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Effects of two different surface sterilization (Sodium Hypochlorite and Mercuric Chloride) agents under *in-vitro* leaf explant in Gerbera (*Gerbera jamesonii* Bolus)

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Abstract
Effect of surface sterilization agents' i.e. Sodium hypochlorite and Mercuric Chloride were tested on the contamination-free establishment of Gerbera leaf under *in-vitro* conditions. Sodium Hypochlorite @ 4 per cent (2, 4, 6 and 8 minutes) performed better results. Mercuric Chloride ($HgCl_2$) @ 0.1 per cent (1, 2, 3 and 4 minutes) and the Sodium hypochlorite @ 4 per cent with Mercuric Chloride ($HgCl_2$) @ 0.1 per cent (2, 3, 4 and 5 minutes) for time duration. Sodium hypochlorite 4 per cent (2, 4, 6 and 8 minutes) was found that the best survival percentage (41.69 %) for 8 minutes. While Mercuric Chloride ($HgCl_2$) @ 0.1 per cent was found the best survival percentage was (62.80%) for 4 minutes However, a combination of Sodium hypochlorite 4 per cent with Mercuric Chloride ($HgCl_2$) 0.1 per cent was found best survival percentage of (73.42%) so, the results found that the best sterilization agent was found to be a combination of mercuric chloride ($HgCl_2$) @ 0.1 percent + Sodium hypochlorite @ 4 percent for 3 minutes.

Keywords: treatment, Gerbera leaf, mercuric chloride ($HgCl_2$), sodium hypochlorite

Introduction
Gerbera (*Gerbera jamesonii* Bolus ex Hook) is the latest sensation to Indian Floriculture, with chromosome number 2n = 50, commercially a wide range of climatic conditions grown in throughout the world Lhoste, (2002) [6]. It is commonly known as Barberton daisy, African daisy, and Translates daisy and is a classic sun tracker like sunflowers. It is a dwarf stem-less herbaceous perennial herb growing in clump with solitary flower heads termed capitulum on a long slender stalk, well above the foliage. The leaves are petioled, lobed, coarse or sometimes tubular and two-lipped and the flowers are daisy-like in appearance available in a wide range of colours Sil *et al.*, (2017) [10]. It ranks 6th among the ornamental flowers in the world from a commercial point of view Barooah and Talukdar, (2009) [3]. It is a perennial herb with deeply lobed leaves covered with silky hairs arising from a crown. Leaves occur in basal rosettes, petioles, oblong-spatulas and deeply lobed to 25 cm long by half as wide and 45 cm in height with a spread of 60 cm wide. It has a symposia rhizome commonly known as the crown. Its internodes are located very close to one another and in close proximity to the substrate. Even the flower receptacles are formed under the ground before they make their way above the soil. This close proximity to the soil in field grown Gerbera makes contamination of explants *in vitro* condition, a major obstacle in the establishment of cultures. The problem is further compounded by the hairy nature of the shoot tip and receptacle the two explants widely used for commercial multiplication of gerbera *in-vitro.* In this order, the new method of growing plants is being used to develop more and more plants in a very short time in many countries, tissue culture is being developed in large quantities and the plants are being transported to another place and country. Sterilization of the surface of explants with chemicals has got better results in tissue culture. It is considered an important study as the applications brings a lot of benefits to mankind. One main application is to produce identical plants which have a high demand to retain the desired quality of the plants (Govindan, 2009) [5].

Material and Methods
Explant source
The gerbera plants were collected from Sheel Biotech Ltd. Gurgaon Haryana-122051 and plants were transferred in the Greenhouse condition at Herbal Garden of Horticulture Research Centre, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut-250110

(Uttar Pradesh). The explants were collected from healthy and disease free plant Young leaves were cleaned thoroughly by repeated washing under running tap water for a period of 30 minutes, and stored in the laboratory at ambient temperature. These leaves were then trimmed and used as explants for culture establishments under *in-vitro* conditions.

Surface sterilization

The culture room was cleaned by gently washing floors and walls with a detergent. This was followed by careful wiping them with 70% ethyl alcohol and fumigation (Potassium permanganate with formaldehyde) and the process of sterilization of culture room was repeated at regular intervals. The transfer area was also sterilized with UV light followed by twice a month by 70% Ethyl alcohol, 30 minutes before starting the transfer work.

The explants were incubated in a culture room where the temperature was maintained at 26ºC, humidity at 60 per cent under a photoperiod of 16 hr. and 8 hr. light/dark respectably.

