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SS Dodake
Wheat Specialist, Agricultural Research Station, MPKV, Niphad, Maharashtra, India

AS Jadhav
State Biotechnology Unit, Department of Agril Botany, PGI, MPKV, Rahuri, Maharashtra, India

SV Pawar
State Biotechnology Unit, Department of Agril Botany, PGI, MPKV, Rahuri, Maharashtra, India

PY Shinde
Senior Research Assistant, Agricultural Research Station, MPKV, Niphad, Maharashtra, India

NM Magar
Senior Research Assistant, Agricultural Research Station, MPKV, Niphad, Maharashtra, India

Corresponding Author:
SS Dodake
Wheat Specialist, Agricultural Research Station, MPKV, Niphad, Maharashtra, India

Standardizing efficient *in vitro* surface sterilization protocol for explants of Kankoda (*Momordica dioica* Roxb.)

SS Dodake, AS Jadhav, SV Pawar, PY Shinde and NM Magar

Abstract
To standardize the sterilization procedure for leaf and apical bud explants of Kankoda (*Momordica dioica* Roxb.), comparison was done between two important sterilizing agents, mercuric chloride (0.1%) and sodium hypochlorite (1.0%) with four time duration 3, 5, 7 and 10 minutes and in combinations. Result showed that amongst the two sterilants i.e. $HgCl_2$ and NaOCl in combination was found better for controlling the infection. Mercuric chloride ($HgCl_2$) for 5 minute and sodium hypochlorite (NaOCl) for 5 minute in combination was selected for suitable sterilization agents after 10 minute of DDW wash, 10 minute dip in bavistin and at last washed with double distilled water for leaf explants with 75.00% survival and apical bud explants with 83.33% survival of explants. This is the first report of successful sterilization and reduced contamination of explants from naturally field grown Kankoda by using $HgCl_2$ and NaOCl sterilizing agents in combination for standardizing efficient *in vitro* surface sterilization protocol.

Keywords: Surface sterilization protocol, explants, Kankoda, *Momordica dioica* Roxb.

Introduction
Kankoda or Spine gourd or Kartoli (*Momordica dioica* Roxb.) belongs to the family *Cucurbitaceae* and under the genus Momordica. This genus is native of tropical regions of Asia with extensive distribution in China, South East Asia, Japan, Polynesia besides tropical Africa and South America. As many of the species of this genus have been found to grow wildly in India, Bangladesh, Sri Lanka, Myanmar, Malaysia, etc. (Hooker, 1879) [7] it indicated that this region might be the origin of *Momordica dioica*. The genus Momordica possesses different sex forms (monoecious, dioecious, hermaphrodite) and x=14 basic chormosome number, *M. dioica* Roxb. (2n = 2x = 28) and its wild relative, *M. cochinchinensis* Spreng. (2n = 4x = 56) are dioecious and mainly propagate vegetatively through tuberous root (Rashid, 1993) [10].

It grows in warm and humid weather and tuberous roots are planted in pits. Plantation is done at ending of the summer when monsoon starts. Flowering starts in July and fruiting ends in October/November. The plants remain dormant in winter. The tubers are left *in situ* and they sprout again in rainy season.

It is an important vegetable in the Indian subcontinent and Bangladesh. It has many advantages, like high market price, good nutritional value and longer keeping quality. The vegetable has been gaining popularity among consumers so its cultivation area has been increasing day by day. The tender green fruits of konkoda are used for culinary purpose. Sometimes fruits are sliced, blanched and dried for further use. In some parts, the young tender leaves are used as vegetable. Besides being a vegetable, the seeds of oil which can be used as an illuminant and also as an admixture with drying oils in formulation of paints and varnishes. Its root froths in water and therefore, is used as washing soap.

The plant was reported to exhibit anti-diabetic, anti-cancer, anti-fertility abortificiant, anti-inflammatory, antioxidant activity, cure jaundice and bleeding pile properties (Bawara *et al.*, 2010) [2]. Owing to its multifold uses, its systematic cultivation would be a boon both for horticulture as well as pharmaceutical industry especially in tropical countries like India. Fruits contain high amounts of protein, calcium, phosphorous, iron, and highest amount of carotene amongst the cucurbitaceous vegetables (Bharathi *et al.*, 2007) [3].

