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Standardization of surface sterilization for *in vitro* propagation of potato (*Solanum tuberosum* L.) variety Kufri Surya

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Abstract
The present investigation entitled "Standardization of surface sterilization for *in vitro* propagation of potato (*Solanum tuberosum* L.) Variety Kufri Surya" was conducted at Plant Tissue Culture Laboratory, College of Horticulture, Dr. Y.S.R Horticultural University, Andhra Pradesh in a Completely Randomized Design. Sprouts were pretreated and surface sterilized with 0.1% mercuric chloride at 4, 6 and 8 minutes and sodium hypochlorite at 5,10 and 15 minutes. Comparison was done between two important sterilants mercuric chloride and Sodium hypochlorite. The observations recorded regularly at 7 days for the fungal, bacterial, total contamination, percent mortality other than microbes and explant survival. Results revealed that between two sterilants, $HgCl_2$ was found better for controlling the contamination though prolonged treatment showed adverse effect on explants due to chemical toxicity causing browning. Mercuric chloride ($HgCl_2$) at 8 min was selected was found as best suitable sterilization chemical as it reduces contamination and increases explant survival.

Keywords: Contamination, surface sterilization and explants

Introduction
Potato (*Solanum tuberosum* L.) is the most important and widely consumed tuber vegetable grown throughout the world and ranks third after rice and wheat. It is native to highland tropics of Andean mountain of Bolivia and Peru in South America, belongs to Solanaceae family. Cultivated potato is tetraploid (2n=4X=48) in nature.

India is the second largest producer of potato in the world and Uttar Pradesh ranks first both in terms of area and production.With its high tuber yield, digestible protein, adequate amount of vitamin B and C, it can effectively contribute to nutritional security in developing countries (Pushkarnath, 1976) [7]. It contains all the dietary substances except fat. It is used as staple diet in many of the countries especially in the West. Potato contains substantial quantity of energy (88 kcal), edible proteins (2.8 g), starch (16.3 g), total sugars (0.6 g), crude fibre (0.5 g), carbohydrates (22.6 g) and vitamin C (25 mg) per 100 g of fresh weight of tubers (Bhuwaneshwari *et al*., 2013) [2].

Potatoes are traditionally propagated by means of asexual propagation. The required seed rate of potato is 2.5 tonnes per hectare (Pandey *et al*., 2013) [6] and the seed tubers account for 50% of the cost of cultivation. Availability of quality seed material is a major constraint in potato production. So micro-propagation is the most effective method and is the alternative to conventional propagation of potatoes. It has been proved to be very efficient method to produce disease-free plantlets of high quality in terms of genetic and physiological consistency (Sathish *et al.*, 2011) [10].

Material and Methods
The present investigation entitled "Standardization of surface sterilization for *in vitro* propagation of potato (*Solanum tuberosum* L.) Variety Kufri Surya" was carried out during December 2021 to April 2022 at Tissue Culture Laboratory, College of Horticulture, Dr. Y.S.R Horticultural University, Venkataramannagudem. The experiment was conducted in Completely Randomized Design seven treatments with three replications. Mercuric chloride ($HgCl_2$) and Sodium hypochloride (NaOCl) chemicals were used for the experimental studies.

Sprouting of potatoes

Disease free healthy potato tubers of kufri surya were collected and and washed under running tap water for 10 minutes. After washing tubers were dipped in a solution of (2 g/L) Bavistin for 10 minutes and again tubers washed thrice with water and dried them in a filter paper to remove additional moisture and were kept in tissue culture inoculation room at 25 ^{0}C. The sprouts were ready for inoculation after 10-12 days of growth. The sprouts of about 0.5-1 cm. were collected from the mother tuber of Kufri surya in beaker and kept under running water for 10 min.

