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Effects of surface sterilization agents under *in vitro* culture of banana (*Musa paradisiaca* L.) variety "Udhayam"

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Abstract
Effect of two different surface sterilization agents i.e. mercuric chloride and ethanol were tested on the contamination-free establishment of banana cv. Udhayam under *in vitro* conditions. All the sterilization agents performed better results when used individually for different time intervals. Minimum contamination percentage of explants after 10 days (31.33%) was noted under $HgCl_2$ 0.1 per cent treating for a period of 8min while; the maximum (73.25%) was noted under the treatment of $HgCl_2$ 0.1 per cent for a period of 2min. followed by 58.13 and 42.28 per cent with the duration of 4 and 6min. A critical observation was recorded as the maximum survival percentage of explants after 25 days (84.74%) was noted under the treatment of $HgCl_2$ 0.1 per cent for a period of 2min. followed by 74.26 and 53.18 per cent with the duration of 4 and 6min.; while the minimum (37.69%) was noted under $HgCl_2$ 0.1 per cent treating for a period of 8min. The combination of 0.1 per cent $HgCl_2$ with 70 per cent ethanol was also found effective for sterilization of banana explants. Best results with lower contamination 0.1 per cent with $HgCl_2$ for a period of 8 min but higher explant survival per cent was observed with ethanol 70 per cent for a period of 2 min. The present study concludes that the use of 0.1 per cent mercuric chloride and 70 per cent ethanol individually for different time intervals was found to be best to generate contamination-free plants in banana cv. Udhayam with higher explant survival percentage i.e. 84.74 and 85.04 per cent respectively.

Keywords: Aseptic technique, explant (Sucker), sterilization

Introduction
Banana, the fourth largest fruit crop of the world, is a general term referring to a type of fruit or herbaceous plant belonging to Kingdom Plantae, Family Musaceae, of the order Zingiberales and Genus *Musa*. It is native to the tropical region of Southeast Asia. Bananas are likely to have been first domesticated in Papua New Guinea. Banana is rich source of energy. (128 Kcal/100g), carbohydrate (27%), crude fibre (0.5%), protein (1.2%) and moisture (70%) and is also rich in vitamins A, B and C, but particularly vitamin B (FAO, 2014). Banana fruit covers an area of 8.41 million hectare, producing 29.13 million tonnes with a productivity of 14.31 MT/ha during the year 2015-16.

In India, Tamil Nadu ranks first in production followed by Gujarat Andhra Pradesh and Uttar Pradesh. However, the productivity was recorded highest in Madhya Pradesh followed by Gujarat, Maharashtra and Tamil Nadu. Banana contributes 32.30 per cent to total production in India. (Review Committee, 16.05.2017., Indian Horticulture N H B Data base 2015-16). It is propagated vegetatively through sword suckers and other types of planting materials like bits, butts and peepers. But the most common limiting factor for enhanced productivity is the non-availability of clean and disease free planting material. To overcome the problem, tissue culture technology is used for the mass production of the planting material. (Uma *et al*. 2010) [10] In India the requirement of tissue culture plantlets is approximately 2500 million but only 60-80 million tissue culture plantlets are produced per year, which accounts only 2.5 per cent of total requirement and suckers constitute 95-97 per cent of the planting material**.** The basic step in micropropagation is the *in vitro* establishment of contamination-free plantlets. This could be easily achieved by using effective chemical sterilization procedures. Therefore, the present study was designed to develop efficient sterilization procedure for *in vitro* clonal propagation of banana with lower contamination and higher explant survival percentages.

