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Research Paper

Standardization of sterilization protocol for *in vitro* propagation of chrysanthemum cvs. "Candor and Local Yellow"

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ABSTRACT

The study aimed at standardization of a sterilization protocol for two most practically functional explants viz. nodal segments and ray florets of *Dendranthema grandiflorum* Kitam cvs. "Candor and Local Yellow". Different sterilant concentrations and combinations were tried for both the explants which differed significantly with each other and showed significant effect on culture asepsis and explant survival in both the cultivars. For nodal explants, sterilization regime consisting of Carbendazim 200 ppm for 30 minutes followed by 0.1% mercuric chloride treatment for 8 minutes followed by a final dip in 70% ethyl alcohol for 10 seconds was concluded to be the standard for both the cultivars for further studies. Moreover, a reduction in Carbendazim concentration to 100 ppm and mercuric chloride concentration to 0.05% also proved to be optimally effective. In contrast, the sterilization treatments consisting of mercuric chloride at 0.05% for 4 minutes and 0.1% for 2 minutes followed by a final rinse with 70% ethyl alcohol for 10 seconds proved to be statistically equally effective for ray florets and were concluded the best with respect both to culture asepsis and explant survival.

Key words: Carbendazim, ethyl alcohol, mercuric chloride, sterilization

Chrysanthemum is a commercially popular flowering plant worldwide next only to roses in importance (Spaargaren and Geest, 2018). The term 'chrysanthemum' comes from the Greek 'krus anthemon', which means gold flower, and was initially used in China (Eisa *et al*., 2022). *Dendranthema grandiflorum* kitam, belonging to the Asteraceae family (Arora, J.S., 1990), is a culturally significant flower with an annual sale of billions of branches. It is available in diverse colors, sizes, and forms. The number of chrysanthemum cultivars is incredibly large with more than 15,000 listed in Japan alone while the National Chrysanthemum Society of Britain lists over 6000 cultivars (Datta, S.K., 2013). Most Chrysanthemum species belong to East Asia. China and Japan have the largest covered areas for production, with 8475 ha (2013) and 5230 ha (2009), respectively. Thailand and India are particularly prominent for domestic market sellers, with 19,000 ha and 2199 ha, respectively. A 2365 ha open production area in Mexico was mentioned in2012 (AIPH International Statistics Flowers and Plants, 2014). Chrysanthemums are usually cultivated using root suckers and shoot cuttings. This conventional technique is relatively slow apart from chances of viral infection and degeneration, thereby increasing the production cost. These hurdles can be effectively managed by applying *in vitro* propagation techniques using small tissue

fragments as explants, thus enhancing reproduction rate apparently impossible under the conventional approach. Micropropagation is a rapid and productive way to generate plants on a larger scale to obtain flowers. Various studies have mentioned using tissue culture to experiment with the large-scale propagation of *Dendranthema grandiflora* by utilizing various novel regeneration pathways (Lim *et al.,* 2012, and Kulus and Zalewska, 2014, Chae, S.C. 2016, Tung *et al.,* 2018, Kim *et al.,* 2019, Rahmy *et al.,* 2019, Jahan *et al.,* 2021, Miller *et al.*, 2021). One of the most serious issues with micropropagation is microbial contamination, which results in the poor quality of plants and the destruction of beneficial stocks. To achieve the desired outcome of any tissue culture procedure, proper sterilization of the explants is the pre-requisite step leading to the development of a successful protocol for *in vitro* propagation. Inadequate sterilization may result in a total loss of explants and the sucrose containing medium as the incubation conditions are highly favourable for microbial growth. A single microbial cell or even a viable spore escaping the sterilization process can destroy all the efforts and lead to a complete failure. Washing explants under running tap water for 30 minutes can assist in obtaining sterile cultures by physically removing some of the contaminants, especially with field-grown material. Mercuric chloride is a potent sterilant but can compromise membrane integrity of the explant tissue (Din *et al*., 2018) which indicates that longer treatment duration will considerably reduce the post sterilization percent survival of the treated explants. Further, ethyl alcohol is a powerful sterilant killing even microbial spores by dehydrating them completely. Moreover, ethyl alcohol removes all the traces of mercury ions from the explant tissue. In this study after washing explants with tap water, different sterilants like mercuric chloride or ethyl alcohol for the disinfection singly or in combination with a fungicide like Carbendazim or Flusilazole have been used. For successful micro-propagation, the first objective is to achieve maximum culture asepsis as possible by using the sterilants. In the process of sterilization of explants, the type, concentration and duration of the sterilant treatment are the important factors not only to achieve a desirable level of asepsis, but also to ensure that explants survive the undesirable toxicity effects. Keeping these factors under consideration, the present investigation attempted to evaluate the safe and effective use of sterilant treatments in order to achieve maximum explant survival.