Pretreatment of sprouts

They were treated with with 1% tween 20, 0.5 g Bavistin, 0.2 g streptomycin each with 5 minutes and followed by distilled water wash. Pretreated sprouts were shifted to laminar air flow for surface sterilization, 20 sec dip in ethanol and at last washed with sterile distilled water and after treated with the following treatments were used during the work:

Standardisation of surface sterilization method

Number of treatments: 7

T_1-70% Ethanol for 20 sec + 0.1% $HgCl_2$ for 4 min
T_2-70% Ethanol for 20 sec + 0.1% $HgCl_2$ for 6 min
T_3-70% Ethanol for 20 sec + 0.1% $HgCl_2$ for 8 min
T_4-70% Ethanol for 20 sec + 4% NaOCl for 5 min
T_5-70% Ethanol for 20 sec + 4% NaOCl for 10 min
T_6-70% Ethanol for 20 sec + 4% NaOCl for 15 min
T_7- Control (Distilled water)

Results and Discussion

4.1a fungal contamination (%)

Data recorded on fungal contamination (Table 4.1) revealed that among the treatments, the minimum fungal contamination was observed when sterilized with 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 8 min (T_3) (10.00 %) which was on par with 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 6 min (T_2) (13.30 %) and 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 4 min (T_1) (16.70%) but highest contamination was observed in Control (Distilled water) (T_7) (60.00 %) followed by 70% Ethanol for 20 sec+4% NaOCl for 5 min (T_4) (30.00%). The overall mean recorded in the present experiment was (25.71%).

4.1b Bacterial contamination (%)

The mean of bacterial contamination was (21.43) (Table 4.1). The data showed significant differences among the treatments. There is no bacterial contamination was observed when sterilized with 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 8 min (T_3) (0.00) followed by 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 6 min (T_2) (6.70 %). The highest bacterial contamination was recorded in Control (Distilled water) (T_7) (40.00 %) followed by 70% Ethanol for 20 sec+4% NaOCl for 5 min (T_4) (33.33 %) and 70% Ethanol for 20 sec+4% NaOCl for 10 min (T_5) (33.33 %).

4.2 Total contamination (%)

Data presented in (Table 4.1) revealed the significant differences among the treatments with a mean value of (36.67%). The minimum contamination was observed in 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 8 min (T_3) (10.00 %) followed by 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 6 min (T_2) (20.00 %) and the maximum contamination was recorded in Control (Distilled water) (T_7) (100.00 %) followed by 70% Ethanol for 20 sec+4% NaOCl for 5 min (T_4) (63.33 %) and 70% Ethanol for 20 sec+4% NaOCl for 10 min (T_5) (60.00 %).

4.3 Explant mortality other than microbial contamination (%)

Data given in (Table 4.2) showed that the mean per cent explant mortality other than microbial contamination in the present study was (2.86). Among the treatments, there is no explant mortality due to toxicity of chemical in sodium hypochlorite so that 0 % toxicity was observed when sterilized with NaOCl. Among the treatments toxicity was observed when sterilized with mercuric chloride. The minimum mortality was observed in 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 4 min (T_1) (3.33%) and the maximum mortality was noticed in 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 8 min (T_3) (10.00 %)

4.4 Explant survival (%)

The mean per cent of explant survival in the present experiment was (49.52 %) (Table 4.2) revealed that the highest explant survival was observed in 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 8 min (T_3) (76.67 %) which was on par with 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 6 min (T_2) (73.33%) and 70% Ethanol for 20 sec+0.1% $HgCl_2$ for 4 min (T_1) (70.00 %). The lowest explant survival was observed in Control (Distilled water) (T_7) (0.00%) followed by 70% Ethanol for 20 sec+4% NaOCl for 5 min (T_4) (36.67 %) which was on par with 70% Ethanol for 20 sec+4% NaOCl for 10 min (T_5) (40.00 %).

The present investigation found that 0.1% $HgCl_2$ alone at lower concentration was not sufficient for sterilization of the explants. Increasing the duration of $HgCl_2$ subsequently decreases the survival. Survival percentage was increased with optimum exposure to mercuric chloride for 8 minutes.

Mercuric chloride is highly poisonous and being a potent sterilant, was found to compromise membrane integrity of live plant tissue. Therefore, survival percentage was lessened with prolonged exposure to it. The same was at the highest in those explants treated for shorter durations. Similar results were obtained by Din *et al*. (2018) [3].