It is mainly propagated by tubers. However, tuberous roots have got dormancy and they sprout only at onset of monsoon. Moreover, the multiplication rate of tubers is very low. Due to lack of technical knowledge, the success of rooting of cuttings is very low. Hard and impermeable seed coat possesses problem in germination of seeds.

Similarly, sex prediction of seed propagated plant is very difficult before flowering because of dioecious and highly cross pollinated nature.

So far not much systematic efforts have been made to overcome the above problems and crop remained neglected from research point of view. Systematic approach can result into some solutions to overcome these problems. Also the natural population of the plant is gradually decreasing due to habitat destruction and hence, conservation of this crop is necessary. The improvement of *M. dioica* through conventional breeding techniques has several limitations because of its dioecious nature and difficulty in seed germination and propagation by tuberous roots is limited due to the low multiplication rate. The successful application of plant tissue culture is requires an efficient shoot regeneration system. Hence, the present investigation was aimed with to develop an efficient explants sterilization protocol in kankoda.

Materials and Methods

Material

The female kankoda genotype was collected from AICRN on Potential Crops, Department of Agricultural Botany, MPKV, Rahuri. This single genotype was taken for regeneration. The two explants namely, leaf and apical bud explants were used for this experiment. These explants were collected from 45 days old plants after sprouting naturally in the field.

The present study was carried out at State Level Biotechnology Centre, Department of Agricultural Botany, MPKV, during *kharif* 2014-15.

Chemicals

Various chemicals of Qualigens Laboratory Pvt. Ltd were used for media preparation and sterilization of explants. Organic supplements like myoinositol were also used.

Cleaning of glassware's

It is important to use cleaned glassware for growth of tissue culture *in vitro*. This was achieved by boiling all glassware's in liquid soap solution for 1 hour, followed by thoroughly rinsing with tap water and immersing in nitric acid (30%) for 2 hours and then removing traces of acid by thoroughly washing with tap water. Then glassware's were rinsed with double distilled water and they were allowed to dry overnight at room temperature.

Sterilization of media, glassware and instruments

The object of sterilization was to make media, glassware and instruments free from microorganisms. For this, glassware such as conical flasks, test tubes, glass bottle etc., were plugged with non-absorbent cotton. Then petri dishes were wrapped with wrapping paper (aluminum foil). Forceps and scalpels were covered with brown paper. Afterwards the mouth ends of graduated pipettes were plugged with non-absorbent cotton. Individually they were wrapped with wrapping paper. All glassware and instruments were then autoclaved at 121 °C and 15 lbs pressure for 20 minutes. Finally they were kept in hot air oven for drying.

Preparation of stock solutions

It was found convenient to prepare the concentrated stock solutions of macro and micro nutrients, vitamins and plant growth regulators. The media used was Murashige and Skoog's (MS media).

Surface sterilization

Sterilization procedure was carried out in three steps. In primary sterilization, all explants were washed with double distilled water for 10 minutes. In secondary sterilization, all the explants were treated with 0.1% bavistin concentration for 10 minutes and washed with double distilled water for 10 minutes. In tertiary sterilization, Mercuric chloride (0.1%) and Sodium hypochlorite (1.0%) were used for study with treatment durations of 3, 5, 7 and 10 minutes with all possible combinations and alone were used and all explants were washed with double distilled water for 10 minutes after every treatment for complete removing of traces of chemicals.

To evaluate the response of different chemicals, implantations of sterilized explants were done using without hormones MS medium. The cultures were placed in culture growth room. The cultures were kept in a growth chamber for one month, at 25 °C, with 16 hours photoperiod and 3500 lux of light intensity. The observations were recorded up to 30 days. Twelve explants were used in each sterilization treatment, and each treatment was replicated thrice. The observations were recorded in percentage on infection per cent, dead per cent and survival per cent of cultures.

Cultural conditions

All the cultural operations including sterilization, inoculation and transfer were carried out in a horizontal laminar air flow chamber using sterilized instruments. Before inoculation, the chamber was smeared with 70% ethyl alcohol and exposed to UV light for 15-20 minutes. The cultured tubes/bottles after inoculation were incubated under dark at 25±1 °C.

Data analysis

All the studies were done in laboratory under well-defined conditions of the medium, growth, temperature and light. Completely Randomized Design (CRD) was employed for the experiment and the data were analyzed in SAS (Statistical analysis system).

