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An alternative safer sterilization method for explants of *Aloe vera barbadensis* Mill.

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Abstract: Utilizing an accurate sterilization procedure in tissue culture techniques can save time and energy. However, the explants must be sterilized and viable while sterilizing. This study aims at providing a new method to replace mercuric chloride. In this study sodium hypochlorite (commercial brand Clorox) with some drops of Tween80 10%, 15%, 20%, 25% and 30% were used. Applying a Kruskal-Walis test (a nonparametric test), revealed that 5% sodium hypochlorite with 20 minutes of hard and constant shaking, gives the highest number (91.7%) of viable and sterilized explants with regeneration potential in Murashige and Skoog medium supplemented with IBA and TDZ and Zeatin. Using Pearson Chi square test (X=37.144, P value = 0.0001), the results revealed a significant relationship between the sodium hypochlorite concentration and percentage of surviving explants in Aloe vera barbadensis Mill.

Keywords: Aloe vera, sterilization, mercuric chloride.

1. Introduction

Aloe vera is an important medicinal plant that belongs to the family of Liliaceae [2]. Liliaceae is the family of perennial tropical plant of African origin. The genus of Aloe has more than 500 species but only few are medicinally important [11]. *Aloe vera* is a xerophytic medicinal plant and can be grown even in rain-fed conditions [1]. *Aloe vera* has been suggested to possess anti-cancer, anti-viral, anti-inflammatory, anti-diabetic and anti-bacterial properties; however, the mechanism(s) of these proposed effects is (are) not fully understood [14]. The plant is a perennial succulent that is characterized by its capacity to store large volumes of water in its tissues. The plant has green fleshy leaves covered by a thick cuticle or rind and an inner clear pulp [14]. The gel of *Aloe vera* makes an excellent treatment for wounds, burns and other skin disorders [2]. Administration of gel extract reduces blood glucose level, blood urea, glycosylated haemoglobin and restores hexokinase, glucose-6- phosphatase and fructose-1, 6-diphosphatase activity close to normal [7].

Industrial demand on production of A*loe vera* gel is increasing every day and due to the slow natural rate of reproduction, the demand of this gel in various industries cannot be met with [11].

Plant tissue culture is a practice used to propagate plants under sterile conditions. Using the tissue culture may offer certain advantages over traditional methods of propagation, including: making exact copies of the plant, quickly producing mature plants, regenerating the plant which has been genetically modified, etc. [13].

Moreover, tissue culture can be use as a method for mass production of *Aloe vera.*Sterilization of explants is one of the very important steps in tissue culture. Utilizing an accurate sterilization method in tissue culture techniques can save time and energy. Whereas, the explants must be sterilized and viable while sterilizing, mercuric chloride is advised by most tissue culturists as a sterilizing agent especially in A*loe vera* in-vitro.

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Mercury is a xenobiotic metal that is a highly deleterious environmental pollutant. The biotransformation of mercury chloride ($HgCl_2$) into methylmercury chloride (CH_3HgCl) in aquatic environments is well-known and humans are exposed by consumption of contaminated fish, shellfish and algae [10]. Chemical stability and harmful effects of mercuric chloride on kidney, liver, adrenal and fertility are reported by WHO in 2005 [12].The increasing demand of *Aloe vera* in industries has forced the producers to improve their methods. The plant tissue culture is one of the best options for mass production of *Aloe vera.* Thus, by increasing the tissue culture, the potential amount of mercuric chloride being released into the environment will rise.

2. Materials and methods

Plants of *Aloe vera* were taken from the experimental garden of the Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia. *Aloe vera* plants were taken out of pots. Soil and dusts were completely washed and roots were cut carefully 1 cm below the transition zone. Leaves were cut from around the nodal region and exuded gel and the parts were washed for 30 minutes under running tap water. Different concentrations of sodium hypochlorite were prepared in 2.5%, 3.75%, 5%, 6.25%, 7.5% (commercial brand Clorox 10%, 15%, 20%, 25%, 30% - Table 1) with distilled water and 5 drops of Tween80 in one litre of solution. The explants were soaked in 70% ethanol for 30 seconds then were exposed to each concentration for a period of 20 minutes with hard and constant shaking – approximately 250 rpm.

The bottles were uncapped under laminar air flow and the explants were washed thrice with sterile distilled water. After a few moments, the explants which were slightly dried were cultured in prepared media in large jam jars then capped and sealed with parafilm and were stored in culture room at 25 ± 2 ºC, under 16 h photoperiod with light intensity of 40 µmol m^{-2} s^{-1} provided by Philips cool white fluorescent tubes and relative humidity of over 70%.

