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Effect of different sterilization agents on *in vitro* culture establishment of guava cv. Arka Kiran

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Abstract
Bacterial and fungal contaminations are major problems during *in vitro* culture stages. Therefore, surface sterilization is an obligatory step prior to *in vitro* culture of any plant tissue and can become a critical point in establishment of certain species, especially when the explant is derived from field grown woody perennial plants. An experiment on "Effect of sterilizing agents on *in vitro* culture establishment of guava cv. Arka Kiran" was carried out at College of Horticulture, Sirsi, Uttar Kannada, Karnataka during 2021-2023. The study revealed significant influence of different sterilizing agents on initiation response, survival, contamination and health parameters. The nodal explants sterilized with $HgCl_2$ (0.1%) for five minutes showed maximum initiation of response (100%) and survival (100%), minimum days taken for regeneration (8.35) and contamination (0%) with better health.

Keywords: Guava, Arka Kiran, sterilizing agent, contamination, survival

Introduction
Guava (*Psidium guajava* L.) is one of the most common and important commercial fruit crops cultivated in both subtropical and tropical regions of the world. The major share of the fruits production in India is utilized for fresh consumption. Genus *Psidium* have about 150 species, most of which are fruit bearing trees. It is native to tropical America, stretching from Mexico to Peru (Singh, 2016) [17]. It is widely adopted and tolerate in frost, drought and saline conditions. It has good amount of ascorbic acid, dietary fibres, vitamin-A (about 250 IU/100 g), pectin, calcium, phosphorus, iron and other nutrients. The roots, bark, leaves and green fruits are used as medicine for gastrointestinal problems, diarrhoea and dysentery (Rathore, 1976) [12]. In India, it has become an important fruit crop, contributing 0.4 percent of total fruit production with estimated production of 4.05 million tons from 0.26 million ha (NHB, 2018) [9]. Major guava producing states are Maharashtra, Bihar, Odisha, Uttar Pradesh, Gujarat, Madhya Pradesh, Karnataka, West Bengal and Tamil Nadu (Singh, 2007) [15].
Arka Kiran variety of guava is gaining importance in nowadays mainly due to semi vigorous, prolific and precocious bearing nature, yields medium sized fruits (200-220 g) with firm, deep red pulp and with high lycopene content (7.14 mg/100 g). Fruits having TSS of 11-12^0 Brix, good vitamin C (190-200 mg/100 g) with medium soft seeds. Crop will come to harvest after two years of planting. Economic yield starts after five years with average fruit yield of 20 ton per acre in a spacing of 4m × 3m (Mitra and Irenaeus, 2018) [7].
Bacterial and fungal contaminations are major problems during *in vitro* culture stages. Therefore, the tissue must be surface sterilized before placing it on nutritional media in order to prevent contamination in cultures especially when the explants collected from a field grown woody perennial plants. The microorganism load in cultures lowered the shoot proliferation rate, compete with plant tissues for nutrients, increased explant mortality, caused tissue necrosis and aberrant growth. In most of the commercial and research plant tissue culture laboratories, contamination caused around 3 to 15% losses for each subculture cycle. As a result, the time, effort and material waste caused by this problem, there were financial losses. In particular it was difficult to get rid of microorganism in woody plant explants (Wolella, 2017) [20]. It is important to choose the right sterilizing agent and exposure duration so that only contaminants are removed from living material not its viability. Hence, the experiment was carried out to select best sterilizing agent and duration of exposure for controlling contamination with better regeneration.

Materials and Methods

The experiment was conducted at College of horticulture, Sirsi, Uttar Kannada, (Karnataka) India. The experiment was laid out in completely randomized design. There were 21 treatments of different sterilizing agents. Each treatment was replicated thrice.

Explant source and treatment

The explants used were nodal explants and were collected from plants of Arka Kiran that were maintained under polyhouse. Explants washed under running tap water for 10 minutes and cut into desired size for sterilization, treated with Bavistin (2 gm/lit) + 0.5 gm/lit Citrimide for 3 hours with Tween 20, then treated with Ascorbic acid and Citric acid at 25 mg/lit each for 15 minutes and then explants were washed with sterile water for three times. After that explants were treated with different sterilizing agents with different duration of exposure as per the treatment details and finally washed with sterile water for five times inside the laminar hood. The MS media was used as basal media along with BAP (3 mg/L) and NAA (0.1 mg/L).