Results and Discussion

Sterilization procedure was carried out in three steps in primary sterilization, secondary sterilization, all the explants were treated with 0.1% bavistin concentration for 10 minutes and washed with double distilled water for 10 minutes along with tertiary sterilization, mercuric chloride (0.1%) and sodium hypochlorite (1.0%) were used for study with duration of 3, 5, 7 and 10 minutes with all possible combinations and alone were used and all explants were washed with double distilled water for 10 minutes.

To evaluate the response of different treatment combinations, implantations of sterilized explants were done using without hormones MS medium and results showed that with increase of time, the infection was decreases in both the sterilizing agents for both the explants when sterilized explants individually or in combination of both sterilizing agents.

The effect of surface sterilizing treatments, on infection per cent, dead per cent and survival per cent of cultures of Kankoda is given in Table 1, 2, Plate 1 and graph 1 and 2.

Effect on infection of cultures (Contamination%)

Among both explants studied the apical buds explants recorded maximum infection percent as compare to leaf explants. The infection per cent was notably much lower in NaOCl as compared to $HgCl_2$ regarding duration of time.

The treatments S_1, S_2, S_3, S_4, S_7 and S_8 recorded 100 per cent infection, while treatments S_{10}, S_{14}, S_{17}, S_{18}, S_{20}, S_{21}, S_{22}, S_{23}, S_{24}, S_{25} and S_{26} recorded zero infection for leaf explants.
The treatments S_1, S_2, S_3, S_4, S_7 and S_8 recorded 100 per cent infection, while treatments S_{10}, S_{14}, S_{17}, S_{18}, S_{21}, S_{22}, S_{23}, S_{24}, S_{25} and S_{26} recorded zero contamination for apical buds explants. The infection of cultures ranged from 0 to 100 per cent for apical buds explants.

Effect on non-growing cultures (Dead%)
Results showed that with increase of time the non-growing/dead per cent increased in both the sterilizing agents for both explants (Table 1 & 2).
The treatments S_{10}, S_{18}, S_{21}, S_{22}, S_{23}, S_{24}, S_{25} and S_{26} recorded 100 per cent non-growing cultures followed by S_{20}, S_{14}, S_{17}, S_6, and S_{19} for leaf explants.
The treatments S_{14}, S_{18}, S_{22}, S_{24}, S_{25} and S_{26} recorded 100 per cent non-growing cultures followed by S_{10}, S_{21}, S_{23}, S_{20} and S_6 for apical buds explants, while treatments S_9, S_{11}, S_{12}, S_5 and S_{16} recorded lowest mortality rate for apical buds explants. The non-growing per cent cultures ranged from 0 to 100 for apical buds explants.

Effect on healthy cultures (Survival%)
Results showed that with incensement of time the survival per cent increased in both the sterilizing agents for both the explants up to limit (Table No.1 & 2).
When $HgCl_2$ was used alone, the highest survival percentage (16.67%) for both explants was recorded in treatment DDW + Bavistin 0.1% + $HgCl_2$ 0.1% (10+10+7 minutes) followed by treatment DDW + Bavistin 0.1% + $HgCl_2$ 0.1% (10+10+10 minutes). Nabi *et al.,* 2002 sterilized Kankoda explants viz. shoot tip, leaves and nodes with 0.1% $HgCl_2$ for 5 minutes. Devendra *et al.,* (2009) [5] sterilized shoot explants of Kankoda with 0.1% (w/v) aqueous mercuric chloride solution for 3 minutes. Thakur *et al.,* (2011) [11] surface sterilized axillary buds of *Momordica balsamina* with 0.1% mercuric chloride solution for 7 minutes.
When NaOCl was used alone, the highest percentage of survival rate was recorded in treatment DDW + Bavistin 0.1% + NaOCl 1.0% (10+10+7 minutes) with 33.33% for leaf explants.
When NaOCl was used alone, the highest percentage of survival rate was recorded in treatment DDW + Bavistin 0.1% + NaOCl 1.0% (10+10+7 minutes) with 41.67% by apical buds explants followed by treatment DDW + Bavistin 0.1% + NaOCl 1.0% (10+10+10 minutes) with only 8.33% survival rate. Badoni and Chauhan (2010) [1] developed *in vitro* sterilization protocol for micropropagation of potato cv. 'Kufri Himalini' by using sterilizing agent 1.0% NaOCl with 8 minutes time duration for explants sterilization. Mihaljevic *el al.,* (2013) [8] used sodium hypochlorite (NaOCl) for sterilization of 'Oblacinska' sour cherry buds for 8 minute was the best for controlling the infection. For leaf explants sterilization, treatments involving combination of $HgCl_2$ and NaOCl, the highest percentage of survivals of cultures were recorded in treatment i.e. DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0% (10+10+5+5 minutes) with 75.00% followed by DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0% (10+10+3+7) with 58.33% by leaf explants.
For apical bud explants sterilization, treatments involving combination of $HgCl_2$ and NaOCl, the highest percentage of survivals of cultures were recorded in treatment i.e. DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0% (10+10+5+5 minutes) with 88.33% followed by DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0% (10+10+3+7) with 66.67% by apical buds explants. Colgecen *et al.,* (2011) [4] analyzed the combinations of two different sterilizing agents i.e 1.25% sodium hypochlorite and Plant Preservative Mixture (PPM) on surface sterilization in *A. densiflora.* Similar results were recorded by Dodake *et al* (2020) [6] in respect to inter node explants of Kankoda.

