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Effects of Mercuric chloride and Ethanol for surface sterilization under *in vitro* plant growth in banana (*Musa paradisiaca* L.) variety "Udhayam"

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Abstract
Present investigation "Effects of Mercuric chloride and Ethanol for surface sterilization under *in vitro* plant growth in banana (*Musa paradisiaca* L.) variety "Udhayam" was carried out at the Tissue Culture Laboratory Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut during the year 2018-2019. 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 (32.24%) was noted under $HgCl_2$ 0.1 per cent treating for a period of 8 min. A critical observation was recorded as the maximum survival percentage of explants after 25 days (67.73%) was noted under the treatment of $HgCl_2$ 0.1 per cent for a period of 8 min. Minimum contamination percentage of explants after 10 days (28.52%) was noted under ethanol 70 per cent treating for a period of 5 min. A critical observation was recorded as the maximum survival percentage of explants after 25 days (70.82%) was noted under the treatment of ethanol 70 per cent for a period of 5 min. 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 4 min. (26.58%). Where as higher explant survival per cent (73.42%) was observed $HgCl_2$ 0.1 per cent with ethanol 70 per cent for a period of 4 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.

Keywords: aseptic technique, explant (Sucker), sterilization

Introduction
Banana, is referring to a type of herbaceous plant belonging to Kingdom Plantae, Family Musaceae, of the order Zingiberales and Genus Musa. 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. India is the largest producer of bananas in the world, followed by China and Indonesia. The world's annual production is 155.2 million tones with an area of 5.6 million hectares (FAOSTAT2018) [2]. Currently, banana is the largest fruit crop accounting for almost 39.40 per cent of total fruit production. In India contributing about 29 per cent of total world production with the production of about 30.807 million tones, covering area of 8.83 million hectares and productivity 34.9 t/ha. 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. 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 micro propagation 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.

Materials 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) 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. All the experiments were conducted in a complete randomized design. The effects of different treatments on various parameters were determined by ANOVA using Window stat 9.2 software.

Results and Discussion

Percentage of explants contaminated and survived after 10 and 25 days (HgCl2)

Table 4.1: Standardization of Hgcl2 (0.1%) treatment period for surface sterilization of explants of banana cv. Udhayam

Treatments	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
2 min	68.30	31.70
4 min	52.18	47.78
6 min	41.69	58.31
8 min	32.24	67.73
S.E.M.	0.58	0.57
CD at 5%	1.94	1.89

The table (4.1) narrates that surface sterilization of explants of banana cv. Udhayam through HgCl2 treatments was significantly increased with increasing of duration from 2 to 8 minutes. Maximum contamination percentage of explants after 10 days (68.30%) was noted under the treatment of $HgCl_2$ 0.1% for a period of 2 minute followed by 52.18 and 41.69 per cent with the duration of 4 and 6 minute respectively; while, the minimum contamination (32.24%) was noted under $HgCl_2$ 0.1% treating for a period of 8 minute. A critical observation was recorded as the maximum survival percentage of explants after 25 days (67.73%) was noted under the treatment of $HgCl_2$ 0.1% for a period of 8 minute followed by 58.31 and 47.78 per cent with the duration of 6 and 4 minute respectively; while, the minimum (31.70%) was noted under $HgC1_2$ 0.1% 69 treating for a period of 2 minute. So, it was observed that survival percentage of explants was directly corelated to the duration of the treatment with $HgCl_2$ (0.1%). So, it was observed that survival percentage of explants was directly corelated to the duration of the treatment with $HgCl_2$ (0.1%); whereas, mercuric chloride gave maximum survival of explants with minimum tissue injury when treated for 4 minutes revealed by Yadav *et al.* (2017) [8] and Jalil *et al.* (2003) [3].

4.2. Percentage of explants contaminated and survived after 10 and 25 days (Ethanol 70%)

Table 4.2: Standardization of Ethanol (70%) treatment period for surface sterilization of banana suckers cv. Udhayam

Treatments	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
2 min	64.55	35.46
3 min	52.74	47.26
4 min	39.85	60.15
5 min	28.52	70.82
S.E.M.	0.80	0.64
CD at 5%	2.65	2.13

The table (4.2) evince that surface sterilization of explants of banana cv. Udhayam through Ethanol (70%) treatments was significantly increased with increasing of duration from 2 to 5 minutes. Maximum contamination percentage of explants after 10 days (64.55%) was noted under the treatment of Ethanol (70%) for a period of 2 minute followed by 52.74 and 39.85 per cent with the duration of 3 and 4 minute respectively; while, the minimum (28.52%) was noted under Ethanol (70%) treating for a period of 5 minute. A critical observation was recorded as the maximum survival percentage of explants after 25 days (70.82%) was noted under the treatment of Ethanol (70%) for a period of 5 minute followed by 60.15 and 47.26 per cent with the duration of 4 and 3 minute respectively; while the minimum (35.46%) was noted under Ethanol (70%) treating for a period of 2 min. So, it was observed that survival percentage of explants was directly corelated with the duration of the treatment with Ethanol (70%). The same pattern was observed to reduce microorganism and sterilize the explant to get a clean material for *in vitro* propagation of banana by Dharmapal *et al.* (2017) [1], Yadav *et al.* (2017) [8] and Rahman *et al.* (2002) [7].

4.3. Percentage of explants contaminated and survived after 10 and 25 days (Hgcl2 with Ethanol 70%)

Table 4.3: Standardization of Mercuric Chloride (0.1%) + Ethanol (70%) treatment period for surface sterilization of explants for banana cv. Udhayam

Treatments	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
1 min	59.15	40.85
2 min	52.70	47.30
3 min	49.26	50.40
4 min	26.58	73.42
S.E.M.	0.69	0.90
CD at 5%	2.30	2.98

The table (4.3) describes that surface sterilization of explants of banana cv. Udhayam through Mercuric Chloride $HgCl_2$ (0.1%) + Ethanol (70%) treatment was significantly increased with increasing of duration from 1 to 4 minute. Maximum contamination percentage explants after 10 days (59.15%) was noted under the treatment of Mercuric Chloride $HgCl_2$ (0.1%) + Ethanol (70%) for a period of 1 minute followed by 52.70 and 49.26 per cent with the duration of 2 and 3 minute respectively; while, the minimum (26.58%) was noted under Mercuric Chloride $HgCl_2$ (0.1%) + Ethanol (70%) treating for a period of 4 minute. A critical observation was recorded as

the maximum survival percentage of explants after 25 days (73.42%) was noted under the treatment of Mercuric Chloride $HgCl_2$ (0.1%) + Ethanol (70%) for a period of 4 minute followed by 50.40 and 47.30 71 per cent with the duration of 3 and 2 minute respectively; while, the minimum (40.85%) was noted Mercuric Chloride $HgCl_2$ (0.1%) + Ethanol (70%) treating for a period of 1 minute. So, it was observed that survival percentage of explants was vice versa to the duration of the treatment with noted Mercuric Chloride $HgCl_2$ (0.1%) + Ethanol (70%). Ethanol with low concentration of $HgCl_2$ have been used by a number of research workers for disinfection purpose. Jalil *et al.* (2003) [3], Onuoha *et al.* (2011) [6] Madhulata *et al.* (2004) [4] and Molla *et al.* (2004) [5] had achieved the contamination free Plantain culture (100%) in explants treated with $HgCl_2$.

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