Treatment details

T_1 - (Control), T_2 - (4% NaOCl for 3 min), T_3 - (4% NaOCl for 5 min), T_4 - (4% NaOCl for 8 min), T_5 - (4% NaOCl for 10 min), T_6 - (0.05% $HgCl_2$ for 3 min), T_7 - (0.05% $HgCl_2$ for 5 min), T_8 - (0.05% $HgCl_2$ for 8 min), T_9 - (0.05% $HgCl_2$ for 10 min), T_{10} - (0.1% $HgCl_2$ for 3 min), T_{11} - (0.1% $HgCl_2$ for 5 min), T_{12} - (0.1% $HgCl_2$ for 8 min), T_{13} - (0.1% $HgCl_2$ for 10 min), T_{14} - (6% H_2O_2 for 3 min), T_{15} - (6% H_2O_2 for 5 min), T_{16} - (6% H_2O_2 for 8 min), T_{17} - (6% H_2O_2 for 10 min), T_{18} - (0.5% NaOH for 3 min), T_{19} - (0.5% NaOH for 5 min), T_{20} - (0.5% NaOH for 8 min) and T_{21} - (0.5% NaOH for 10 min).

Observations

The observations taken were initiation response, days taken for regeneration, survival (%), contamination (%) and health of explant at first, second, third and fourth week of culture. For initiation of response, the explants that were healthy greenish and exhibiting some degree of shoot initiation as bulged buds or increase in length of explants were treated as response and their percentage were expressed. The average number of days it took for the first bud to sprout to form explant was recorded to calculate days taken for regeneration. The explants were scaled as +++ for least health (Dead or explants with maximum browning and deformation), ++ for moderate health (Moderate green with less browning and less deformation) and +++ for good healthy (Fully green and without any browning and deformation) based on health of explant. The survival and contamination (%) was calculated using below formula.

$$\text{Survival (\%)} = \frac{\text{Total number of explants survived}}{\text{Total number of explants inoculated}} \times 100$$

$$\text{Contamination (\%)} = \frac{\text{Total number of explants contaminated}}{\text{Total number of explants inoculated}} \times 100$$

Statistical analysis

The data recorded for all the parameters was statistically analysed (ANOVA) by following the completely randomized design (CRD) at 1% level of significance. The analysis has been done in Web Agri-Stat Package (WASP 2.0) developed by ICAR Research Complex, Goa.

Results and Discussion

Effect on initiation response in explants (%)

The different surface sterilants showed significant influence on the initiation response in nodal segments (Table 1). In the first week, there was no initiation response noticed in all the treatments. Whereas, the initiation response started during the second week of culture, the highest response (100% at first, second and third week of culture) was noticed in T_{11} (0.1% $HgCl_2$ for 5 min) which was followed by T_9 (0.05% $HgCl_2$ for 10 min) (88.21%, 88.38% and 88.38% at first, second and third week of culture respectively) and the lowest (0%) response was noticed in T_1 (Control), T_2 (4% NaOCl for 3 min), T_3 (4% NaOCl for 5 min), T_4 (4% NaOCl for 8 min), T_5 (4% NaOCl for 10 min), T_6 (0.05% $HgCl_2$ for 3 min), T_{13} (0.1% $HgCl_2$ for 10 min), T_{14} (6% H_2O_2 for 3 min), T_{17} (6% H_2O_2 for 10 min), T_{18} (0.5% NaOH for 3 min), T_{19} (0.5% NaOH for 5 min), T_{20} (0.5% NaOH for 8 min) and T_{21} (0.5% NaOH for 10 min). The highest (100%) initiation response was recorded in treatment where explants treated with $HgCl_2$ (0.1%) for five minutes. The response was noticed only in treatment where explants treated with $HgCl_2$ (0.05% for 5, 8 and 10 minutes), $HgCl_2$ (0.1% for 3, 5 and 8 minutes) and H_2O_2 (6% for 5 and 8 minutes). No response was noticed in NaOCl (4% for 3, 5, 8 and 10 minutes), (0.1%) $HgCl_2$ at 10 minutes, H_2O_2 (6% for 3 and 10 minutes) and NaOH (0.5% for 3, 5, 8 and 10 minutes). Similarly, Zamir *et al*. (2007) [22], reported highest initiation of response (72%) when explants were surface sterilized with $HgCl_2$ (0.05%) for five minutes in shoot tips of guava cv. Safeda. Yadav and Singh (2011) [21] reported maximum *in vitro* germination in *Albizia lebbeck*, when the seeds were treated with $HgCl_2$ (0.1%) for five minutes.