Suitable surface sterilization agents
While comparing the effect of $HgCl_2$ and NaOCl alone and in all possible combination with varying times, the treatment DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0% (10+10+5+5 minutes time respectively) was always found better than any other treatments for suitable sterilization combination to both the explants of kankoda used in this investigation.
This is the first report of successful sterilization and reduced contamination of explants from naturally field grown Kankoda (*Momordica dioica* Roxb., by using $HgCl_2$ and NaOCl sterilizing agents in combination for standardizing efficient *in vitro* surface sterilization protocol. These results were found better for controlling the infection and it had not any adverse effect on explants even in long duration.

Table 1: Percentage of contamination, non grown culture and survival in leaf explants after sterilization with various concentrations of sterilizing agents for different time duration

Treatment No.	Treatment details	Time (min.)	Infection% (% contamination) in leaf explants	Non-grown% cultures or % death in leaf explants	% Survival in leaf explants
S1	Control (DDW washing)	10	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S2	DDW + Bavistin 0.1%	10+10	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S3	DDW + Bavistin 0.1% + $HgCl_2$ 0.1%	10+10+3	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S4	DDW + Bavistin 0.1% + $HgCl_2$ 0.1%	10+10+5	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S5	DDW + Bavistin 0.1% + $HgCl_2$ 0.1%	10+10+7	75.00 (60.00)	8.33 (16.77)	16.67 (24.10)
S6	DDW + Bavistin 0.1% + $HgCl_2$ 0.1%	10+10+10	25.00 (30.00)	66.67 (54.74)	8.33 (16.77)
S7	DDW + Bavistin 0.1% + NaOCl 1.0%	10+10+3	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S8	DDW + Bavistin 0.1% + NaOCl 1.0%	10+10+5	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S9	DDW + Bavistin 0.1% + NaOCl 1.0%	10+10+7	58.33 (49.79)	8.34 (16.80)	33.33 (35.26)
S10	DDW + Bavistin 0.1% + NaOCl 1.0%	10+10+10	0.00 (0.00)	100.00 90.00)	0.00 (0.00)
S11	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+3+3	91.67 (73.23)	0.00 (0.00)	8.33 (16.77)
S12	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+3+5	75.00 (60.00)	0.00 (0.00)	25.00 (30.00)
S13	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+3+7	16.67 (24.10)	25.00 (30.00)	58.33 (49.79)
S14	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+3+10	0.00 (0.00)	83.33 (65.90)	16.67 (24.10)
S15	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+5+3	66.67 (54.73)	0.00 (0.00)	33.33 (35.27)
S16	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+5+5	5.55 (11.18)	19.45 (26.07)	75.00 (60.00)
S17	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+5+7	0.00 (0.00)	75.00 (60.00)	25.00 (30.00)
S18	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+5+10	0.00 (0.00)	100.00 90.00)	0.00 (0.00)
S19	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+7+3	13.87 (21.65)	61.13 (51.45)	25.00 (30.00)