Table 1: Concentrations of sterilization treatments

Treat	Sodium hypochlorite	Cloro
A	2	10
B	3.	15
C	5	20
D	6.	25
E	7	30

3. Results and discussion

In the earlier studies [9, 8, 5, 1, 4, 2 and 11] *Aloe vera* has been sterilized using the mercuric chloride which is harmful for environment. Our method is easy and new for explants sterilization for in-vitro culture of *Aloe vera.*The number of contaminated explants kept increasing until the fourth week in A and B treatments (sodium hypochlorite 2.5% and 3.75%). In treatments C and D few contaminations were seen but no contamination was seen in treatment E (Figure 1).

After four weeks of culture, some of the contaminated explants died, however, because of its fungal resistance property, some *Aloe vera* explants survived. The number of survived explants was counted after 8 weeks (Table 2).

Table 2: Percentages of Sterilized and survived

Trt.	Sterile %	Viable %
A	8.3	12.5
B	37.5	45.8
C	87.5	91.7
D	95.8	66.7
E	100	29.2

Percentages of dead explants due to contamination effect in treatments A, B and C were 95.45%, 86.66% and 66.66% respectively and percentages of survived explants were 12.5%, 45.8% and 91.7% as it illustrated in Figure 1 and Figure 2.

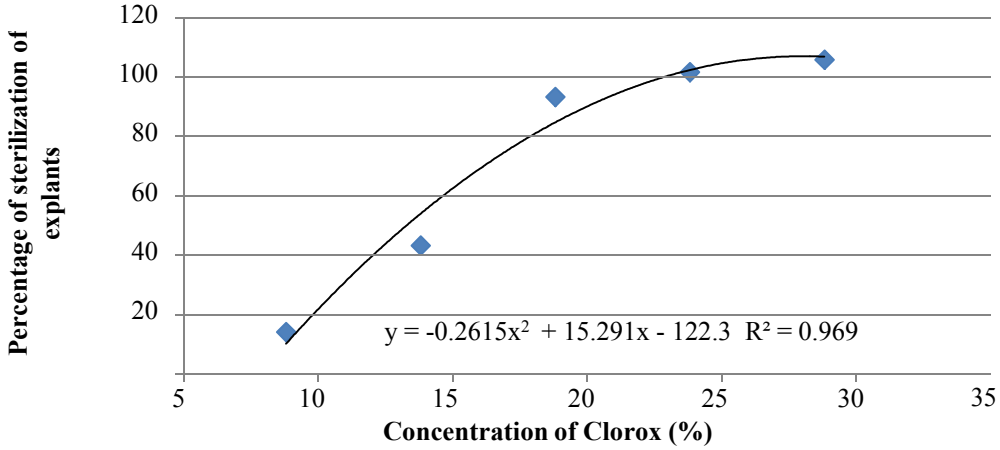

Figure 1: Effects of concentrations of sodium hypochlorite on sterilization of segment nodal discs explants

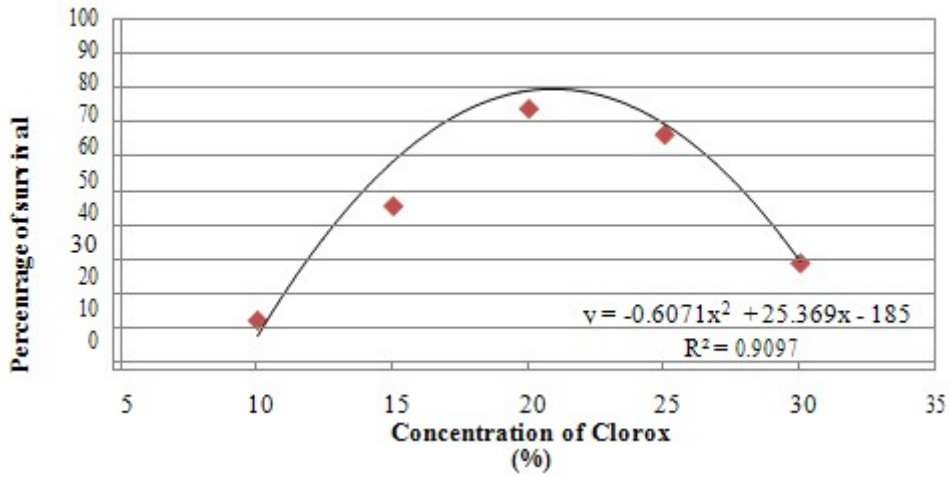

Figure 2: Potential of viability in *A. vera* explants derived from nodal segment after contact of different concentrations of sodium hypochlorite