Material and Methods
The present study was carried out in the Tissue Culture Laboratory, Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram,

Meerut, Uttar Pradesh for developing efficient sterilization procedure for *in vitro* establishment of contamination-free plantlets of Banana cv. Udhayam. The sword suckers of Banana cv. Udhayam were used as explants to investigate the effects of different surface sterilization agents. The suckers were washed by Hi Spark cleaning solution (Hi media) under tap water for 30 min and the outer layer was removed carefully. Then it is rinsed 3 times by double distilled water. The explants were washed with sterilized double distilled water for three times rinsed for a period of 5 minutes, followed by soaking in Mercuric chloride (0.1%) and Ethanol (70%) for different time intervals. At the final step, the suckers were again washed by sterilized distilled water for three times, and were trimmed, cut and cultured in MS media. All needed glasswares, equipments and distilled water were autoclaved at a pressure of 15 psi at 121.6 ºC for 25 minutes. The inside surface of laminar flow was wiped by 70 per cent ethanol and was sterilized through Ultra Violet rays for 30 min prior to explant sterilization. Finally, all explants inoculated on basal MS media (Murashige and Skoog, 1962) [6] supplemented with 2, 4-D with different concentration were incubated in culture room at the temperature was maintained at 26 ºC, humidity at 60 per cent at 16h/8h light/dark photoperiods under white fluorescent tubes providing light intensity of 4000 lux. The contamination percentage and explant survival were recorded at weekly time intervals and the contaminated cultures were discarded immediately. All the experiments were conducted in a complete randomized design (CRD) with ten replicates (n=10) per treatment and repeated thrice. The effects of different treatments on various parameters were determined by ANOVA using Window stat 9.2 software.

Results and Discussion

Maximum contamination percentage of explants after 10 days (73.25%) was noted under the treatment of $HgCl_2$ 0.1 per cent for a period of 2min. followed by 58.13 and 42.28 per cent with the duration of 4 and 6min.; while the minimum (31.33%) was noted under $HgCl_2$ 0.1 per cent treating for a period of 8min. A critical observation was recorded as the maximum survival percentage of explants after 25 days (84.74%) was noted under the treatment of $HgCl_2$ 0.1 per cent for a period of 2min. followed by 74.26 and 53.18 per cent with the duration of 4 and 6min.; while the minimum (37.69%) was noted under $HgCl_2$ 0.1 per cent treating for a period of 8min. So, it was observed that survival percentage of explants was *vice versa* to the duration of the treatment with $HgCl_2$ 0.1 per cent (Table-1).

Maximum contamination percentage of explants after 10 days (77.48%) was noted under the treatment of Ethanol (70%) for a period of 2min. followed by 61.56 and 47.30 per cent with the duration of 3 and 4min.; while the minimum (38.38%) was noted under Ethanol (70%) treating for a period of 5min. A critical observation was recorded as the maximum survival percentage of explants after 25 days (85.04%) was noted under the treatment of Ethanol (70%) for a period of 2min. followed by 75.27 and 56.78 per cent with the duration of 3 and 4min., while the minimum (42.77%) was noted under Ethanol (70%) treating for a period of 5min. So, it was observed that survival percentage of explants was again *vice versa* to the duration of the treatment with Ethanol 70 per cent (Table-2).

Maximum contamination percentage of explants after 10 days (83.66%) was noted under the treatment of Mercuric Chloride (0.1%) + Ethanol (70%) for a period of 1min. followed by 62.00 and 41.09 per cent with the duration of 2 and 3min., while the minimum (40.57%) was noted under Mercuric Chloride (0.1%) + Ethanol (70%) treating for a period of 4min. A critical observation was recorded as the maximum survival percentage of explants after 25 days (72.34%) was noted under the treatment of Mercuric Chloride (0.1%) + Ethanol (70%) for a period of 1min. followed by 32.19 and 31.14 percent with the duration of 2 and 3min., while the minimum (10.41%) was noted under Mercuric Chloride (0.1%) + Ethanol (70%) treating for a period of 4min. So, it was observed that survival percentage of explants was also *vice versa* to the duration of the treatment with Mercuric Chloride (0.1%) + Ethanol (70%). The present study, between the two chemicals i.e. Mercuric Chloride ($HgCl_2$) and Ethanol (70%) were found better for controlling the infection and had no any adverse effect on explants even in long duration (1-2 minutes) exposure. However, a treatment combination of Mercuric Chloride (1.0%) with Ethanol (70%) for 1 minute followed by 2 minutes resulted the highest percentage (72.34 and 32.19%) of aseptic culture establishment in banana cv. Udhayam (Table-3). The same pattern was observed to reduce microorganism and sterilize the explant to get a clean material for *in-vitro* propagation of banana by Molla *et al.*, (2004) [5]; Titov *et al.*, (2006) [9]; Rahman *et al*, (2002) [8]; Madhulatha *et al*, (2004) [4]; Dharmapal *et al.*, (2017) [2] and Yadav *et al*, (2017) [11], Ethanol with low concentration of $HgCl_2$ have also been used by a number of research workers for disinfection purposes Jalil *et al*, (2003) [3]; Onuoha *et al*, (2011) [7] achieved the contamination free Plantain culture (100%) in the explants treated with $HgCl_2$ for 6min.