Effect on days taken for regeneration

The different surface sterilants showed significant influence on days taken for regeneration in nodal segments (Table 1). In the first week, there was no regeneration observed in all the treatments. Whereas, regeneration started during the second week of culture, the significant lower number of days (8.35) was taken in T_{11} (0.1% $HgCl_2$ for 5 min) which was on par with T_{12} (0.1% $HgCl_2$ for 8 min) (9.46) and T_9 (0.05% $HgCl_2$ for 10 min) (9.76). Whereas, the highest number of days (15.36) was taken in T_{16} (6% H_2O_2 for 8 min) which was on par with T_{15} (6% H_2O_2 for 5 min) (14.35). The average number of days taken for regeneration ranged from 8.35 to 15.36 days among the treatments. Similar to this, Mishra *et al.* (2007) [6] reported that, when explants treated with $HgCl_2$ (0.1% for 6 minutes) took five days for bud induction in shoots of guava.

Effect on survival (%)

As the culture time increases, survival percentage of nodal segments was decreased in almost all the treatments (Table 2). The surface sterilants showed significant influence on survival (%) of explants. At first week of culture, the highest (100%) survival was recorded in T_2, T_3, T_5, T_6, T_7, T_8, T_9 T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, T_{15}, T_{16} and T_{18} followed by T_{20} (53.20%) which was on par with T_4 (55.50%). Whereas, the (0%) survivability was noticed in T_1, T_{19} and T_{21}. At second week of culture, the highest (100%) survival was recorded in T_2, T_5, T_7, T_8, T_9, T_{10}, T_{11}, T_{12}, T_{16} and T_{18} followed by T_3 (73.58%) which was on par with T_6 (68.42%). Whereas, the lowest (0%) survivability was noticed in T_1, T_{13} T_{17}, T_{19} and T_{21}. At third week of culture, highest (100%) survival was noticed in T_8,

T_9, T_{11} and T_{12} followed by T_{10} (76.42%). Whereas, the lowest (0%) survivability was noticed in T_1, T_4, T_5, T_{13}, T_{17}, T_{19} and T_{21}. At fourth week of culture, the highest (100%) survival was observed in T_8, T_9, T_{11} and T_{12} followed by T_{10} (71.35%). Whereas, the lowest (0%) survivability was observed in T_1, T_2, T_3, T_4 T_5, T_6 T_{13}, T_{17}, T_{18}, T_{19}, T_{20} and T_{21}.