Whole leaf segments were used as explants. The young leaf from the top of the plant was selected. The cutting size of the leaf segments with mid rib was 2-4 cm. The explants were then treated with 6-7 minutes in 4 per cent Sodium hypochlorite After 3 times rinsing with sterile distilled water and it was again treated with 0.1 per cent Mercuric Chloride ($HgCl_2$) for 5 minutes, After 3 times rinsing sterile distilled water under a laminar airflow cabinet. The cutting of these explants was placed on a filter paper to absorb the extra water of the surface. The leaf segments were placed in to the test tube containing without touching the surface of the wall and then placed them in the growth room are temperature 26^0C and 55- 60 per cent RH.

Results and Discussion

The table reported that the surface sterilization (15 days) of leaves explants in Gerbera alone and with the combination of through sodium hypochlorite 4 per cent and mercuric chloride 0.1 per cent treatments were significantly increased with increasing of duration from 1 to 8 minutes (Table-1).

Maximum survival percentage 73.42 per cent of explants of Gerbera after 15 days was noted under the combination of Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent for a period of 3 min. followed by 68.30, 66.60, 65.84, 62.80, 58.60, 55.62, 52.18, 41.69, 37.93 and 35.77 per cent with treatment and time period in Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent in 5 min, Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent 4 min, Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent 2 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 4 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 3 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 2 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 1 min, Sodium Hypochlorite @ 4 per cent for 8 min, Sodium Hypochlorite @ 4 per cent for 6 min and Sodium Hypochlorite @ 4 per cent for 4 min; while the minimum survival percentage 32.24 per cent was noted under Sodium Hypochlorite 4 per cent treating for a period of 2 minutes.

A critical observation was recorded as the minimum contamination percentage 26.58 per cent explants after 15 days was noted under the treatment of Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent 3 min. followed by 31.70, 32.40, 33.49, 37.20, 41.67, 44.34, 47.78, 58.31 and 62.07 per cent with treatment and time period in Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent 4 min, Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent 5 min, Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent 2 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 4 min, Mercuric Chloride ($HgCl_2$) @ 0.1per cent for 3 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 2 min,

Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 1 min, Sodium Hypochlorite @ 4% for 8 min and Sodium Hypochlorite @ 4 per cent for 6 min; respectively While the maximum 64.23 per cent was noted under Sodium Hypochlorite 4 per cent treating for a period of 2 minute So, it was observed that the survival percentage of explants was *vice versa* to the duration of the treatment with ($HgCl_2$) 0.1 per cent.

After 40 days survival percentage, maximum Survival Percentage 69.78 per cent explants of Gerbera after 40 days was noted under the combination treatment of Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent for a period of 3 min. followed by 66.39, 65.32, 64.54, 59.15, 54.24, 52.74, 49.26, 39.85, 34.27 and 33.36 per cent with the treatment and time period in Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent in 5 min, Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent 4 min, Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent 2 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 4 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 3 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 2 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 1 min, Sodium Hypochlorite @ 4 per cent for 8 min, Sodium Hypochlorite @ 4 per cent for 6 min and Sodium Hypochlorite @ 4 per cent for 4 min.; while the minimum survival percentage 28.52 per cent was noted under Sodium Hypochlorite 4 per cent treating for a period of 2 minutes.

A critical observation was recorded as the maximum contamination percentage 30.22 per cent explants of Gerbera after 40 days was noted under the treatment of Sodium Hypochlorite 4 per cent treating for a period of 2 min. followed by 33.61, 34.68, 35.46, 40.85, 45.76, 47.26, 50.40, 60.15, 65.73 and 66.64 per cent with treatment and time period in Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent 4 min, Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent 5 min, Mercuric Chloride ($HgCl_2$) 0.1 per cent + Sodium Hypochlorite 4 per cent 2 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 4 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 3 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 2 min, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent for 1 min, Sodium Hypochlorite @ 4 per cent for 8 min and Sodium Hypochlorite @ 4 per cent for 6 min.; while the maximum contamination percentage 70.82 per cent was noted under Sodium Hypochlorite 4 per cent treating for a period of 2 min. So, it was observed that the survival percentage of explants was *vice versa* to the duration of the treatment with ($HgCl_2$) 0.1 per cent. The present finding is akin with Modh *et al.* (2002) [7]; Aswath and Choudhary (2001) [2] treated with 0.1 per cent (w/v) HgCl2 for 3 minutes while Shailaja. (2002) [11]; Mohanty *et al.* (2005) [8]; Warar *et al.* (2008) [14]; Altaf *et al.* (2009) [1]; Shabanpour *et al.* (2011) [12]; Son *et al.* (2011) [13] Daud *et al.* (2012) [4] Majid *et al.* (2014) [9]; Majid *et al.* (2014) [9] treated with 1 per cent sodium hypochlorite (NaOCI) (+ 2-3 drops of Tween 20) for 15 min proved most effective for maximum survival percentage *viz*., 99 per cent and 0.1 percent mercuric chloride (HgCl2) for 5 min proved to be more effective for maximum survival percentage 96 per cent of leaf explant.