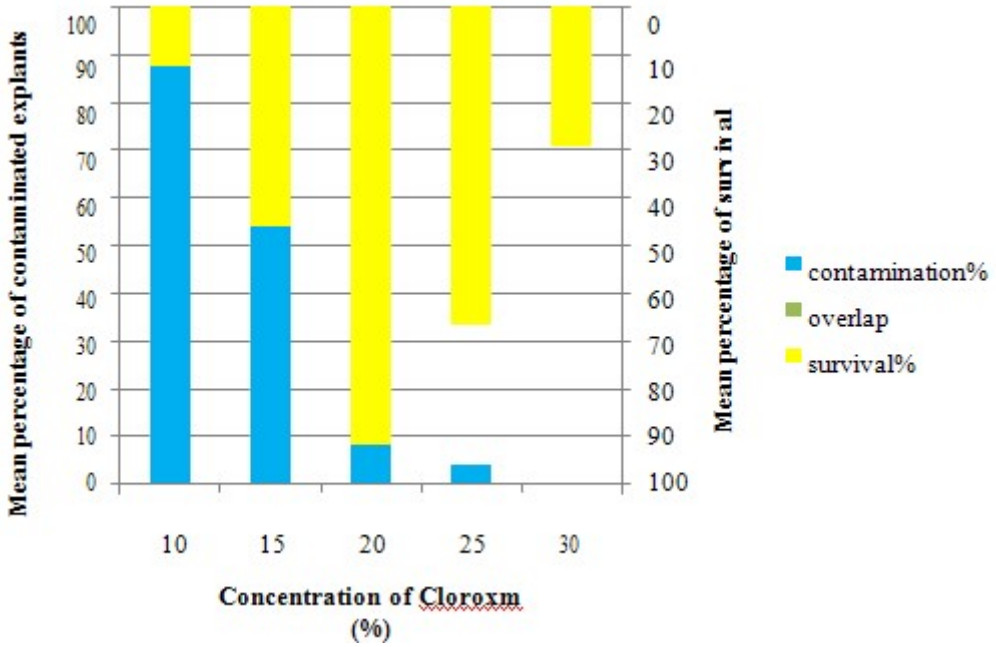

Figure 3: effects of concentrations of sodium hypochlorite on percentage of survived and contaminated explants

It is interesting to mention that, some infected explants, survived. In addition, disparity of percentage of sterile and survived explants in treatments A, B and C, and overlapping of bars in Figure 3 are causes of this matter. While, in high concentrations of sodium hypochlorite contamination was lower (treatment D= 4.2%),

yet the percentage of survived explants decreased (D= 66.70%). Based on the results, in treatment E which has the highest concentration among the treatments, no contaminations was observed. At the same time, only 29% of explants regenerated in treatment E.

Analysis the results showed that, polynomial tender line as illustrated in the chart, has 96.9% similarity with the chart line of the results as it appears in Figure 1. Also, through the equation placed in Figure 1 it is possible to calculate the percentage of sterilization for the other concentrations between the tested treatments, with almost 97% confidence in the range of 10 to 30 percent aqueous solution of Clorox.

After drawing the confidence curve, beside the graphing line of the results, 90.9% similarity were observed. Although, effect of all of concentrations of sodium hypochlorite was not studied, but through the equation showed in the Figure 2, percentage of survival for the other points between the tested amounts can be calculated. The graph has a $R^2 \geq 0.9097$ indicating that survival rate and viability with 20% Clorox is the best treatment.

Applying a Kruskal-Walis test (a nonparametric test), revealed that 5% sodium hypochlorite (Treatment C) with 20 minutes of hard and constant shaking, gives the highest number (91.7%) of viable and sterilized explants with regeneration potential in MS medium enriched by PGR. Using Pearson Chi square test (X=37.144, P value = 0.0001), the results revealed a significant relationship between the Clorox concentration and percentage of surviving explants in *Aloe vera barbadensis* Mill

4. Conclusion

From the results, it can be concluded that for in-vitro culture of *Aloe vera barbadensis* Mill explants can be sterilized without using mercuric chloride. The most suitable concentration of sodium hypo chloride for sterilization is 5% (20% Clorox).

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