The survival percent in this study was depends on concentration and exposure time of sterilizing agents and contamination percent. The 100 percent survival was noticed in treatments where explants treated with $HgCl_2$ (0.05% for 8 and 10 minutes) and $HgCl_2$ (0.1% for 5 and 8 minutes). Whereas, zero survival was noticed in control, NaOCl (4% for 3, 5, 8 and 10 minutes), $HgCl_2$ (0.05% for 3 minutes), $HgCl_2$ (0.1% for 10 minutes), H_2O_2 (6% for 10 minutes) and NaOH (0.5% for 3, 5, 8 and 10 minutes) at fourth week of culture. The results are similar with Jan *et al*. (2013) [2]. They reported highest survival (37.77 and 31.11% in runner tip and nodal segment, respectively) when explants treated with $HgCl_2$ (0.1%) for four minutes in strawberry. Zamir *et al*. (2004) [23] reported that, maximum (67%) and minimum survival (22%) with HgCl2 (0.05%) for five minutes and NaOCl (2%) for 10 minutes, respectively in shoot tip explants of guava. Similar results were also reported by Khattak *et al*. (1990) [3] in guava. Survival in this study was reduced mainly by two factors *i.e.* contamination percent and high concentration of sterilizing agents. In this study, $HgCl_2$ (0.1%) for 10 minutes have showed zero percent survival even though the same sterilizing agent was effective at other exposure times (3, 5 and 8 minutes). The results are similar with Meghwal *et al*. (2000) [4]. They reported maximum survival (67%) at $HgCl_2$ (0.05%) for five minutes but when exposure time increased from five to ten minutes the survival rate reduced to 37 percent. The probable reason for the death of explants may be the heavy metal contamination of mercury present in the $HgCl_2$, causing detrimental effect for the survival of the explant when they are treated for a long time (Papadatou *et al*., 1990) [10]. The explants treated with NaOCl (4%) and NaOH (0.5%) gave zero survival at all exposure time. Which may be due to high contamination (100%) at these treatments. The contamination spread rapidly all over the explants and thereby reduces the survival rate in explants. In some studies the combination of sterilizing agents gave superior results than individual sterilants in guava (Mishra *et al*., 2010 and Devi and Yadav, 2022) [5, 1].

Effect on contamination (%)

As the culture time increases, contamination percentage in nodal segments was increased in almost all the treatments (Table 3). The surface sterilants showed significant influence on contamination (%) of explants. At first week of culture, the highest (100%) contamination was observed in T_1, T_2, T_3 and T_{21} followed by T_{20} (61.37%). Whereas, the lowest (0%) contamination was observed in T_6, T_7, T_8, T_9 T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, T_{15} and T_{19}. At second week of culture, the highest (100%) contamination was noticed in T_1, T_2, T_3, T_4, T_5, T_6, T_{14}, T_{18} and T_{21} followed by T_{20} (65.68%). Whereas, the lowest (0%) contamination was noticed in T_7, T_8, T_9, T_{10}, T_{11}, T_{12} and T_{15}. At third week of culture, highest (100%) contamination was recorded in T_1, T_2, T_3, T_4, T_5, T_6, T_{14}, T_{18}, T_{20} and T_{21} followed by T_{17} (63.29%). Whereas, the lowest (0%) contamination was recorded in T_7, T_8, T_9, T_{10}, T_{11}, T_{12} and T_{15}. At fourth week of culture, the highest (100%) contamination was found in T_1, T_2, T_3, T_4, T_5, T_6, T_{14}, T_{18}, T_{19}, T_{20} and T_{21} followed by T_{17} (68.45%). Whereas, the lowest (0%) contamination was found in T_8, T_9, T_{10}, T_{11}, T_{12} and T_{15}.