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MATERIALS AND METHODS

The present investigation was carried out in the Plant Tissue Culture Laboratory of the Division of Floriculture and Landscape Architecture, SKUAST-Kasmir, during the year 2021-2022. Chrysanthemum cultivars “Candor” (Mauve) and “Local Yellow” (Bright Yellow) were used in the current study (Fig. 1).



A. *Dendranthema grandiflorum kitam.* cv Candor” B. *Dendranthema grandiflora Kitam.* cv. “Local Yellow”

Fig. 1: Chrysanthemum cultivars

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The stock plants of the two cultivars were grown in pots under poly-house at experimental farm of the Division of Floriculture and Landscape Architecture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar. Two types of explants i.e., nodal segments and ray florets of *two cultivars viz.,* "Candor" and "Local Yellow" were taken from actively growing young plants and fully opened flowers, respectively. The shoots of mature plant were excised in the morning of a sunny day and placed in a beaker containing distilled water while carrying them to the laboratory. The leaves were removed and shoots cut to make smaller nodal cuttings in the laboratory. The cuttings were placed in a beaker and put under running tap water for about 30 minutes and washed thoroughly to remove any adhering dirt and contamination. The explants were then placed in clean flasks containing distilled water before further processing. Similarly fully opened flower heads were excised from the plants and the ray florets were collected in a beaker in the laboratory. The beaker was covered with muslin cloth with the help of a rubber band and kept under running tap water for about 20-30 minutes. The florets were then transferred to clean flaks containing distilled water for further treatments. The nodal explants were then soaked in fungicide solution with few drops of tween-20 for 15 and 30 minutes, respectively. Further treatment with mercuric chloride (0.05% or 0.1%) followed by 70% ethyl alcohol dip for 10 seconds was done under laminar air flow. Explants were given final 3 washings with sterile water before placing on the medium. Nodal cuttings were cut to the size of 1.0-2.0 cm by giving both top and basal re-cut to the segments in order to remove the sterilant damaged tissues. The explants were properly prepared and placed on the medium for aseptic inoculation conforming to their original polarity. Ray florets were sterilized with mercuric chloride at two concentrations; 0.05% and 0.1% for 2 and 4 minutes alone or in combination with 100 ppm Carbendazim for 15 minutes and ethyl alcohol dip for 10 seconds (Table 2). A 5-7 mm vertical cut extending right above the basal end was given before placing them on the inoculation medium. While making incisions, knives and forceps were flame sterilized to avoid spread of contamination between different parts of the explants. In this experiment, 12 cultures per treatment were maintained, each replicated three times. Data recorded was subjected to statistical analysis using OPSTAT. Observations with regard to aseptic cultures (without infection) and explant survival (surviving explants) were made upto 4 weeks of inoculation. Data for following parameters was recorded during the course of investigation.

i) Asepsis (%) = Aseptic cultures per treatment per replication / total number of cultures per treatment per replication X 100.

ii) Survival (%) = Surviving cultures out of aseptic cultures per treatment per replication / total number of aseptic cultures per treatment per replication X 100.

RESULTS AND DISCUSSION

The results of the present investigation regarding the explant sterilization have been discussed in the following sections.

Culture asepsis of nodal segments and ray florets: Significant differences were noticed for all the sterilant treatments in both the cultivars. Data pertaining to the effect of various sterilant treatments and their interaction on culture asepsis of nodal explants of Chrysanthemum cultivars "Candor" and "Local Yellow" is presented in Table 1. Both the cultivars followed almost a similar trend. All the sterilant treatments recorded a significant effect on culture asepsis. Among the individual sterilization treatments, S_2 (0.1% mercuric chloride for 8 minutes) resulted in maximum culture asepsis of 74.33% in cv. Candor and 61.99% in cv. Local Yellow. Combined treatment of sterilants was found more effective and yielded better results. Maximum culture asepsis of 91.55% and 81.88% were obtained with S_{10} (Flusilazole 0.02% for 20 minutes followed by mercuric chloride 0.1% for 8 minutes followed by ethyl alcohol 70% for 10 seconds) and 90.22% and 78.44 % with S_6 (Carbendazim 200 ppm for 30 minutes followed by mercuric chloride 0.1% for 8 minutes followed by ethyl alcohol 70% for 10 seconds), respectively.

