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A novel aseptic technique for micropropagation of *Aloe vera* mill

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ABSTRACT

Background and aims: *Aloe vera* has been used medicinally for several thousands of years in many cultures from Egypt, Greece, and Rome to China, India, and etc. Although *Aloe barbadensis* propagates vegetative manner in its natural state, but propagation is too slow for commercial plant production. To overcome slow propagation rate, micropropagation will be a very useful technique for mass multiplication of *Aloe vera*. The aim of this study was to investigate and establish an effective method for aseptic micropropagation of *Aloe vera*.

Methods: This research was an experimental study that was conducted in tissue culture lab, Department of Genetics and Plant Breeding, BHU, Varanasi, India to develop a protocol for surface sterilization of *Aloe vera* explants. 42 treatments carried out for the shoot tip, apical meristem and rhizome discs of *Aloe* explant.

Results: Using of Tween 20 (5 drops) for 10 mints, Bavistin 1% for 10 mints and NaOCl (1.0%) for 6 minutes followed by rinsing sterilized distilled water showed the higher survival explants (70%) and asepsis was 81% and just 11% percent of the explants contaminated after 2 weeks. But the highest survived explants was for Tween 20 (5 drops) 10 minutes, Bavistin 1%; 10 minutes and $Ca(ClO)_2$ (3.25.0%) 6 minutes (77% survival) and asepsis was 81% and only 11% of the explants contaminated after 2 weeks.

Conclusion: In the present study, calcium hypochlorite with the highest asepsis, survival of explant and normally growth were more suitable than sodium hypochlorite for surface sterilization of *Aloe vera*.

Keywords: Aseptic technique, Micropropagation, *Aloe vera.*

Original article

INTRODUCTION

Aloe vera has been used medicinally for several thousands of years in many cultures from Egypt, Greece, and Rome to China, India etc.[1] Although *Aloe barbadensis* propagates vegetative manner in its natural state, but propagation is too slow for commercial plant production.[2] Concern in the utilization of medicinal and aromatic plants as pharmaceuticals, herbal remedies, flavourings, perfumes and cosmetics, and other natural products has considerably increased in the recent years.[3-7] *Aloe vera*

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Linn. (*Aloe barbadensis miller*) belongs to family Liliaceae, is a supernatural plant with great medicinal value.[8] A number of biological activities of *Aloe vera* have been claimed; such as antiseptic [saponins and anthraquinones], anti-tumoral [mucopolysaccharides], anti-inflammatory [steroids and salicylic acid], antioxidant [vitamins], and immune-regulator [glucomannans] effects.[9] Although *Aloe barbadensis* propagates vegetative manner in its natural state, but natural propagation is too slow for commercial plant production.[10] The *in vitro* preservation of medicinal plants contains the selection of explants, aseptic culture establishment, multiplication of micro shoots and rooting followed by acclimatization of the plantlets. Across these stages, the challenging step is standardization of sterility of explants for aseptic culture establishment. On the average, fails due to contamination under *in vitro* conditions were between 3-15% in the large number of commercial and scientific plant tissue culture laboratories, that the most of them are caused by bacterial, fungal, and yeast contaminants.[11,12] The prior good care of stock plants may reduce the amount of contamination that is present on explants. Plants grown in the garden and field are typically more "dirty" than those grown in a greenhouse or growth chamber, particularly in humid areas. The most important step for aseptic culture establishment is sterilization of explants. Prospering tissue culture of all plant species depends on the removal of exogenous and endogenous contaminated micro-organisms.[13-15] There are no special proper techniques on surface sterilization of *Aloe vera*, which is the prerequisite for further micropropagation techniques like organogenesis for any genetic manipulation and for preservation of this medicinal plant. The most conclusive step in explant preparation for further processes is keeping the explant survivability and overcoming the problem of contamination.[16-17] Therefore, in this investigation, an attempt has been made to set up an efficient surface sterilization protocol for the *in vitro* multiplication of *Aloe vera* Linn, using different types of sterilizing agents and differing their concentrations and duration of exposure as the best alternative for conservation of this medicinal important plant.

METHODS

This study was an experimental research that was conducted in tissue culture lab, Department of Genetics and Plant Breeding, BHU, Varanasi, India. The *Aloe vera* plant material for *in vitro* culture study was collected from the Aurvedic garden of Banaras Hindu University, Varanasi, U.P., India, and shoot tip, apical meristem and rhizome discs from mature plants (one- year old) were applied in this work. First of all, *Aloe vera* plants were taken out of pots. Soil and dusts were thoroughly washed and roots were cut completely. Leaves were cut from near the nodal region (5 mm-1 cm above the central leaves). Extruded gel and the parts were washed completely for 30 minutes under running tap water.