Table 1: Standardization 15 days and 40 days Survival Percentage with Contamination Percentage of leaves explants in Gerbera alone and with the combination of Sodium hypochlorite 4 per cent and Mercuric Chloride ($HgCl_2$) 0.1 per cent in different time duration treatment.

Treatment	Treatment detail	Survival Percentage	Contamination Percentage	Survival Percentage	Contamination Percentage
		15 days		40 days	
T_1	Mercuric Chloride ($HgCl_2$) @ 0.1% for 1 min	52.18	47.78	49.26	50.40
T_2	Mercuric Chloride ($HgCl_2$) @ 0.1%for 2 min	55.62	44.34	52.74	47.26
T_3	Mercuric Chloride ($HgCl_2$) @ 0.1% for 3 min	58.60	41.67	54.24	45.76
T_4	Mercuric Chloride ($HgCl_2$) @ 0.1% for 4 min	62.80	37.20	59.15	40.85
T_5	Sodium Hypochlorite @4% for 2 min	32.24	67.73	28.52	70.82
T_6	Sodium Hypochlorite @4% for 4 min	35.77	64.23	33.36	66.64
T_7	Sodium Hypochlorite @4% for 6 min	37.93	62.07	34.27	65.73
T_8	Sodium Hypochlorite @ 4% for 8 min	41.69	58.31	39.85	60.15
T_9	Mercuric Chloride ($HgCl_2$)0.1% + Sodium Hypochlorite 4% 2 min	65.84	33.49	64.54	35.46
T_{10}	Mercuric Chloride ($HgCl_2$)0.1% + Sodium Hypochlorite 4% 3 min	73.42	26.58	69.78	30.22
T_{11}	Mercuric Chloride ($HgCl_2$)0.1% + Sodium Hypochlorite 4% 4 min	68.30	31.70	65.32	34.68
T_{12}	Mercuric Chloride ($HgCl_2$)0.1% + Sodium Hypochlorite 4% 5 min	66.60	32.40	66.39	33.61
SE(m)		0.411	0.469	0.482	0.548
C.D.		1.206	1.378	1.415	1.608

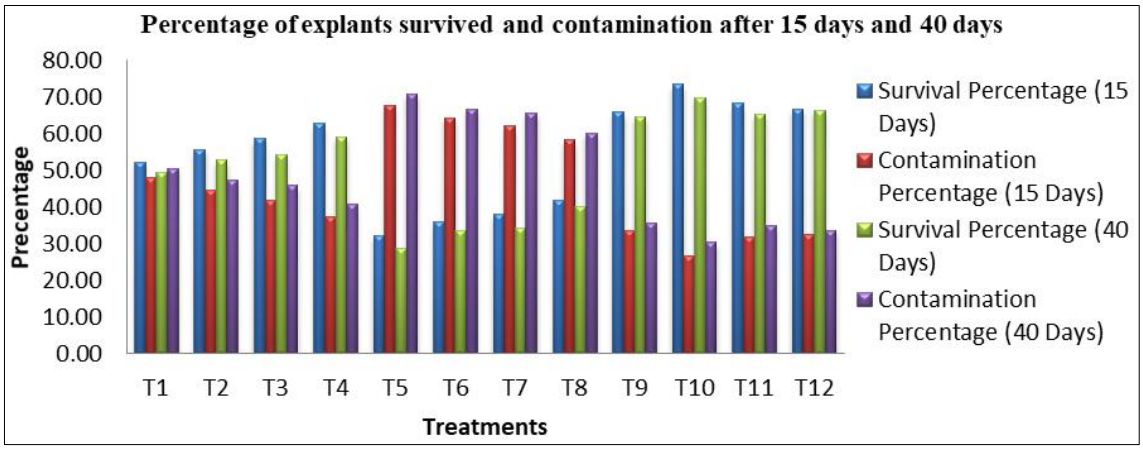

Fig 1: Standardization 15 days and 40 days Survival Percentage with Contamination Percentage of leaves explants in Gerbera alone and with the combination of Sodium hypochlorite 4 per cent and Mercuric Chloride ($HgCl_2$) 0.1 per cent in different time duration treatment.

Conclusion

The present investigation explained that the behavior of two different surface sterilization agents such as Sodium hypochlorite @ 4 per cent, Mercuric Chloride ($HgCl_2$) @ 0.1 per cent and their combination successive behavior of survival and contamination percentage on 15 and 40 days of Gerbera leaf

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