Table1: Influence of various sterilant treatments on culture asepsis (%) of nodal segments of Chrysanthemum Cultivars "Candor" and "Local Yellow"

Treatment	Sterilant used	Concentration	Treatment duration	Culture asepsis (%)		Mean
				V1 (Candor)	V2 (Local Yellow)	
S_1	Mercuric chloride	0.05 %	8 minutes	**71.11 (8.49) ^{g}**	**59.11 (7.75) ^{h}**	**65.11 (8.12)**
S_3	Mercuric chloride	0.10 %	8 minutes	74.33 (8.67)^{fg}	61.99 (7.93)gh	68.16 (8.30)
S_3	Carbendazim Mercuric chloride	100 ppm 0.05 %	30 minutes 8 minutes	77.55 (8.86)^{ef}	64.66 (8.10)^{fg}	71.10 (8.48)
S_4	Carbendazim Mercuric chloride	200 ppm 0.10 %	30 minutes 8 minutes	82.22 (9.12) ^{cd}	69.44 (8.39) ^{de}	75.83 (8.75)
S_5	Carbendazim Mercuric chloride Ethyl alcohol	100 ppm 0.05 % 70.00 %	30 minutes 8 minutes 10 seconds	86.88 (9.37) ^{ab}	73.33^{cd} (8.62)	80.10 (8.99)
S_6	Carbendazim Mercuric chloride Ethyl alcohol	200 ppm 0.10 % 70.00 %	30 minutes 8 minutes 10 seconds	90.22 (9.55) ^{a}	78.44^{ab} (8.91)	84.33 (9.23)
S_7	Flusilazole Mercuric chloride	0.01% 0.05 %	20 min 8 minutes	79.66 (8.98) ^{de}	67.33 (8.26)^{ef}	73.49 (8.62)
S_8	Flusilazole Mercuric chloride	0.02% 0.10 %	20 min 8 minutes	84.44 (9.24)^{bc}	71.11 (8.49) ^{de}	77.77 (8.86)
S_9	Flusilazole Mercuric chloride Ethyl alcohol	0.01% 0.05 % 70.00 %	20 min 8 minutes 10 seconds	88.10 (9.44) ^{ab}	75.99 (8.77)^{bc}	82.04 (9.10)
S_{10}	Flusilazole Mercuric chloride Ethyl alcohol	0.02% 0.10 % 70.00 %	20 min 8 minutes 10 seconds	**91.55 (9.62) ^{a}**	**81.88 (9.10) ^{a}**	**86.71 (9.36)**
Mean				**82.60** (9.14)	**70.33** (8.43)	

The lowest culture asepsis of 71.11% and 59.11% in cultivars Candor and Local Yellow, respectively was recorded in S_1 (0.05% mercuric chloride for 8 minutes). The interaction effect of sterilant treatments on per cent culture asepsis was not significant. Waseem *et al.* (2011) used nodal segments of chrysanthemum (*Chrysanthemum morifolium* L) and were sterilized with 1.0% mercuric chloride for three minutes. Mishra *et al.* (2006) standardized an efficient protocol for large scale multiplication of chrysanthemum cv. Yellow Bungalow and obtained good success for nodal segment explants when pretreated with 0.2% carbendazim + 200 ppm 8- hydroxy quinnoline citrate (HQC) for 2 h, followed by 3 minutes agitation in 0.1% $HgCl_2$. As per the data presented in (Table 2), combined treatment of sterilants was found more effective and yielded better results. Maximum culture asepsis of 94.33% and 97.99% was recorded with S_{12} (Carbendazim 100 ppm for 15 minutes followed by mercuric chloride 0.1% for 4 minutes followed by 70% ethyl alcohol dip for 10 seconds). Among individual sterilant treatments, S_4 (0.1% mercuric chloride for 4 minutes) resulted in maximum culture asepsis of 74.99% and 77% for cultivars Candor and Local Yellow, respectively. Also, an increase in sterilization interval of mercuric chloride from 2 to 4 minutes showed a positive impact on culture asepsis along-with increase in concentration from 0.05% to 0.1%. Mercuric chloride being a reliable disinfectant has been successfully used by most of the researchers singly for the surface sterilization of chrysanthemum explants. The results obtained are in accordance with Din *et al.,* 2018 in Chrysanthemum cultivars "Candid and Flirt". Hiremath *et al.*