S20	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+7+5	0.00 (0.00)	83.33 (65.91)	16.67 (24.09)
S21	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+7+7	0.00 (0.00)	100.00 90.00)	0.00 (0.00)
S22	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+7+10	0.00 (0.00)	100.00 90.00)	0.00 (0.00)
S23	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+10+3	0.00 (0.00)	100.00 (90.00)	0.00 (0.00)
S24	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+10+5	0.00 (0.00)	100.00 (90.00)	0.00 (0.00)
S25	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+10+7	0.00 (0.00)	100.00 (90.00)	0.00 (0.00)
S26	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+10+10	0.00 (0.00)	100.00 (90.00)	0.00 (0.00)
		Minimum	0.00	0.00	0.00
		Maximum	90.00	90.00	60.00
		Mean	35.56	42.60	14.47
		SE±	1.20	0.49	0.001
		CD at 5%	3.42	1.40	0.004
		CV%	5.87	2.00	0.02

* Figures in parenthesis showing arc sine values

Table 2: Percentage of contamination, non grown culture and survival in apical bud after sterilization with various concentrations of sterilizing agents for different time duration

Treatment No	Treatment details	Time (min.)	Contamination in apical bud explants (%)	Non-grown cultures or % death in apical bud	Survival% in apical bud explants
S1	Control (DDW washing)	10	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S2	DDW + Bavistin 0.1%	10+10	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S3	DDW + Bavistin 0.1% + $HgCl_2$ 0.1%	10+10+3	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S4	DDW + Bavistin 0.1% + $HgCl_2$ 0.1%	10+10+5	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S5	DDW + Bavistin 0.1% + $HgCl_2$ 0.1%	10+10+7	75.00 (60.00)	8.33 (16.77)	16.67 (24.10)
S6	DDW + Bavistin 0.1% + $HgCl_2$ 0.1%	10+10+10	25.00 (30.00)	50.00 (45.00)	25.00 (30.00)
S7	DDW + Bavistin 0.1% + NaOCl 1.0%	10+10+3	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S8	DDW + Bavistin 0.1% + NaOCl 1.0%	10+10+5	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)
S9	DDW + Bavistin 0.1% + NaOCl 1.0%	10+10+7	58.33 (49.79)	0.00 (0.00)	41.67 (40.21)
S10	DDW + Bavistin 0.1% + NaOCl 1.0%	10+10+10	0.00 (0.00)	91.67 (73.23)	8.33 (16.77)
S11	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+3+3	91.67 (73.23)	0.00 (0.00)	8.33 (16.77)
S12	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+3+5	75.00 (60.00)	0.00 (0.00)	25.00 (30.00)
S13	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+3+7	16.67 (24.10)	16.67 (24.09)	66.66 (54.73)
S14	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+3+10	0.00 (0.00)	100.00 (90.00)	0.00 (0.00)
S15	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+5+3	66.65 (54.73)	0.00 (0.00)	33.35 (35.27)
S16	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+5+5	8.33 (16.77)	8.35 (16.80)	83.33 (65.90)
S17	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+5+7	0.00 (0.00)	41.67 (40.21)	58.33 (49.79)
S18	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+5+10	0.00 (0.00)	100.00 (90.00)	0.00 (0.00)
S19	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+7+3	16.65 (24.08)	33.35 (35.27)	50.00 (45.00)
S20	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+7+5	2.77 (5.58)	55.56 (48.20)	41.67 (40.20)
S21	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+7+7	0.00 (0.00)	91.67 (73.23)	8.33 (16.77)
S22	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+7+10	0.00 (0.00)	100.00 (90.00)	0.00 (0.00)
S23	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+10+3	0.00 (0.00)	83.33 (65.90)	16.67 (24.10)
S24	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+10+5	0.00 (0.00)	100.00 (90.00)	0.00 (0.00)
S25	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+10+7	0.00 (0.00)	100.00 (90.00)	0.00 (0.00)
S26	DDW + Bavistin 0.1% + $HgCl_2$ 0.1% + NaOCl 1.0%	10+10+10+10	0.00 (0.00)	100.00 (90.00)	0.00 (0.00)
		Minimum	0.00	0.00	0.00
		Maximum	90.00	90.00	65.90
		Mean	36.09	37.64	18.83
		SE±	1.09	0.31	0.001
		CD at 5%	3.11	0.89	0.004
		CV%	5.25	1.44	0.01

