration of pathogens.

In conclusion, selection of explants from upper position of pinkish-colored suckers, sterilization of them with 100 mg/L $HgCl_2$ for 5 min and their culture in media containing 150 mg/L 8-hydroxyquinoline reduced bacterial and fungal contamination.

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کنترل آلودگی در ریزازدیادی زرشک بیدانه ایرانی

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چکیده

زرشک بیدانه ایرانی گونه‌ای با درجه آلودگی بالا در کشت بافت می‌باشد. مطالعه حاضر با هدف بررسی تأثیر کلرید جیوه، هیپوکلرید سدیم + کلرید جیوه، کلرید جیوه + ۸ هیدروکسی کینولین، سفوتاکسیم + استرپتومایسین، کلرید جیوه + نوع شاخه، کلرید جیوه + موقعیت ریزنمونه و کلرید جیوه + رنگ شاخه بر کاهش آلودگی اجرا گردید. بر اساس نتایج حاصل، استفاده ترکیبی از هیپوکلرید سدیم و کلرید جیوه مؤثرتر از استفاده یگانه کلرید جیوه بود، اما میزان آلودگی نسبتاً بالا بود. نتایج به‌طور شگفت‌انگیزی نشان داد که ریزنمونه‌های ضدعفونی‌شده با ۱۰۰ میلی‌گرم بر لیتر کلرید جیوه و کشت آن‌ها در محیط حاوی ۱۵۰ میلی‌گرم بر لیتر ۸ هیدروکسی کینولین کمترین میزان آلودگی قارچی (۱۵٪) را داشتند، درحالی‌که ریزنمونه‌های تیمار شده با ۵۰ میلی‌گرم کلرید جیوه و کشت آن‌ها در محیط فاقد ۸-هیدروکسی کینولین بیشترین میزان آلودگی قارچی (۹۶٪) را دارا بودند. همچنین، در مقایسه با تیمار کنترل با ۵۸٪ آلودگی باکتریایی، تیمار ۷۵ میلی‌گرم بر لیتر سفوتاکسیم + ۷۰ میلی‌گرم بر لیتر استرپتومایسین فاقد هر گونه آلودگی باکتریایی بود. ریزنمونه‌های تهیه‌شده از پاجوش و تیمار شده با ۱۵۰ میلی‌گرم بر لیتر کلرید جیوه کمترین میزان آلودگی قارچی (۱۶٪) و باکتریایی (۱۳٪) را نشان دادند. همچنین نتایج حاکی از آن بود که ریزنمونه‌های جمع‌آوری‌شده از موقعیت بالایی، از کمترین میزان آلودگی قارچی (۲۰/۸۷٪) و باکتریایی (۱۸/۴٪) برخوردار بودند. ریزنمونه‌های تهیه شده از شاخه‌های صورتی‌رنگ به‌طور شگفت‌انگیزی کمترین میزان آلودگی قارچی (۱۳/۳۳٪)، آلودگی باکتریایی (۱۰٪) و مرگ جوانه (۳۶/۴۷٪) را نشان دادند. به‌طورکلی، انتخاب ریزنمونه‌ها از موقعیت بالایی پاجوش با رنگ صورتی، ضدعفونی آن‌ها با ۱۰۰ میلی‌گرم بر لیتر کلرید جیوه به مدت ۵ دقیقه و کشت آن‌ها در محیط حاوی ۱۵۰ میلی‌گرم بر لیتر ۸-هیدروکسی کینولین برای کاهش آلودگی پیشنهاد می‌گردد.

واژه‌های کلیدی: ۸-هیدروکسی کینولین؛ آلودگی؛ جوانه؛ ریزازدیادی؛ زرشک بیدانه ایرانی؛ کلرید جیوه؛ نوع شاخه