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Studies on the effects of different surface sterilization agents under *in vitro* culture of Pepino (*Solanum muricatum* Ait.) cv. Valentia

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Abstract
An experiment was conducted at the Tissue culture Lab Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh, India during the year 2018-20. Effect of different sterilization agent for surface sterilization under *in-vitro* culture of pepino with completely randomized design (CRD) having four treatment duration (1.5, 3.0, 4.5, 6.0 minutes) and used mercuric chloride ($HgCl_2$ 0.1%), ethanol (70%), mercuric chloride ($HgCl_2$ (0.1%) + ethanol (70%), sodium hypochlorite (NaOCl) (70%) and mercuric chloride ($HgCl_2$ 0.1%) + fungicide (Bavistin) (0.15%) for surface sterilization agent. The response of different sterilization agents was evaluated on the basis on contamination percentage at 10 days after and survived percentage at 25 days after. Minimum contamination percentage (26.35) percent and maximum survival percentage (73.13) per cent was noted under $HgCl_2$ (0.1%) treating for a period of 4.5 minute. Minimum contamination percentage (39.55) percent and maximum survival percentage (60.37) per cent was noted under ethanol (70%) treating for a period of 4.5 minutes. Minimum contamination percentage (31.55) percent and maximum survival percentage (68.07) per cent was noted under mercuric chloride (0.1%) + ethanol (70%) treating for a period of 4.5 minutes. Minimum contamination percentage (38.53) percent and maximum survival percentage (61.46) per cent was noted under sodium hypochlorite (NaOCl) (70%) treating for a period of 4.5 minutes. Minimum contamination percentage (21.76) percent and maximum survival percentage (77.36) per cent was noted under mercuric chloride (HgCl2 0.1%) + fungicide (bavistin) treating for a period of 4.5minutes. On the basis of results obtained present investigations, it can be concluded that contamination and survival percentage of explants was vice versa to the duration of the treatment.

Keywords: Contamination, fungicide, *in-vitro* survival, tissue culture

1. Introduction
The pepino (*Solanum muricatum* Aiton), which is also referred to as pepino dulce in Spanish language, has been described as a succulent, juicy, and sweet fruit that is used mainly in desserts, although some cultivars have been used in salads due to their higher acidity content and grassy flavor notes (Rodriguez-Burruezo *et al.*, 2011) [17]. In the pepino was proposed as a physiological model of the texture or firmness changes that occur during maturation and ripening (Heyes *et al.*, 1994) [8]. The pepino fruit is a diploid (2n = 24) subtropical species and is also known as melon pear, melon shrub, or sweet cucumber. Native species from South America, more specifically from the Andes area of Peru and Chile, is widely distributed from Colombia to Bolivia.

The pepino fruit served as an important crop in Pre-Columbian Andean cultures, and it is a member of the Solanaceae family. Pepino is one of the few that is domesticated and cultivated for food purposes (Daunay *et al.*, 1995) [4]. Interestingly, most pepino research has been conducted in New Zealand, Spain, and Israel through their respective breeding programs (Rodriguez-Burruezo *et al.*, 2011) [17]. Taxonomically, pepino is placed within Solanum subgenus Potatoe section Basarthrum (Correll, 1962; Anderson and Bernardello, 1991 and Anderson *et al.*, 1996) [1, 3]. This section, characterized by the basal pedicel articulation (i.e., flowers fall off with pedicels attached, leaving only scars on the inflorescence axis), includes 11 species, the cultivated pepino and 10 wild species distributed through Central and South America.

Pepino is normally self-pollinated, but insect pollination can boost up the fruit set. Flowering continues throughout the year but fruit set during winter would not attain the proper size. Apart from low temperature affecting fruit set, high temperature leads to shriveling of flower

buds and loss of pollen viability. Pepino takes 150-160 days between anthesis to fruit ripening in spring-summer season. However, more time can be taken if cool weather is prolonged. Fruits are harvested when its colour changes from pale green to yellowish green with purple stripes on their surface. Fruit weight 150-700 g and one plant produces 10-15 fruits. The fruits mature irregularly, hence many pickings are required. Fruits should be plucked with pedicel for market supply. Fruits can be stored for 15-20 days under ambient conditions Rana and Verma (2011) [15]. The pepino fruit has been described as a berry that develops on a cymose inflorescence (Gould *et al.*, 1990) [7]. The fruit presents a simple sigmoid growth curve, and its maximum fruit size is reached 60 day after anthesis.