After thoroughly washing with tap water (30 minutes), the explants were shifted to laminar air flow and continuation works were done under laminar air flow which at first was cleaned with cotton impregnated by absolute alcohol, and then, for 15 minutes, UV light was on to disinfect the chamber. In front of flame, a solution containing (5-10%) Tween 20 (5 drops) (Himedia, laboratory of India) on ddH2O was prepared and added to the sterile flask. Then, it was swirled the flask containing the explants and bleach solution.

Explants were sterilized for 10 minutes then removed the bleachable solution and rinse the explants with ddH2O, 3 times. The explants were washed with surface sterilized in a bottle by dipping in 1% Bavistin for 10 minutes. Afterwards, explants were washed thoroughly with ddH_2O, 3 times. In the first, 3 experiments tested to find out how many explants bottles prevent contamination by using of these 2 sterilants (Table 1).

Three types of treatments have done: 1. Tween 20 (5 drops) 5-10%-10 mins; 2. Bavistin 1%-10 mins; 3. Tween 20 (5 drops) 5-10% in 10 mins + Bavistin 1% in 10 minutes.

Sodium hypochlorite (NaOCl) is the most repeated choice for surface sterilization. It is easily available and can be diluted to proper concentrations. Commercial laundry bleach is 5.25% sodium hypochlorite. It is usually diluted to 10%-20% of the original concentration; finally, a final concentration is 1.0% sodium hypochlorite. In the present study, different exposure time of NaClO was tested because of effectiveness of NaClO in controlling of microbial contamination and survival of explants (Figure 2). The healthy explants for surface sterilization contained shoot tip with young leaves, nodal explants, inter nodal stem segments; rhizome discs, and leaf bases that were immersed in 1.0% sodium hypochlorite (NaOCl) solution for (2,4,6,8,10,12,14,18,20) minutes (Table 2).

To find out the effect of 70% ethanol for 30 seconds in combination with sodium hypochlorite, after different exposure of 1.0% sodium hypochlorite, explants were washed thoroughly with ddH_2O 3 times. Then, they were immerged in 70% ethanol for 30 seconds, again, 3 times, and once more properly were washed with ddH_2O (Table 2).

Calcium hypochlorite ($Ca(ClO)_2$) is one of the frequentative choices for surface sterilization. It has shown less harmful to plant tissues than sodium hypochlorite as sterilants in tissue culture studies. In the present study, calcium hypochlorite powder dissolved in double distilled water and made 3.5% solution, then filtered the solution by filter paper. This ($Ca(ClO)_2$) (3.25.0%) solution added to the container. Then the container comprising the explants was swirled and immersed in this solution for (2,4,6,8,10,12,14,18,20) minutes (Table 3).

After the exposure of $Ca(ClO)_2$ (3.25.0%) solution, to check out the effect of 70% ethanol for 30 seconds in combination with ($Ca(ClO)_2$), explants were washed thoroughly with ddH_2O, 3 times, and then were immerged in 70% ethanol for 30 seconds. After that again theywere properly washed with ddH_2O 3 times, and their results were observed (Table 3).

Mercuric (II) chloride is advised by most tissue culturists as a sterilizing agent especially in *Aloe vera in vitro*. Mercuric (II) chloride ($HgCl_2$) has been used as a disinfectant, although it is extremely toxic. Here we applied mercuric chloride in limited treatments to compare with other sterilants. After treatment with Mercuric (II) Chloride ($HgCl_2$), the explants properly were washed 4 times with ddH_2O (Table 7).

After treatment with Mercuric (II) Chloride ($HgCl_2$), to investigate the effect of KCl (1.0%) with Mercuric (II) Chloride ($HgCl_2$), the explants properly were washed 4 times with ddH_2O. Then, all the explants were immersed in KCl (1.0%) for 1 min and again the explants were eluted 3 times with ddH_2O (Table 7).

RESULTS

In the first and the second treatments, (treatment by Tween 20/ Bavistin alone) (Table 1), all the plants contaminated in the presence of Tween 20/ Bavistin 1% for 10 minutes and no survival of explants after 30 days was observed. In the third treatment,

10% of the explants did not contaminate within 2 weeks of culturing and out of 10% aseptic explants, only 3% survived and the others contaminated and after 4 weeks necrosed.