In the present study, no contamination was noticed in treatments where explants treated with $HgCl_2$ (0.05% for 8 and 10 minutes), $HgCl_2$ (0.1% for 3, 5 and 8 minutes), H_2O_2 (6% for 5 minutes). However, 100 percent contamination was noticed control, NaOCl (4% for 3, 5, 8 and 10 minutes), $HgCl_2$ (0.05% for 3 minutes), H_2O_2 (6% for 3 minutes) and NaOH (0.5% for 3, 5, 8 and 10 minutes) at fourth week of culture. The bacterial contamination was not noticed much in all explants because the growth of fungal contamination was very quick in the culture and it has covered entire surface of explants. The results are similar in studies by Shah *et al*. (2008) [14]. They reported that surface sterilization of guava seeds with $HgC1_2$ (0.05%) for five minutes gave maximum aseptic plants. In an another study, Singh and Patel (2016) [18] reported that, surface sterilization with $HgCl_2$ (0.1%) for five minutes resulted in reduced contamination of 23.50 percent in nodal segment and $HgCl_2$ (0.1%) for three minutes resulted in contamination of 12.33 percent in shoot tips of pomegranate. Among different $HgC1_2$ treatments the maximum aseptic cultures (43.33 and 40.00% in runner tip and nodal segment, respectively) were obtained at 0.1 percent for four minutes in strawberry (Jan *et al*., 2013) [2]. Rattanpal *et al*. (2011) [13] reported least (20%) contamination when explants were treated with $HgCl_2$ (0.1%) for four minutes in strawberry. Singh *et al*. (2007) [15] obtained maximum aseptic culture (74.0%) when the explants treated with $HgCl_2$ (0.1%) for 10 minutes in guava. Yadav and Singh (2011) [21] reported minimum contamination (20.0%) in *Albizia lebbeck*, when the seeds were treated with $HgCl_2$ (0.1% for 8 minutes). Puchooa (2004) [11] reported no contamination in leaf explants of lychee treated with $HgC1_2$ (0.1% at 10, 15 and 30 minutes). In this study, the explants treated with NaOCl (4%) and NaOH (0.5%) gave 100 percent contamination at all exposure time which indicates these sterilizing agents alone were not efficient in controlling contamination. Similar to this Meghwal *et al*. (2000) [4] also reported 100 percent contamination in NaOCl treatments when it was used alone. In contrast to this, Nelson *et al*. (2015) [8] reported that NaOCl (2% for 20 minutes) was effective for sterilization of axillary bud explants in pineapple. In Gisela-5 (*Prunus cerasus* x *Prunus canescens*) NaOCl (2.0% for 3 minutes) showed very less contamination (13.33%) in auxiliary bud explants (Thakur *et al*., 2016) [19].

Effect on health of explant Health (+++, ++, +)

As the culture time increases, health of nodal segments was decreased in almost all the treatments (Table 4). At first week of culture, the explants in T_2, T_7, T_8, T_9, T_{10}, T_{11} T_{12}, T_{13} and T_{14} showed good health (+++), T_3, T_4, T_5, T_6, T_{15}, T_{16} T_{17} and T_{18} showed moderate health (++) and explants in T_1, T_{20} and T_{21} showed least health (+). At second week of culture, the explants in T_2, T_7, T_8, T_9, T_{10}, T_{11}, T_{12} and T_{13} expressed good health (+++), T_3, T_4, T_6, T_{14}, and T_{15} expressed moderate health (++) and explants in T_1, T_5, T_{16}, T_{17}, T_{18}, T_{19}, T_{20} and T_{21} expressed least health (+). At third week of culture, the explants in T_2, T_7, T_8, T_9, T_{10}, T_{11}, T_{12} and T_{13} showed good health (+++), T_3, T_4, T_6, T_{10}, T_{13}, T_{14} and T_{15} showed moderate health (++) and explants in T_1, T_4, T_5, T_6, T_{16} T_{17}, T_{18}, T_{19}, T_{20} and T_{21} showed least health (+). At fourth week of culture, the explants in T_9, T_{11} and T_{12} showed good health (+++), T_7, T_8, and T_{10} showed moderate health (++) and explants in T_1, T_2, T_3, T_4, T_5, T_6, T_{13}, T_{14}, T_{15}, T_{16}, T_{17}, T_{18}, T_{19}, T_{20} and T_{21} showed least health (+).

In this study, the good health (+++) was recorded in treatments where explants treated with $HgCl_2$ (0.05% for 5, 8 and 10 minutes) and $HgCl_2$ (0.1% for 3, 5 and 8minutes). The treatment with H_2O_2 (6% for 5 and 8 minutes) showed moderate health (++) and all other treatments showed least health (+). The results were similar with Jan *et al*. (2013) [2]. They reported lowest necrotic cultures (6.66 and 11.11% in runner tip and nodal segment, respectively) when explants treated with $HgCl_2$ (0.1% for 4 minutes) in strawberry and thereby maintaining good health in explants.