Table 1: Standardization of $HgCl_2$ (0.1%) treatment duration for surface sterilization of shoot tips of banana cv. Udhayam

Treatments	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
2 min	73.257 a	84.743 a
4 min	58.130 b	74.260 b
6 min	42.283 c	53.180 c
8 min	31.337 d	37.690 d
Gen. Mean	51.252 ***	62.468 ***
C.V.	2.023	1.292
S. E. M.	0.599	0.466
C.D.@ 5%	1.952	1.520
C.D.@ 1%	2.840	2.211

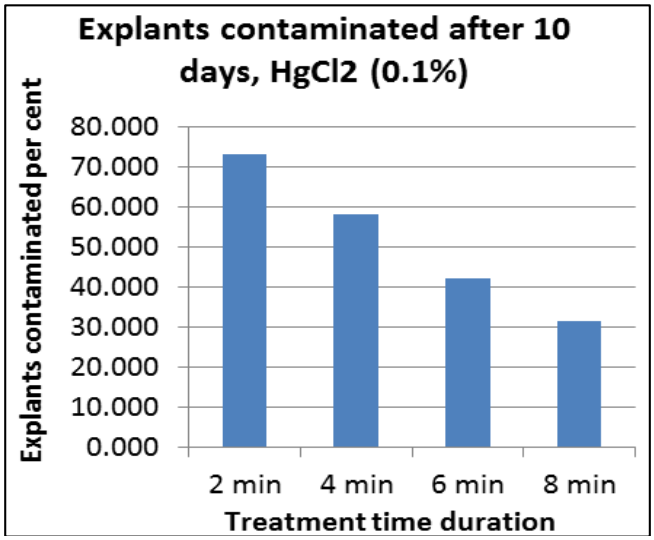

Fig 1: Explants contaminated after 10 days, HgCl2 (0.1%)

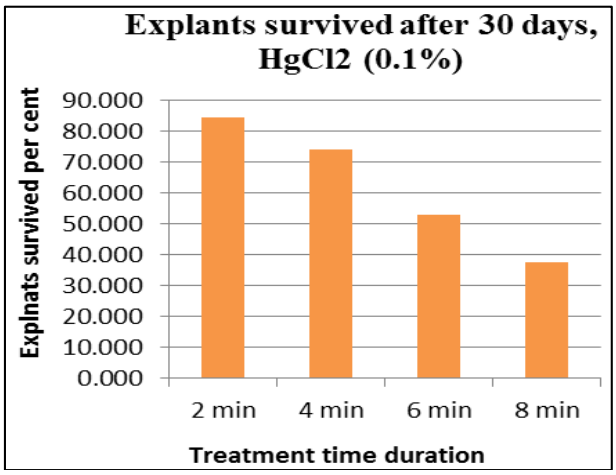

Fig 2: Explants survived after 30 days, HgCl2 (0.1%)

Table 2: Standardization of Ethanol (70%) treatment period for surface sterilization of shoot tip of banana cv. Udhayam