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(2004) reported use of mercuric chloride 0.1 % as surface disinfectant of nodal segment, leaf and flower bud explants of carnation for a 3 minute dip. Devi and Gupta (2004), also employed mercuric chloride 0.1% dip for 2-3 minutes in carnation explants of shoot tip, nodal stem, inter-nodal and leaf segments. Sharma and Srivastava (2005) employed a 10 minute 0.1% mercuric chloride dip

Table 2: Influence of various sterilant treatments on culture asepsis (%) of ray florets of Chrysanthemum Cultivars "Candor" and "Local Yellow"

Treatment	Sterilant used	Concentration	Treatment duration	Culture asepsis (%)		Mean
				V1 (Candor)	V2 (Local Yellow)	
S_1	Mercuric chloride	0.05 %	2 minutes	**67.00 (8.24)^{i}**	**69.33 (8.38)^{i}**	**68.16 (8.31)**
S_2	Mercuric chloride	0.05 %	4 minutes	70.33 (8.44)^{hi}	72.66 (8.58)^{hi}	71.49 (8.51)
S_3	Mercuric chloride	0.10 %	2 minutes	72.66 (8.58)^{gh}	75.00 (8.71)^{gh}	73.83 (8.65)
S_4	Mercuric chloride	0.10 %	4 minutes	74.99 (8.71)^{fg}	77.00 (8.83)^{fg}	75.99 (8.77)
S_5	Mercuric chloride Ethyl alcohol	0.05 % 70.00 %	2 minutes 10 seconds	77.00 (8.83)^{ef}	80.00 (9.00)^{f}	78.50 (8.91)
S_6	Mercuric chloride Ethyl alcohol	0.05 % 70.00 %	4 minutes 10 seconds	79.99 (8.99)^{de}	83.66 (9.20)^{e}	81.82 (9.10)
S_7	Mercuric chloride Ethyl alcohol	0.10 % 70.00 %	2 minutes 10 seconds	81.33 (9.07)^{d}	86.00 (9.32)^{de}	83.66 (9.20)
S_8	Mercuric chloride Ethyl alcohol	0.10 % 70.00 %	4 minutes 10 seconds	83.00 (9.16)^{cd}	89.33 (9.50)^{cd}	86.16 (9.33)
S_9	Carbendazim Mercuric chloride Ethyl alcohol	100 ppm 0.05 % 70.00 %	15 minutes 2 minutes 10 seconds	85.33 (9.29)^{bc}	91.66 (9.62)^{bc}	88.49 (9.45)
S_{10}	Carbendazim Mercuric chloride Ethyl alcohol	100 ppm 0.05 % 70.00 %	15 minutes 4 minutes 10 seconds	87.99 (9.43)^{b}	93.33 (9.71)^{b}	90.66 (9.57)
S_{11}	Carbendazim Mercuric chloride Ethyl alcohol	100 ppm 0.10 % 70.00 %	15 minutes 2 minutes 10 seconds	92.00 (9.64)^{a}	95.00 (9.79)^{ab}	93.50 (9.72)
S_{12}	Carbendazim Mercuric chloride Ethyl alcohol	100 ppm 0.10 % 70.00 %	15 minutes 4 minutes 10 seconds	**94.33 (9.76)^{a}**	**97.99 (9.95)^{a}**	**96.16 (9.85)**
Mean				**80.49** (9.01)	**84.24** (9.22)	

Survival of Nodal Segment and Ray florets: The treatment combinations for sterilization of nodal segments with carbendazim fungicide showed higher explant survival in comparison to the treatment combinations using Flusilazole hence implying higher toxicity of Flusilazole over Carbendazim (Table 3). Maximum explant survival of 87.77 % and 74.88% was recorded with S_6 (Carbendazim 200 ppm for 30 minutes followed by mercuric chloride 0.1% for 8 minutes followed by ethyl alcohol 70% for 10 seconds) followed by S_5 (Carbendazim 100 ppm for 30 minutes followed by mercuric chloride 0.05% for 8 minutes followed by ethyl alcohol 70% for 10 seconds) with explant survival of 84.66% and 71.55%. Both these treatments were statistically at par with each other but significantly different from all other treatments. Minimum explant survival of