* Figures in parenthesis showing arc sine values

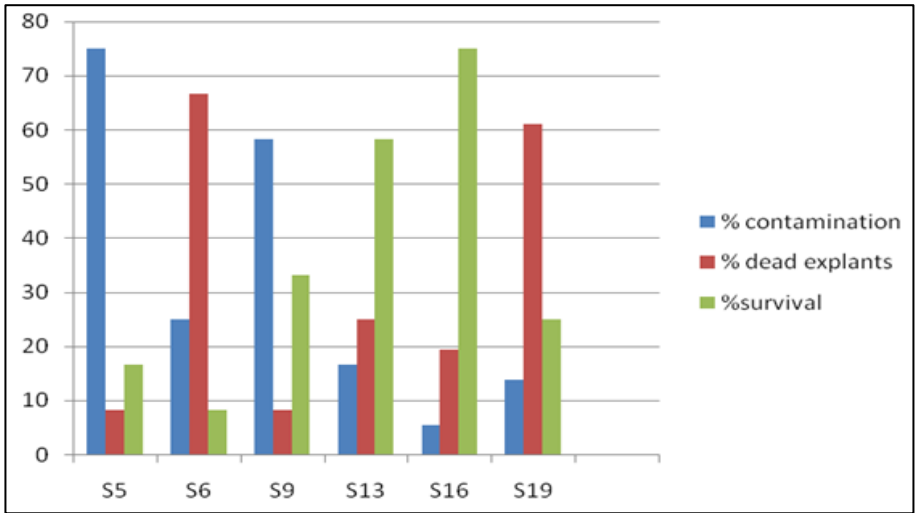

Fig 1: Promising treatment combination for % contamination, % dead explants and% survival in leaf explants after sterilization with various concentrations of sterilizing agents for different time duration

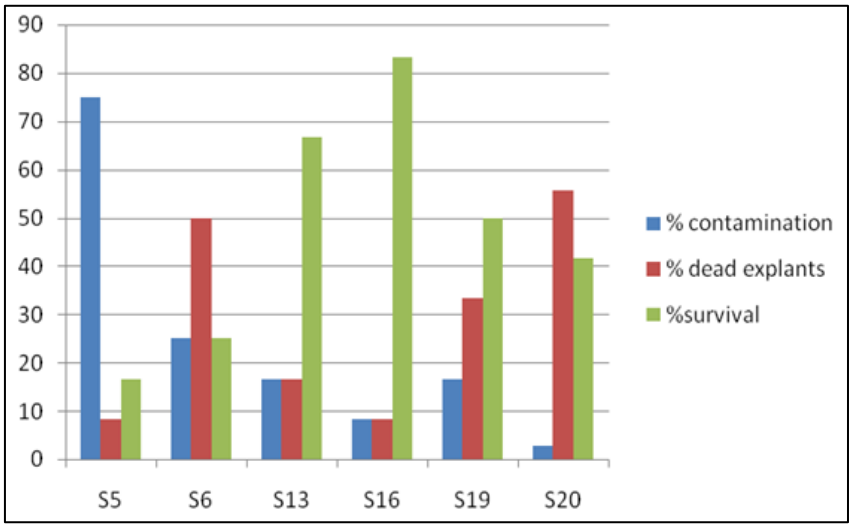

Fig 2: Promising treatment combination for% contamination, % dead explants and % survival in apical bud explants after sterilization with various concentrations of sterilizing agents for different time duration