The present investigation revealed that both the chemicals used for sterilization reduced per cent contamination with increase in the exposure time. The exposure time with $HgCl_2$ increases resulted toxicity death of explants recording a lesser survival due to damage of sprout tissues. The results showed harmful effect of $HgCl_2$ at high concentration and longer duration is agreement with other reports (Bamel *et al.*, 2007 and Singh *et al.*, 2007) [1, 11]. Surface sterilization should not kill or break off the biological activity of explants, but the contaminants only should be eliminated. Explants must be surface sterilized only by treatment with disinfectant solution at suitable concentrations for a specified period (Oyebanji *et al.*, 2009) [5].

Lower concentrations or lesser duration of exposure of chemicals were found to be poor in checking contamination. This might be due to the insufficiency of the concentration of chemicals short exposure time to kill the microorganisms from cultured explants as opined by Wegayehu *et al.* (2015) [12].

In the present investigation was $HgCl_2$ at 0.1% for 8 minutes may be due to bleaching action of two chloride atoms and

also ions that combines strongly with proteins and causing the death of organisms with respect to low contamination rate, which is in accordance with the findings of Raju *et al.* (2005) [9]. This might be due to the most useful radical in $HgCl_2$ probably the chlorite, commonly present as bichloride of mercury. (Rahman *et al.* (2004) [8].

Yasmin *et al.* (2003) [13] reported that for surface sterilization, isolated sprouts of potato were first sterilized with 70% (v/v) ethanol for few seconds. The sprouts were then rinsed twice with sterile distilled water then immersing in 0.1% $HgCl_2$ solution for 2 min then washed several times with sterile distilled water.

Koleva *et al.* (2012) [4] reported that the sprouts were pretreated by washing under flow of tab water for 10-15 min. After washing, the sprouts are surface sterilized by dipping in 70% alcohol for 2 min, followed by 0.1 $HgCl_2$ solution for 3-5 min, then were washed several times with sterilized distilled water.

It can be concluded that sterilization treatment (T_3) comprising 70% ethanol for 20 seconds followed by $HgCl_2$ (0.1%) for 8 minutes was found to be the best amongst all the sterilization treatment methods used for culture establishment.

Table 1: Effect of surface sterililization on contamination mortality other than microbes and explant survival in potato sprouts.

Treatment details	Extent of microbial Contamination (%)		Total contamination (%)	Explant mortality other than microbial contamination (%)	Explant Survival (%)
	Fungal	Bacterial			
T_1-70% Ethanol (20 sec)+0.1% $HgCl_2$ (4 min)	16.70 (23.84)	10.00 (18.42)	26.67 (30.98)	3.33 (1.77)	70.00 (56.97)
T_2-70% Ethanol (20 sec)+0.1% $HgCl_2$ (6 min)	13.30 (21.13)	6.70 (12.28)	20.00 (30.28)	6.67 (2.54)	73.33 (59.68)
T_3-70% Ethanol (20 sec) + 0.1% $HgCl_2$, (8 min)	10.00 (18.42)	0.00 (0.00)	10.00 (21.13)	10.00 (3.31)	76.67 (61.19)
T_4-70% Ethanol (20sec) + 4% NaOCl, (5 min)	30.00 (32.98)	33.33 (35.20)	63.33 (18.42)	0.00 (1.00)	36.67 (37.21)
T_5-70% Ethanol (20sec) + 4% NaOCl, (10 min)	26.67 (30.98)	33.33 (35.20)	60.00 (52.75)	0.00 (1.00)	40.00 (39.13)
T_6-70% Ethanol (20sec) + 4% NaOCl, (15 min)	23.33 (28.76)	26.67 (30.98)	50.00 (51.48)	0.00 (1.00)	50.00 (44.98)
T_7- Control(Distilled water)	60.00 (50.83)	40.00 (39.13)	100.00 (48.82)	0.00 (1.00)	0.00 (0.00)
Mean	25.71 (29.56)	21.43 (24.46)	36.67 (36.27)	2.86 (1.66)	49.52 (42.74)
SE m	3.98	3.33	3.53	1.78	5.34
CD at 5%	12.20	10.20	10.83	5.45	16.37