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70.66% and 56.11% was recorded with S_1 (0.05% mercuric chloride for 8 minutes) followed by S_2 (0.1% mercuric chloride for 8 minutes) with 71.66% and 58.55%. Both these treatments were statistically at par with each other but differed significantly from all other treatments. In this study, treatment of nodal segment explants of chrysanthemum with combination of three disinfectants at higher concentrations yielded maximum culture asepsis (91.55% and 81.88%) but largely affected the culture survival (84.44 and 70.66%).

In case of ray florets, the treatment combinations S_6 (0.05% mercuric chloride for 4 minutes followed by 70% ethyl alcohol dip for 10 seconds) and S_7 (0.1% mercuric chloride for 2 minutes followed by 70% ethyl alcohol dip for 10 seconds) showed optimum culture asepsis for both the varieties (78% and 78.66% for S_6 and 76% and 77.33% for S_7 respectively).

Table 3: Influence of various sterilant treatments on explant survival (%) of nodal segments of Chrysanthemum Cultivars "Candor" and "Local Yellow"

Treatment	Sterilant used	Concentration	Treatment duration	Explant survival (%)		Mean
				V1 (Candor)	V2 (Local Yellow)	
S_1	Mercuric chloride	0.05 %	8 minutes	**70.66 (8.46)^{g}**	**56.11 (7.55)^{f}**	**63.38 (8.01)**
S_3	Mercuric chloride	0.10 %	8 minutes	71.66 (8.52)^{fg}	58.55 (7.71)^{f}	65.10 (8.12)
S_3	Carbendazim Mercuric chloride	100 ppm 0.05 %	30 minutes 8 minutes	76.11 (8.78)^{ef}	63.44 (8.02)^{de}	69.77 (8.40)
S_4	Carbendazim Mercuric chloride	200 ppm 0.10 %	30 minutes 8 minutes	79.55 (8.97)^{cde}	67.55 (8.27)^{bcd}	73.55 (8.62)
S_5	Carbendazim Mercuric chloride Ethyl alcohol	100 ppm 0.05 % 70.00 %	30 minutes 8 minutes 10 seconds	84.66 (9.25)^{ab}	71.55 (8.51)^{ab}	78.10 (8.88)
S_6	Carbendazim Mercuric chloride Ethyl alcohol	200 ppm 0.10 % 70.00 %	30 minutes 8 minutes 10 seconds	**87.77 (9.42)^{a}**	**74.88 (8.71)^{a}**	**81.33 (9.06)**
S_7	Flusilazole Mercuric chloride	0.01% 0.05 %	20 min 8 minutes	75.66 (8.75)^{efg}	60.66 (7.85)^{ef}	68.16 (8.30)
S_8	Flusilazole Mercuric chloride	0.02% 0.10 %	20 min 8 minutes	78.99 (8.94)^{de}	66.22 (8.19)^{cd}	72.60 (8.57)
S_9	Flusilazole Mercuric chloride Ethyl alcohol	0.01% 0.05 % 70.00 %	20 min 8 minutes 10 seconds	82.33 (9.12)^{bcd}	69.44 (8.39)^{bc}	75.88 (8.76)
S_{10}	Flusilazole Mercuric chloride Ethyl alcohol	0.02% 0.10 % 70.00 %	20 min 8 minutes 10 seconds	84.44 (9.24)^{abc}	70.66 (8.46)^{abc}	77.55 (8.85)
Mean				**79.18** (8.94)	**65.90** (8.17)	

The maximum explant survival (90.33% and 91.22%) was recorded with lower concentration of mercuric chloride (0.05%) for shorter duration (2 minutes). Thus the data clearly reveals that longer duration of mercuric chloride (0.1%) exposure had a significant detrimental effect on survival of explants (Table 4). Mercuric chloride being a potent sterilant is also known to compromise membrane integrity of live plant tissue. Therefore, longer duration resulted in decreased explant survival as against shorter one. Similar results were recorded by Farooq *et al.* (2022) and Bhat et al. (2022) with Lilium LA hybrids "Indian Summerset" and "Nashville" and *Jasminum nudiflorum* respectively. Ethyl alcohol is also a well known sterilant that acts by killing microorganisms and

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their spores by desiccating them out. It is also known to remove the traces of Hg^{2+} ions from the explant. Many researchers recommended using ethyl alcohol as a final wash when using mercuric chloride for surface sterilization to draw out the traces of Hg^{2+} remaining thereby. The results coincide with those of Verma *et al*., 2012, Verma *et al*., 2019 and Anjum *et al.,* 2023.