Table 1: Three treatments with Tween 20 (5 drops), Bavistin 1% for 10 minutes alone and in combination together

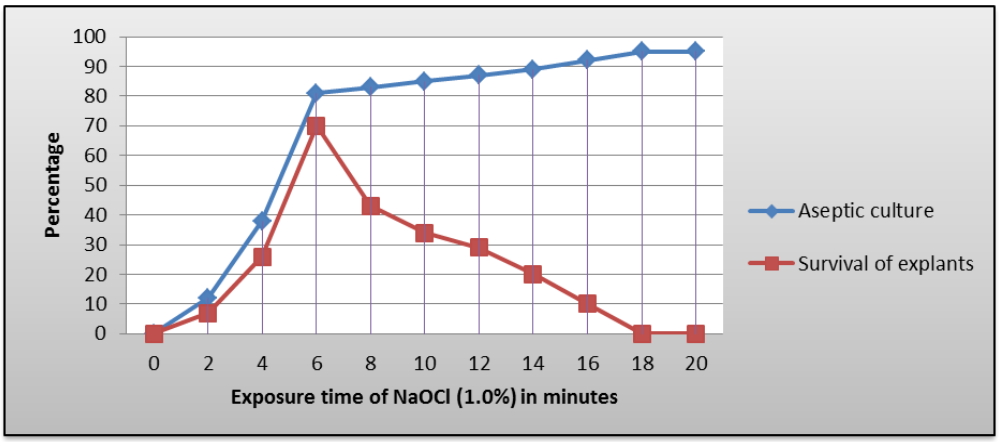
Treatment No.	Exposure time in minutes		Aseptic culture %	Survival of explants %	Dead explants %
	Tween 20 (5 drops)	Bavistin 1%			
T1	10	0	0	0	0
T2	0	10	0	0	0
T3	10	10	10	3	7

At the next, 10 treatments, in the presence of Tween 20 and Bavistin 1% for 10 minutes and sodium hypochlorite (NaOCl) with different exposure time, result on asepsis of shoot tip cultures and survival of explants was observed (Table 2).

Table 2: Treatments from (T1-T11) indicated in the presence of Tween 20 (5 drops), Bavistin 1% for 10 mins and NaOCl (1.0%) and the treatments (T12-T22) in presence of NaOCl (1.0%) and 70% ethanol for 30 sec

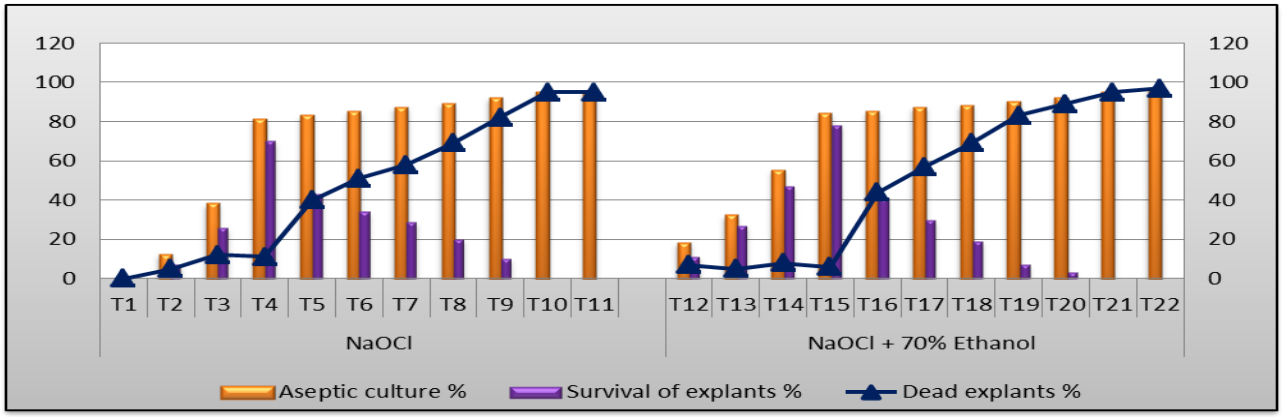
Treatment No.	Exposure time in minutes				Aseptic culture %	Survival of explants %	Dead explants %
	Tween 20 (5 drops)	Bavistin 1%	NaOCl (1.0%)	Ethanol 70%			
T1	10	10	0	0	10	3	7
T2	10	10	2	0	12	7	5
T3	10	10	4	0	38	26	12
T4	10	10	6	0	81	70	11
T5	10	10	8	0	83	43	40
T6	10	10	10	0	85	34	51
T7	10	10	12	0	87	29	58
T8	10	10	14	0	89	20	69
T9	10	10	16	0	92	10	82
T10	10	10	18	0	95	0	95
T11	10	10	20	0	95	0	95
T12	10	10	0	0.5	18	11	7
T13	10	10	2	0.5	32	27	5
T14	10	10	4	0.5	55	47	8
T15	10	10	6	0.5	84	78	6
T16	10	10	8	0.5	85	41	44
T17	10	10	10	0.5	87	30	57
T18	10	10	12	0.5	88	19	69
T19	10	10	14	0.5	90	7	83
T20	10	10	16	0.5	92	3	89
T21	10	10	18	0.5	95	0	95
T22	10	10	20	0.5	97	0	97