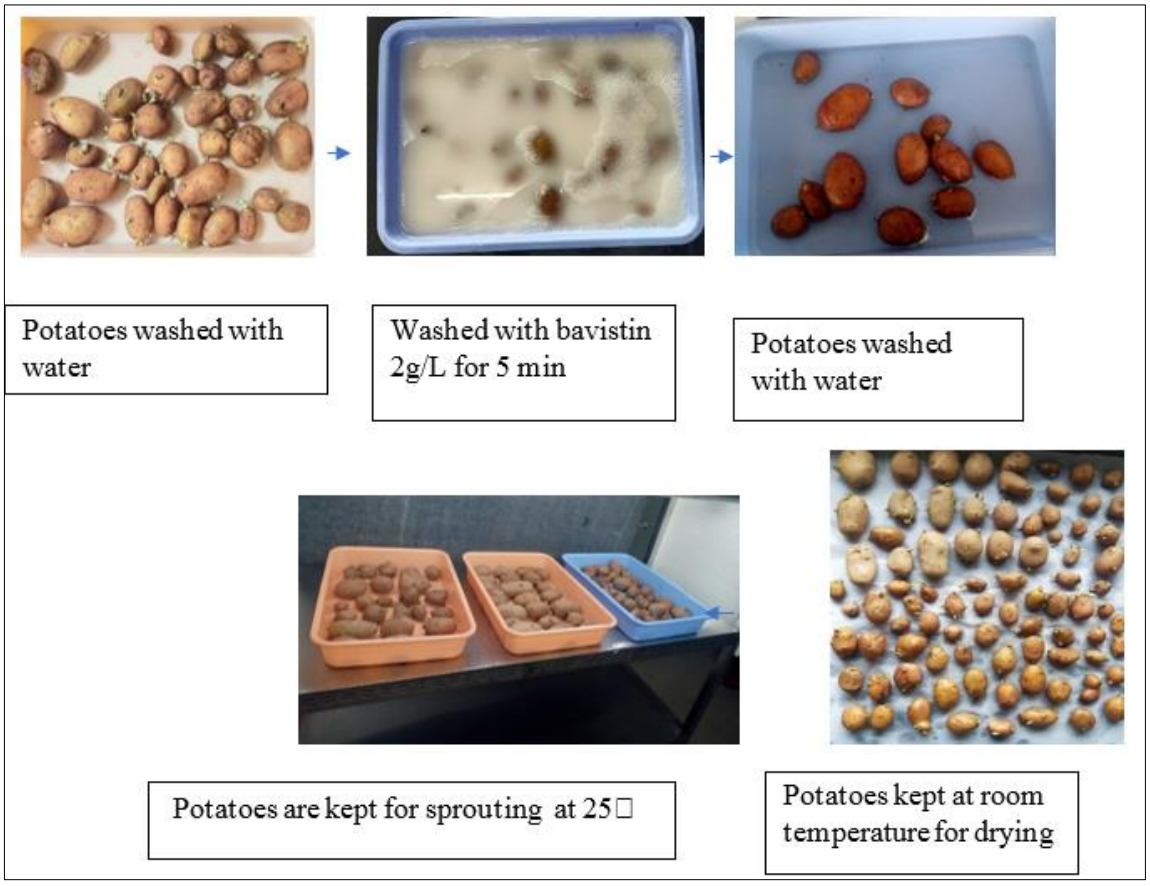
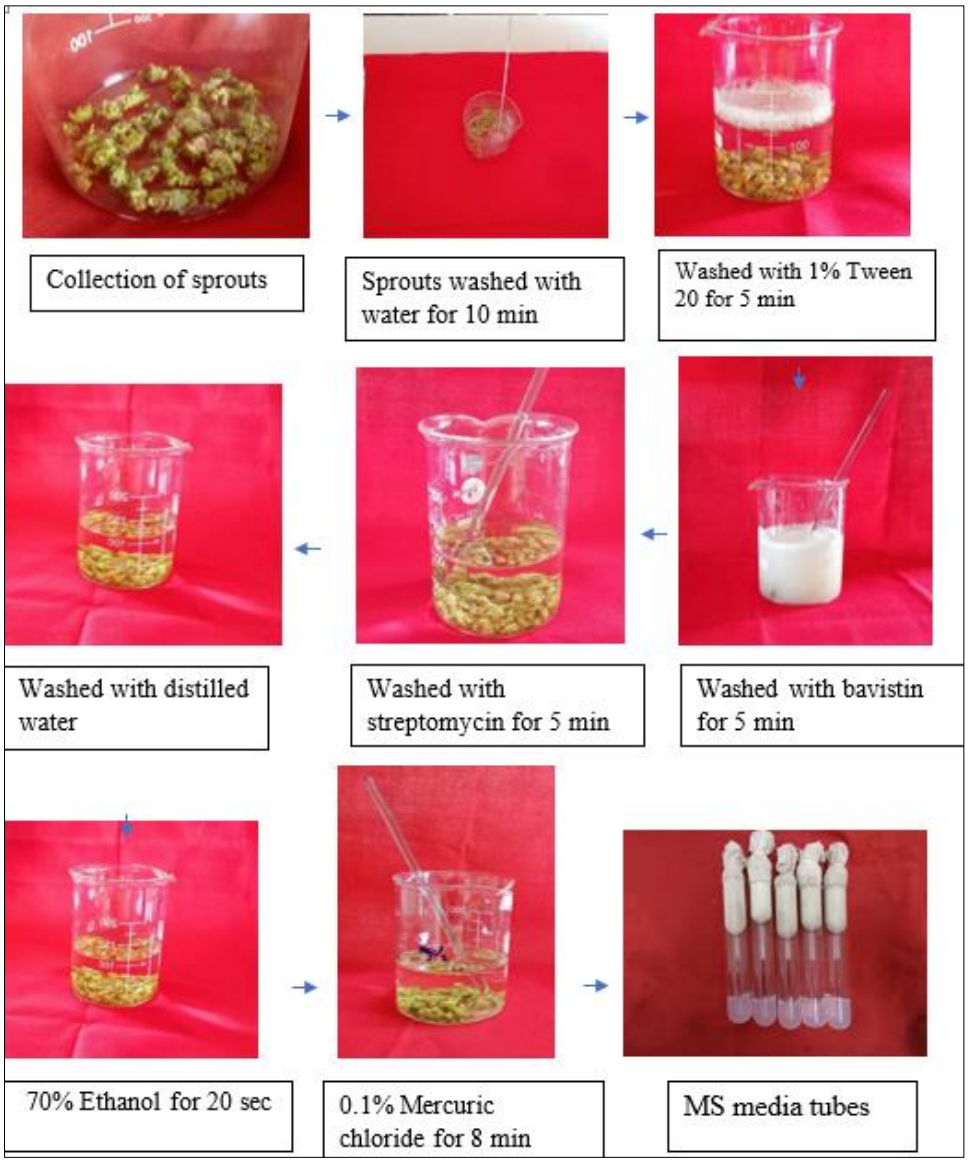