Table 1: Effect of different sterilizing agents on initiation response and days taken for regeneration in guava cv. Arka Kiran

Sl. No.	Treatments		Initiation response (%)			Days taken for regeneration
			15 Days	21 Days	30 Days	
1.	T_1	Control	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
2.	T_2	4% NaOCl for 3 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
3.	T_3	4% NaOCl for 5 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
4.	T_4	4% NaOCl for 8 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
5.	T_5	4% NaOCl for 10 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
6.	T_6	0.05% $HgCl_2$ for 3 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
7.	T_7	0.05% $HgCl_2$ for 5 min	51.48 (45.85)^{d}	55.45 (48.13)^{d}	55.45 (48.13)^{d}	12.42 (3.65)^{cd}
8.	T_8	0.05% $HgCl_2$ for 8 min	81.33 (64.48)^{c}	86.37 (68.47)^{b}	86.37 (68.34)^{c}	11.10 (3.47)^{bc}
9.	T_9	0.05% $HgCl_2$ for 10 min	88.21 (70.01)^{b}	88.38 (70.11)^{b}	88.38 (70.09)^{b}	9.76 (3.27)^{ab}
10.	T_{10}	0.1% $HgCl_2$ for 3 min	26.51 (30.95)^{e}	31.38 (34.06)^{e}	33.61 (35.43)^{e}	10.28 (3.35)^{abc}
11.	T_{11}	0.1% $HgCl_2$ for 5 min	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}	8.35 (3.05)^{a}
12.	T_{12}	0.1% $HgCl_2$ for 8 min	79.71 (63.29)^{c}	81.43 (64.52)^{c}	85.38 (67.53)^{c}	9.46 (3.22)^{a}
13.	T_{13}	0.1% $HgCl_2$ for 10 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
14.	T_{14}	6% H_2O_2 for 3 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
15.	T_{15}	6% H_2O_2 for 5 min	20.33 (26.69)^{f}	23.38 (28.86)^{f}	23.38 (28.91)^{f}	14.35 (3.91)^{de}
16.	T_{16}	6% H_2O_2 for 8 min	13.23 (21.12)^{g}	17.47 (24.62)^{g}	17.47 (24.70)^{g}	15.36 (4.04)^{e}
17.	T_{17}	6% H_2O_2 for 10 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
18.	T_{18}	0.5% NaOH for 3 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
19.	T_{19}	0.5% NaOH for 5 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
20.	T_{20}	0.5% NaOH for 8 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
21.	T_{21}	0.5% NaOH for 10 min	0 (0.28)^{h}	0 (0.28)^{h}	0 (0.28)^{h}	0 (1)
SEm ±			1.01	0.83	0.25	0.009
LSD at 0.01%			3.87	3.02	0.96	0.33
CV (%)			8.87	6.67	2.10	7.83

The values given in parenthesis are arc sine transformed in initiation of response (%) and square root transformed in days taken for regeneration. Values with the same letter are stastically non-significant at LSD ($p \leq 0.01$).

NaOCl - Sodium hypochlorite, $HgCl_2$ - Mercuric chloride, H_2O_2 - Hydrogen peroxide and NaOH - Sodium hydroxide.

Table 2: Effect of different sterilizing agents on survival percent of explants in guava cv. Arka Kiran