The treatment of T9 and T10, sodium hypochlorite (NaOCl) exposed for 18 and 20 minutes, gave the highest aseptic culture (95% asepsis), but all the explants due to exposure in long time of chemical necrosed at the first week of culturing. In the presence of Tween 20 and Bavistin 1% for 10 minutes and sodium hypochlorite (NaOCl) for 6 minutes indicated the higher survival explants (70%) and asepsis was 81% and only 11% of the explants after 2 weeks contaminated. The results indicated that exposure time of NaOCl (1.0%) has straight linear relation with asepsis of culture and by increasing the exposure time, asepsis percentage also are increasing (Graph 1). It continued till 6 minutes and after that it was converted to negative relation. So, when the exposure time of NaOCl (1.0%) increases, the survival of explants decreases. It has to be considered that after NaOCl (1.0%) treatment, it is important that (3-4) times the explants are washed with ddH_2O properly. Otherwise, the survival percentage of explants will decrease clearly in the first week of culturing.



Graph 1: Effect of different exposure time of sodium hypochlorite treatment at surface sterilization of *Aloe vera*

Ethanol is a powerful sterilizing agent but also highly phytotoxic. Therefore, plant material is usually exposed to it for only seconds or a minute. The more adjacency of the tissue, the more it will be damaged by alcohol. Some tissues like seeds, dormant buds, or unopened flower buds can be treated for couple of time still the tissue that will be explanted or that will develop is indeed within the structure that is being surface-sterilized. Generally, 70% ethanol is used earlier to treatment with other compounds. The results of treatments with NaOCl (1.0 %) for 6 mins in the presence of Tween 20 and Bavistin 1% for 10 minutes and 70% ethanol for 30 second indicated the highest percentage of the aseptic culture (84%). The percentage of survival of explants was 78% that in comparison with the treatment without 70% ethanol improved 3% and only 6% of the all explants died after culturing which was 5% less than similar treatment without ethanol (Table 2). One more treatment (T12) which was in the presence of Tween 20 and Bavistin 1% for 10 minutes and 70% ethanol for 30 seconds indicated the better performance in comparison with T1 which was Tween 20 and Bavistin 1% without ethanol, by 18% asepsis and 11% of explants survival (Table 2, Graph 2).



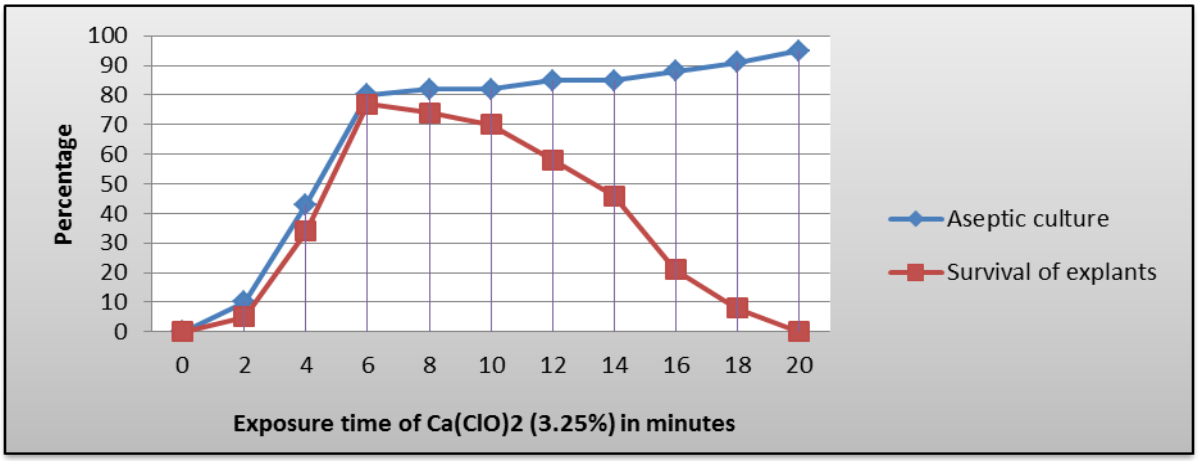
Graph 2: Effect of 70% ethanol along with sodium hypochlorite treatment on surface sterilization of *Aloe vera*