Treatments	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
2 min	77.487 a	85.047 a
3 min	61.563 b	75.277 b
4 min	47.307 c	56.783 c
5 min	38.383 d	42.777 d
Gen. Mean	56.185 ***	64.971 ***
C.V.	1.273	1.026
S. E. M.	0.413	0.385
C.D.@ 5%	1.347	1.256
C.D.@ 1%	1.960	1.827

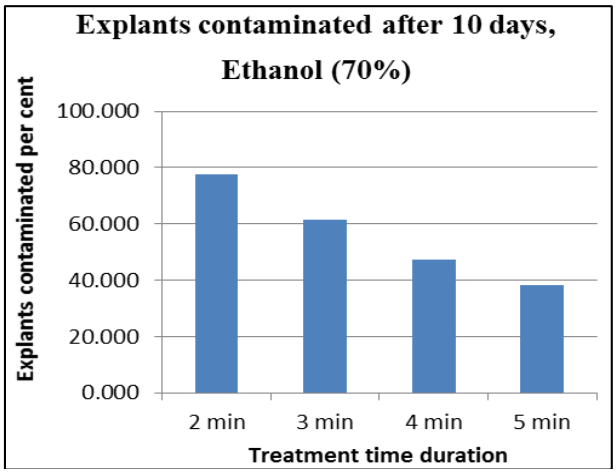

Fig 3: Explants contaminated after 10 days, Ethanol (70%)

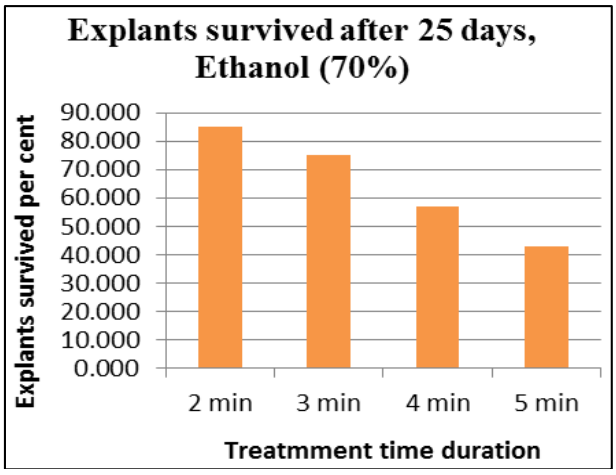

Fig 4: Explants survived after 25 days, Ethanol (70%)

Table 3: Standardization of Mercuric Chloride (0.1%) + Ehanol (70%) treatment period for surface sterilization for banana explant cv. Udhayam

Mercuric Chloride (0.1%) + Ethanol (70%)	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
1 min	83.660 a	72.340 a
2 min	62.003 b	32.197 b
3 min	41.090 c	31.140 b
4 min	40.570 c	10.417 c
Gen. Mean	**56.831 *****	**36.523 *****
C.V.	1.027	1.992
S.E.M.	0.337	0.420
C.D.@ 5%	1.099	1.370
C.D.@ 1%	1.599	1.994

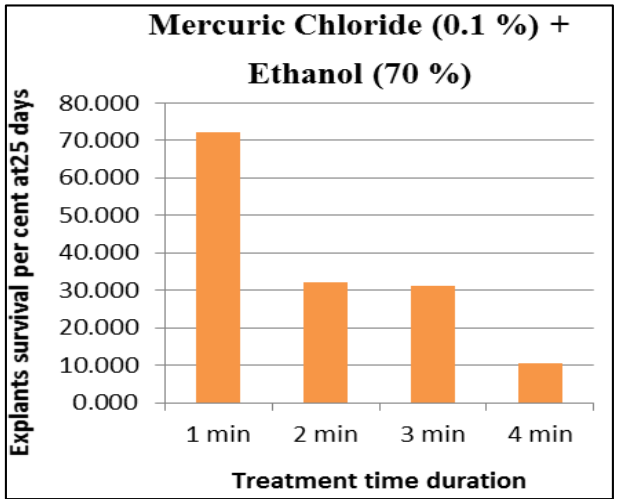

Fig 5: Mercuric Chloride (0.1%) + Ethanol (70%)

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