Table 4: Influence of various sterilant treatments on explant survival (%) of ray florets of Chrysanthemum Cultivars "Candor" and "Local Yellow"

Treatment	Sterilant used	Concentration	Treatment duration	Survival (%)		Mean
				V1 (Candor)	V2 (Local Yellow)	
S_1	Mercuric chloride	0.05 %	2 minutes	**90.33 (9.55)[a]**	**91.22 (9.60)[a]**	**90.77 (9.58)**
S_2	Mercuric chloride	0.05 %	4 minutes	88.33 (9.45)[a]	89.88 (9.53)[a]	89.10 (9.49)
S_3	Mercuric chloride	0.10 %	2 minutes	85.00 (9.27)[b]	86.00 (9.32)[b]	85.50 (9.30)
S_4	Mercuric chloride	0.10 %	4 minutes	82.00 (9.11)[c]	83.33 (9.18)[c]	82.66 (9.14)
S_5	Mercuric chloride Ethyl alcohol	0.05% 70.00 %	2 minutes 10 seconds	80.33 (89.01)[c]	81.66 (9.09)[c]	80.99 (9.05)
S_6	Mercuric chloride Ethyl alcohol	0.05 % 70.00 %	4 minutes 10 seconds	78.00 (8.88)[d]	78.66 (8.92)[d]	78.33 (8.90)
S_7	Mercuric chloride Ethyl alcohol	0.10 % 70.00 %	2 minutes 10 seconds	76.00 (8.77)[d]	77.33 (8.85)[d]	76.66 (8.81)
S_8	Mercuric chloride Ethyl alcohol	0.10 % 70.00 %	4 minutes 10 seconds	73.00 (8.60)[e]	74.00 (8.66)[e]	73.50 (8.63)
S_9	Carbendazim Mercuric chloride Ethyl alcohol	100 ppm 0.05 % 70.00 %	15 minutes 2 minutes 10 seconds	71.33 (8.50)[e]	72.00 (8.54)[e]	71.66 (8.52)
S_{10}	Carbendazim Mercuric chloride Ethyl alcohol	100 ppm 0.05 % 70.00 %	15 minutes 4 minutes 10 seconds	68.33 (8.32)[f]	69.00 (8.36)[f]	68.66 (8.34)
S_{11}	Carbendazim Mercuric chloride Ethyl alcohol	100 ppm 0.10 % 70.00 %	15 minutes 2 minutes 10 seconds	66.00 (8.18)[g]	66.66 (8.22)[g]	66.33 (8.20)
S_{12}	Carbendazim Mercuric chloride Ethyl alcohol	100 ppm 0.10 % 70.00 %	15 minutes 4 minutes 10 seconds	**62.00 (7.93)[h]**	**63.00 (8.00)[h]**	**61.50 (7.96)**
Mean				**76.72** (8.80)	**77.73** (8.86)	

*Figures in the parenthesis are square root transformed values of percentage data; **Data recorded after 6 weeks of culture on MS medium; ***Data are a mean of three replicates. Same superscript letters in a column do not differ significantly when compared by Duncan's MRT at 5% level of significance.

CONCLUSION

It is concluded that sterilization method involving the use of Carbedazim 200 ppm for 30 minutes followed by 0.1% mercuric chloride for 8 minutes followed by 70% ethyl alcohol dip for 10 seconds was appropriate in obtaining optimum culture asepsis as well as explant survival in nodal segments. Whereas, treatment combination involving the use mercuric chloride 0.05 % for 4 minutes followed by 70% ethyl alcohol dip for 10 seconds was the most appropriate treatment in achieving highest explant survival and adequate culture asepsis in ray florets of both the cultivars.

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CONFLICT OF INTEREST

All the authors affirm that there is no conflict of interest among them. All research activities comply with relevant legal, institutional and ethical standards.

AUTHOR CONTRIBUTION

All the authors contributed equally in this research article.

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