In the next 10 treatments with $Ca(ClO)_2$ solution, the highest percent of aseptic culture was obtained in the presence of Tween 20 and Bavistin 1% for 10 minutes and $Ca(ClO)_2$ for 20 minutes (Table 3).

Table 3: Treatments from (T1-T10) indicated the Tween 20, Bavistin 1% and $Ca(ClO)_2$ treatments and the other treatments (T11-T20) indicated the treatments in presence of Tween 20, Bavistin 1%, $Ca(ClO)_2$ and 70% ethanol

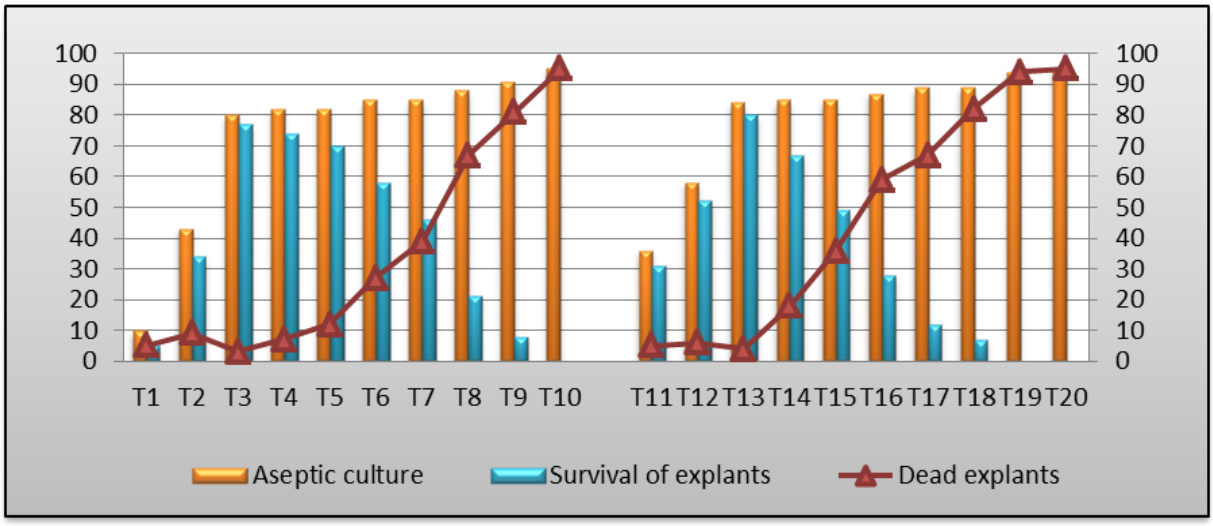
Treatment No.	Exposure time in minutes				Aseptic culture %	Survival of explants %	Dead explants %
	Tween 20 (5 drops)	Bavistin 1%	$Ca(ClO)_2$ (3.25.0%)	Ethanol 70%			
T1	10	10	2	0	10	5	5
T2	10	10	4	0	43	34	9
T3	10	10	6	0	80	77	3
T4	10	10	8	0	82	74	7
T5	10	10	10	0	82	70	12
T6	10	10	12	0	85	58	27
T7	10	10	14	0	85	46	39
T8	10	10	16	0	88	21	67
T9	10	10	18	0	91	8	81
T10	10	10	20	0	95	0	95
T11	10	10	2	0.5	36	31	5
T12	10	10	4	0.5	58	52	6
T13	10	10	6	0.5	84	80	4
T14	10	10	8	0.5	85	67	18
T15	10	10	10	0.5	85	49	36
T16	10	10	12	0.5	87	28	59
T17	10	10	14	0.5	89	12	67
T18	10	10	16	0.5	89	7	82
T19	10	10	18	0.5	94	0	94
T20	10	10	20	0.5	95	0	95

The best treatment for highest survival of explants indicated in the presence of Tween 20 and Bavistin 1% for 10 minutes and $Ca(ClO)_2$ for 6 minutes (77% survival). By the increasing the exposure time of $Ca(ClO)_2$ asepsis was increased but percentage of survival explants decreased. Increases in the exposure time of $Ca(ClO)_2$ surface sterilants drastically affected the survival of explants. However, its effectiveness was less than NaOCl treatments (Graph 3).