Sl. No.	Treatments		Survival (%)			
			7 Days	15 Days	21 Days	30 Days
1.	T_1	Control	0 (0.28)^{d}	0 (0.28)^{e}	0 (0.28)^{h}	0 (0.28)^{g}
2.	T_2	4% NaOCl for 3 min	100 (90.00)^{a}	100 (90.00)^{a}	65.31 (53.93)^{cd}	0 (0.28)^{g}
3.	T_3	4% NaOCl for 5 min	100 (90.00)^{a}	73.58 (59.13)^{b}	61.30 (51.55)^{de}	0 (0.28)^{g}
4.	T_4	4% NaOCl for 8 min	55.50 (48.17)^{b}	53.42 (46.96)^{d}	0 (0.28)^{h}	0 (0.28)^{g}
5.	T_5	4% NaOCl for 10 min	100 (90.00)^{a}	100 (90.00)^{a}	0 (0.28)^{h}	0 (0.28)^{g}
6.	T_6	0.05% $HgCl_2$ for 3 min	100 (90.00)^{a}	68.42 (55.85)^{b}	45.30 (42.29)^{f}	0 (0.28)^{g}
7.	T_7	0.05% $HgCl_2$ for 5 min	100 (90.00)^{a}	100 (90.00)^{a}	65.35 (53.97)^{cd}	61.24 (51.50)^{c}
8.	T_8	0.05% $HgCl_2$ for 8 min	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}
9.	T_9	0.05% $HgCl_2$ for 10 min	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}
10.	T_{10}	0.1% $HgCl_2$ for 3 min	100 (90.00)^{a}	100 (90.00)^{a}	76.42 (61.00)^{b}	71.35 (57.65)^{b}
11.	T_{11}	0.1% $HgCl_2$ for 5 min	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}
12.	T_{12}	0.1% $HgCl_2$ for 8 min	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}
13.	T_{13}	0.1% $HgCl_2$ for 10 min	100 (90.00)^{a}	0 (0.28)^{e}	0 (0.28)^{h}	0 (0.28)^{g}
14.	T_{14}	6% H_2O_2 for 3 min	100 (90.00)^{a}	61.25 (51.53)^{c}	56.82 (48.93)^{e}	43.30 (41.15)^{f}
15.	T_{15}	6% H_2O_2 for 5 min	100 (90.00)^{a}	69.45 (56.49)^{b}	55.32 (48.06)^{e}	50.33 (45.19)^{d}
16.	T_{16}	6% H_2O_2 for 8 min	100 (90.00)^{a}	100 (90.00)^{a}	59.35 (50.40)^{de}	45.56 (42.45)^{e}
17.	T_{17}	6% H_2O_2 for 10 min	48.26 (44.03)^{c}	0 (0.28)^{e}	0 (0.28)^{h}	0 (0.28)^{g}
18.	T_{18}	0.5% NaOH for 3 min	100 (90.00)^{a}	100 (90.00)^{a}	68.32 (55.76)^{c}	0 (0.28)^{g}
19.	T_{19}	0.5% NaOH for 5 min	0 (0.28)^{d}	0 (0.28)^{e}	0 (0.28)^{h}	0 (0.28)^{g}
20.	T_{20}	0.5% NaOH for 8 min	53.20 (46.84)^{b}	50.18 (45.10)^{d}	33.57 (35.37)^{g}	0 (0.28)^{g}
21.	T_{21}	0.5% NaOH for 10 min	0 (0.28)^{d}	0 (0.28)^{e}	0 (0.28)^{h}	0 (0.28)^{g}
SEm ±			0.50	0.93	1.03	0.33
LSD at 0.01%			1.90	3.55	3.92	1.26
CV (%)			1.21	2.78	4.32	2.01

The values given in parenthesis are arc sine transformed in survival (%). Values with the same letter are stastically non-significant at LSD ($p \leq 0.01$).

NaOCl - Sodium hypochlorite, $HgCl_2$ - Mercuric chloride, H_2O_2 - Hydrogen peroxide and NaOH - Sodium hydroxide.

Table 3: Effect of different sterilizing agents on contamination percent of explants in guava cv. Arka Kiran

Sl. No.	Treatments		Contamination (%)			
			7 Days	15 Days	21 Days	30 Days
1.	T_1	Control	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]
2.	T_2	4% NaOCl for 3 min	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]
3.	T_3	4% NaOCl for 5 min	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]
4.	T_4	4% NaOCl for 8 min	61.33 (51.55)[b]	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]
5.	T_5	4% NaOCl for 10 min	53.30 (46.89)[d]	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]
6.	T_6	0.05% $HgCl_2$ for 3 min	0 (0.28)[f]	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]
7.	T_7	0.05% $HgCl_2$ for 5 min	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]	10.27 (18.63)[e]
8.	T_8	0.05% $HgCl_2$ for 8 min	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]
9.	T_9	0.05% $HgCl_2$ for 10 min	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]
10.	T_{10}	0.1% $HgCl_2$ for 3 min	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]
11.	T_{11}	0.1% $HgCl_2$ for 5 min	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]
12.	T_{12}	0.1% $HgCl_2$ for 8 min	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)
13.	T_{13}	0.1% $HgCl_2$ for 10 min	0 (0.28)[f]	30.33 (33.37)[e]	35.36 (36.45)[e]	40.35 (39.43)[d]
14.	T_{14}	6% H_2O_2 for 3 min	0 (0.28)[f]	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]
15.	T_{15}	6% H_2O_2 for 5 min	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]	0 (0.28)[f]
16.	T_{16}	6% H_2O_2 for 8 min	52.21 (46.27)[d]	54.53 (47.61)[c]	55.38 (48.10)[c]	58.89 (50.12)[c]
17.	T_{17}	6% H_2O_2 for 10 min	55.52 (48.17)[c]	59.96 (50.77)[c]	63.29 (52.74)[b]	68.45 (55.83)[b]
18.	T_{18}	0.5% NaOH for 3 min	48.31 (44.03)[e]	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]
19.	T_{19}	0.5% NaOH for 5 min	0 (0.28)[f]	38.37 (38.25)[d]	48.52 (44.15)[d]	100 (90.00)[a]
20.	T_{20}	0.5% NaOH for 8 min	61.37 (51.57)[b]	65.68 (54.17)[b]	100 (90.00)[a]	100 (90.00)[a]
21.	T_{21}	0.5% NaOH for 10 min	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]	100 (90.00)[a]
SEm ±			0.18	0.84	0.73	0.36
LSD at 0.01%			0.70	3.21	2.81	1.36
CV (%)			1.02	2.95	2.47	1.12