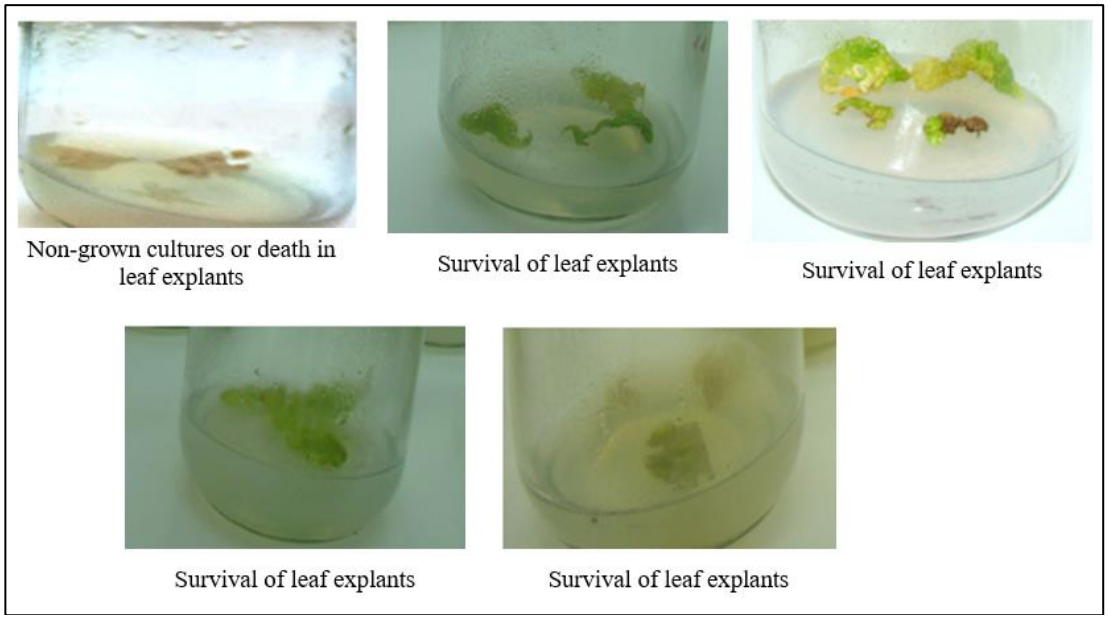

Plate 1: Standardizing efficient *in vitro* surface sterilization protocol through DDW + Bavistin 0.1% + HgCl2 0.1% + NaOCl 1.0% (10+10+5+5) for leaf explants

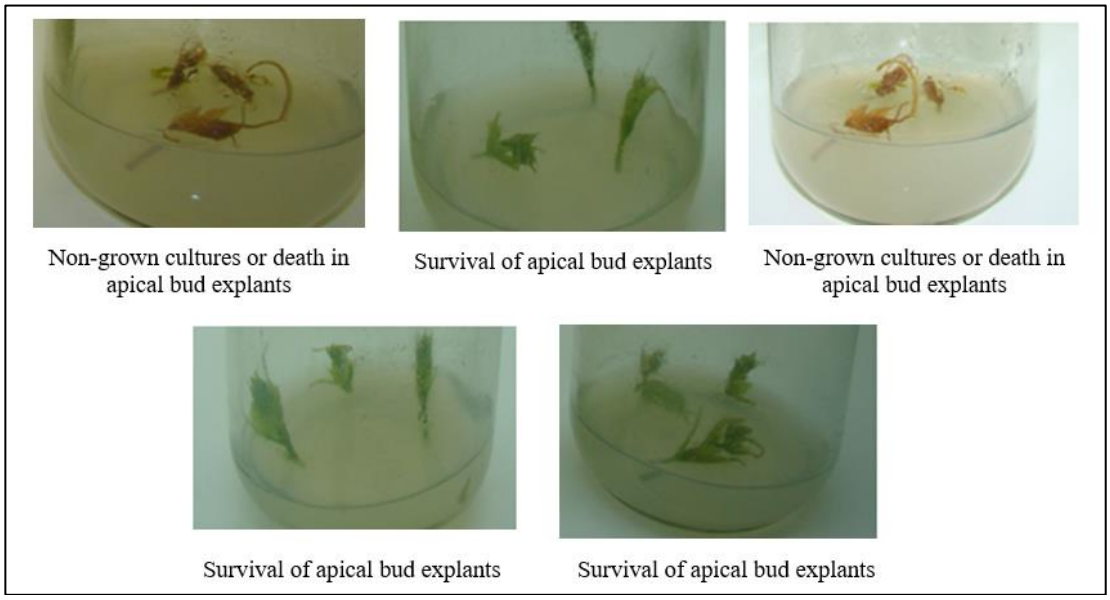

Plate 2: Standardizing efficient *in vitro* surface sterilization protocol through DDW + Bavistin 0.1% + HgCl2 0.1% + NaOCl 1.0% (10+10+5+5) for apical bud explants

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