Graph 3: Effect of different treatments with calcium hypochlorite on surface sterilization of *Aloe vera*

The results of 20 treatments indicated the higher percentage of aseptic culture (84%) is in present of $Ca(ClO)_2$ (3.25.0%) for 6 minutes exposure time but in additional treatment with 70% ethanol for 30 seconds, percentage of survival explant showing 3% addition. Asepsis of the cultures 4 % increased but percentage of explant which died after culturing, 1% increased (4%) (Table 3, Graph 4).



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Graph 4: Effect of 70% ethanol along with calcium hypochlorite treatment on surface sterilization of *Aloe vera*

The ANOVA and MANOVA results of ethanol treatments along with sodium hypochlorite or calcium hypochlorite in different exposure time (Tables 4,5 and 6). The result of variance analysis indicated that in the first 11 groups (T1-T11), there was a significant (P<0.05) positive relationship between sodium hypochlorite with survival of explants and aseptic culture of the explants. In other words, by increasing of sodium hypochlorite, the survival of explants and the aseptic culture also has been increased (Table 4).

Table 4: Correlations between different exposure time of sodium hypochlorite, asepsis of the culture and survival of explants

		NaOCl (1.0%)	**Aseptic culture**	**Survival of explants**
NaOCl (1.0%)	Pearson Correlation	1		
	Sig. (2-tailed)			
	N	11		
Aseptic culture	Pearson Correlation	0.837**	1	
	Sig. (2-tailed)	0.001		
	N	11	11	
Survival of explants	Pearson Correlation	0.647*	0.932**	1
	Sig. (2-tailed)	0.031	0.000	
	N	11	11	11

***: Correlation is significant at the 0.01 level (2-tailed); *: Correlation is significant at the 0.05 level (2-tailed).*

The result of MANOVA (multi analysis of variance) demonstrated the effect of sodium hypochlorite in combination with alcohol and without alcohol in surviving of explants and asepsis of the cultures. MANOVA analysis considered that, there is no significantly differences between each similar exposure times of the sodium hypochlorite in combination with and without alcohol at the levels of the explant surviving (F=1.336, sig=0.262; F=1.293, sig=0.270). The results indicated significantly differences (P<0.05) between different exposed times of sodium hypochlorite in combination with alcohol at the levels of aseptic culture, (F=26.170, F=124.068, sig=0.000). Due to the positive statistic value F, it was concluded that by increasing the amount of sodium hypochlorite in combination with alcohol, aseptic level amount increases (Table 5).

Table 5: MANOVA tests of between-subjects effects (for significantly different means at the different levels of NaOCl alone or along with 70% ethanol P<0.05)

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Asepsis	14636.914[a]	2	7318.457	66.055	0.000
	Survival of explants	1159.273[b]	2	579.636	1.314	0.292
Intercept	Asepsis	2899.432	1	2899.432	26.170	0.000
	Survival of explants	4582.286	1	4582.286	10.389	0.004
NaOCl	Asepsis	13746.005	1	13746.005	124.068	0.000
	Survival of explants	589.091	1	589.091	1.336	0.262
70% Ethanol	Asepsis	890.909	1	890.909	8.041	0.011
	Survival of explants	570.182	1	570.182	1.293	0.270
Error	Asepsis	2105.086	19	110.794		
	Survival of explants	8380.000	19	441.053		
Total	Asepsis	98604.000	22			
	Survival of explants	17330.000	22			
Corrected Total	Asepsis	16742.000	21			
	Survival of explants	9539.273	21			

a: R Squared= 0.874 (Adjusted R Squared= 0.861); b: R Squared= 0.122 (Adjusted R Squared= 0.029).

The result of MANOVA of 20 groups treatments (different exposure time of calcium hypochlorite in combination with alcohol or without alcohol) (Table 3), indicated significant differences between effects of calcium hypochlorite exposure time, asepsis and survival of explants (F=7.362, sig=0.015; F=56.707, sig=0.000). Due to the signification of F value (P<0.05), it was concluded that by increasing the calcium hypochlorite levels, the amount of aseptic culture and survival explants increases. While the different level of exposure time of calcium hypochlorite in combination with alcohol has not shown any significance changes in asepsis and survival of explants (F=1.464, sig=0.243; F=0.631, sig=0.438) (Table 6).