Plate 1: Washing of potatoes for sprouting



Types of contamination observed in potato sprouts cultured on MS media

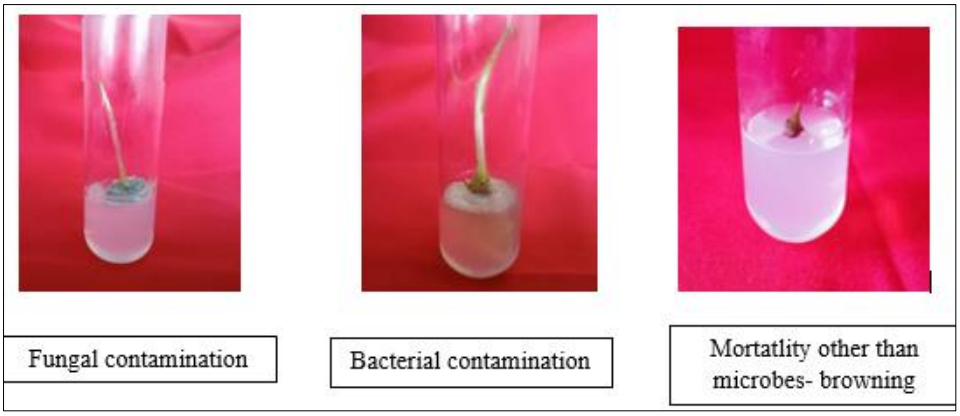


Plate 2: Procedure for pre-treatment and surface sterilization of potato sprouts

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