The values given in parenthesis are arc sine transformed in contamination (%). Values with the same letter are stastically non-significant at LSD ($p \leq 0.01$).

NaOCl - Sodium hypochlorite, $HgCl_2$ - Mercuric chloride, H_2O_2 - Hydrogen peroxide and NaOH - Sodium hydroxide.

Table 4: Effect of different sterilizing agents on health of explant in guava cv. Arka Kiran

Sl. No.	Treatments		Health			
			7 Days	7 Days	7 Days	7 Days
1.	T_1	Control	+	+	+	+
2.	T_2	4% NaOCl for 3 min	+++	+++	++	+
3.	T_3	4% NaOCl for 5 min	++	++	++	+
4.	T_4	4% NaOCl for 8 min	++	++	+	+
5.	T_5	4% NaOCl for 10 min	++	+	+	+
6.	T_6	0.05% $HgCl_2$ for 3 min	++	++	+	+
7.	T_7	0.05% $HgCl_2$ for 5 min	+++	+++	+++	+++
8.	T_8	0.05% $HgCl_2$ for 8 min	+++	+++	+++	+++
9.	T_9	0.05% $HgCl_2$ for 10 min	+++	+++	+++	+++
10.	T_{10}	0.1% $HgCl_2$ for 3 min	+++	+++	++	+++
11.	T_{11}	0.1% $HgCl_2$ for 5 min	+++	+++	+++	+++
12.	T_{12}	0.1% $HgCl_2$ for 8 min	+++	+++	+++	+++
13.	T_{13}	0.1% $HgCl_2$ for 10 min	+++	+++	++	+
14.	T_{14}	6% H_2O_2 for 3 min	+++	++	++	+
15.	T_{15}	6% H_2O_2 for 5 min	++	++	++	++
16.	T_{16}	6% H_2O_2 for 8 min	+	+	+	++
17.	T_{17}	6% H_2O_2 for 10 min	++	+	+	+
18.	T_{18}	0.5% NaOH for 3 min	++	+	+	+
19.	T_{19}	0.5% NaOH for 5 min	+++	+	+	+
20.	T_{20}	0.5% NaOH for 8 min	+	+	+	+
21.	T_{21} -	0.5% NaOH for 10 min	+	+	+	+

Good health (+++), moderate health (++) and least health (+).

NaOCl - Sodium hypochlorite, $HgCl_2$ - Mercuric chloride, H_2O_2 - Hydrogen peroxide and NaOH - Sodium hydroxide.

Conclusion

Among the various sterilizing agents, $HgCl_2$ was showed superior results when compare to other sterilizing agents. Among various $HgCl_2$, the $HgCl_2$ (0.5%) at 8 and 10 minutes

and $HgCl_2$ (1.0%) at 5 and 8 minutes showed superior results. Overall, $HgCl_2$ (0.1%) at five minutes was performed better with respect to all parameters like zero contamination, maximum survival and initiation response, early regeneration and good health.

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