Table 6: MANOVA tests of between subjects effects (for significantly different means at the different levels of $Ca(ClO)_2$ alone or along with 70% ethanol $P<0.05$)

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	Asepsis	6220.306a	2	3110.153	28.669	0.000
	Survival of explants	3074.597b	2	1537.298	4.413	0.029
Intercept	Asepsis	5574.876	1	5574.876	51.388	0.000
	Survival of explants	8691.433	1	8691.433	24.950	0.000
$Ca(ClO)_2$	Asepsis	6151.856	1	6151.856	56.707	0.000
	Survival of explants	2564.547	1	2564.547	7.362	0.015
70% Ethanol	Asepsis	68.450	1	68.450	0.631	0.438
	Survival of explants	510.050	1	510.050	1.464	0.243
Error	Asepsis	1844.244	17	108.485		
	Survival of explants	5921.953	17	348.350		
Total	Asepsis	105087.000	20			
	Survival of explants	19901.000	20			
Corrected Total	Asepsis	8064.550	19			
	Survival of explants	8996.550	19			

a: R Squared= 0.771 (Adjusted R Squared= 0.744); b: R Squared= 0.342 (Adjusted R Squared= 0.264).

The highest percentage of aseptic culture in next four Mercuric (II) chloride ($HgCl_2$) treatments was obtained in present of Mercuric (II) chloride for 10 minutes (92% and 93%). But due to high toxicity of Mercuric (II) chloride, all the explants necrosed within 2 weeks. Treatment of $HgCl_2$ (0.1%) for 5 minutes and Tween 20 + Bavistin 1% for 10 minutes, was the best treatment for the highest survival of explants (68%) and only 12% of explants has been contaminated after 2 weeks. In addition, $HgCl_2$ treatment improved survival percentage of explants when the exposure time maintained at 5 minutes. Increasing of the exposure time of surface sterilants drastically is affecting the survival of explants (Table 7).

Table 7: Effect of different exposure time of $HgCl_2$ (0.1%) treatment lonely and along with KCl (1.0%)

Treatment No.	Exposure time in minutes				Aseptic culture %	Survival of explants %	Dead explants %
	Tween 20 (5 drops)	Bavistin 1%	$HgCl_2$ (0.1%)	KCl (1.0%)			
T1	10	10	5	0	78	68	10
T2	10	10	5	1	78	75	3
T3	10	10	10	0	92	0	92
T4	10	10	10	1	93	41	42

The result of treatments with mercuric chloride and KCl (1.0%) (Table 7) indicated that the highest percentage of aseptic culture (93%) obtained from $HgCl_2$ (0.1%) for 10 minutes and KCl (1.0%) for 1 minutes. The best treatment for highest survival of explants (75%) observed on $HgCl_2$ (0.1%) for 5 minutes and KCl (1.0%) for 1 minute. In addition, treatment of $HgCl_2$ (0.1%) for 5 minutes and KCl (1.0%) for 1 minute represented the less necrosis of explants. 1.0% KCl for 1 minute improved survival percentage of explants by removing excess mercury (Hg^{++}) ions and only 3% of explant necrosed after 2 weeks of culturing. When exposure time of $HgCl_2$ was increased, even in the presence of KCl (1.0%) drastically survival of explants affected (Figure 1).

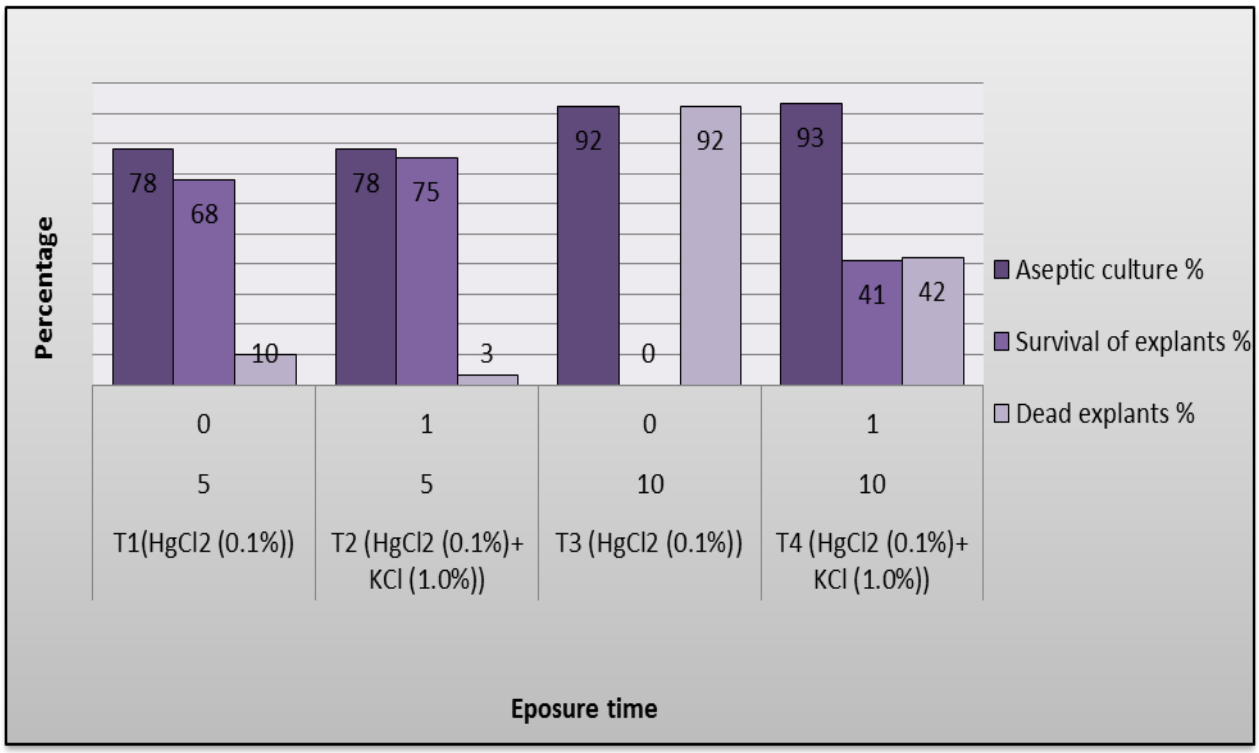

Figure 1: Interactive effect of mercuric chloride and KCl (1.0%) on disinfect the *Aloe vera* explants

Figure 2: *In vitro* grown of *Aloe vera*

(A) Rhizome disc for callus induction on four-week-old culture, (B) Asepsis of shoot media on four-week-old culture, (C) three-week-old plantlets cultured in bud culture media, (D) Tissue culture lab, (E) aseptic and alive plantlet, (F) aseptic culture media but explant died, and (G) infected explant 3-week-old culture.

DISCUSSION

In the present study, it was examined different treatments which were less toxic for environment such as *Aloe vera* plant and obtained the maximum aseptic cultures. Mercuric chloride is a chemical easy to use, but due to highly toxicity to environment and biology should be used instead of chemicals with non-toxicity.

Treatment with Tween 20 or Bavistin alone was not effective, but in combination some cultures showed asepsis at least. To make the higher aseptic culture we need more compounds to prevent infection of explants through the growth stage. Calcium Hypochlorite and sodium hypochlorite both are appropriate chemicals for *Aloe vera* tissue culture. Out of 42 different treatments, although treatments with sodium hypochlorite were showing acceptable asepsis but it was harmful for explants. Calcium hypochlorite along with Tween 20, Bavistin and 70% ethanol indicated the highest asepsis, survival of explant and normal growth which was suitable for surface sterilization of *Aloe vera*. These results are in close conformity with Aarifa Jan et al. in strawberry that they found sodium hypochlorite for 20 minutes plus ethyl alcohol 70% for 30 seconds. The surviving percentage decreased because this treatment resulted in necrosis and tissue injury of explants.[18] Results in the current study are in line with those of Nidhi Srivastava et al. who micropropagated Aconitum heterophyllum through nodal segments and seeds and found that treating explants with mercuric chloride (0.1%) for 5 min was the most effective surface sterilization procedure for 100% healthy shoots were obtained with minimum tissue injury.[19] Likewise, Gautam et al. also found that treating the explants of strawberry with 0.1% mercuric chloride for 3 minutes gave minimum contamination with maximum culture establishment.[20]

CONCLUSION

The *Aloe vera* explants were collected from Ayurvedic Department, Banaras Hindu University, India for tissue culture studies. In the present research, different chemicals and their effect on surface sterilization of *Aloe vera* explant for safer and effective surface sterilization were studied. Our results indicated that calcium hypochlorite is more effective than sodium hypochlorite. These differences can probably be due to less toxicity of calcium hypochlorite on shoot tip, apical meristem and rhizome discs of *Aloe vera* explant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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