It's also prized for its medicinal applications. Aqueous extract of its fruit could attenuate the progression of diabetes due to its anti-inflammatory, anti-glycative and antioxidant effects (Hsu *et al*., 2011) [9]. A medium serving (100 g) of its fruit provides 80 calories of energy and 5 g of dietary fibres similar to oatmeal, which helps to lower cholesterol and it's easy to digest. The fibre also helps with constipation and it tends to sooth away gastric ulcers too. The fruit is rich in minerals and vitamin C but low in starch, sugars and free from oxalates. The minerals contained in Pepino fruits are Fe, Zn, Cu, Mn, Ca & P. It has been observed that level of glucose and fructose decreases during ripening, whereas, sucrose concentration increases as the ripening progresses. A discernible reduction has also been noticed in contents of protein and fat as the fruit turns from raw to mature (Huyskens-Keil *et al.* 1999) [10]. Pepino is known as a source of beta-carotene, 27 mg per 100 grams of fruit flesh. The crop is also considered as a sucrose accumulator during final ripening stage. Fruits picked when immature are flavourless and non-aromatic. The fruit is juicy (more than 40% juice) with very mild flavour. Fruit acidity is low (0.04-0.10%) and citric acid is predominant. Vitamin C content varies among cultivars from 30 to 70 mg/100 g fresh weight. Pepino fruits contain about 9.5% soluble solids, 4.06 g/100 g sugars, 0.06 g/100 g acids, and 34.25 mg/100 g vitamin C (De Arriola *et al.*, 1976) [5]. Redgwell and Turner (1986) [16] reported that ripe pepino fruits of 'El Camino' contained 6.8– 8.2% dry matter, 0.1% protein, 4.9-6.4 g/100 g sugars, 48-68 mg/100 g vitamin C, 119-153 mg/100 g organic acids, and 52-70 mg/100 g amino acids. Uses of the pepino fruit include juices, preserves, ice-cream, and jam. Fruits at the "green" and "turning stage" before ripening may have a cucumber-like scent and can be used in green salads or as a vegetable in stews, and it can be consumed as a refreshing dessert fruit or as an ingredient of fruit salads (Gonzalez *et al.*, 2000) [6].

The plant is cultivated very recently in areas other than South America. It has been possible to develop new improved materials for such traits. Although most pepino cultivars are sexually fertile and produce viable seeds, but their high level of heterozygosis. Pepino tissue culture regeneration systems can achieve a rapid propagation true to type of plantlets within short period of time for successful, aseptic and contamination free plant production, surface sterilization and determination duration of treatment is very important.

2. Materials and Methods

Details of experiments

2.1. Details of Pepino cultivar

The pepino cultivar Valentia was collected from ICAR-NBPGR Regional Station-Phagli, Shimla, - 171004, Himachal Pradesh, (INDIA), it is suitable for cultivation under subtropical region. Valentia takes 150-160 days between anthesis to fruit ripening in spring-summer season fruit weight 150-700 g and one plant produces 10-15 fruits.

2.2. Location of experiment

The experiments were carried out in the Tissue Culture Laboratory, Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, U.P. (India), from November, 2018 to March, 2020 to fulfill the objectives.

2.3. Source of explants material

The leaves, internode explants were collected from healthy and disease free plants and cleaned thoroughly by repeated washing under running tap water for period of 20 minutes, air dried and stored in the laboratory at ambient temperature. These leaves and internode were then trimmed and used as explants for culture establishments under *in-vitro* conditions.

2.4. Treatments and experimental design

Five types of chemical treatments such as Mercuric Chloride, Ethanol, Sodium Hypochlorite (NaOCl), fungicide (Bavistin) each with different combination and duration of treatment, three replication, ten containers or pots/treatment CRD design were tested.

The contamination and survival percentage of explants were calculated using fallowing formula

A. Contaminated percentage: Percentage of explants contamination was calculated using the following formula:

$$\text{Percentage of explants contaminated} = \frac{\Sigma Xi}{N} \times 100$$

Where, Σ = Summation, Xi = Number explants contaminated
N = Number of explants cultured

B. Survival percentage: Percentage of explants Survived was calculated using the following formula:

$$\text{Percentage of explants Survived} = \frac{\Sigma Xi}{N} \times 100$$

Where, Σ = Summation, Xi = Number explants survived,
N = Number of explants cultured

3. Statistical analysis

The data recorded were subjected to analyze as per the design. The experiment was arranged in Completely Randomized Design (CRD). Each treatment was performed with three replicates. Cultures were observed routinely. For statistical analysis of data generated through various experiments was qualified and the significance of difference among means were determined by ANOVA using Window stat 9.2 software.

4. Results and Discussions

4.1 Percentage of explants contaminated and survived after 10 and 25 days with Mercuric Chloride ($HgCl_2$ 0.1%) treatment period for surface sterilization of pepino fruit

Mercuric chloride is antimicrobial, with action against both fungi and bacteria, but frequently also kills the explants. At low concentrations (up to 0.1%) it is the most effective dis-infective agent for explants with soil-borne and the epiphytic fungi and bacteria. So this was the reason to prefer use of

mercuric Chloride ($HgCl_2$) as (0.1%) for surface sterilization of explants during tissue culture.

In the present study, (0.1%) $HgCl_2$ was found to be effective for sterilization of internodal explants. The Maximum contamination percentage of explants (96.22) per cent at 10 days after was noted under the treatment of control followed by (51.66) and (40.62) per cent under $HgCl_2$ (0.1%) with duration of 1.5 and 3.0 minutes respectively; while the minimum (26.35) percent was noted under $HgCl_2$ (0.1%) treating for a period of 4.5 minutes and maximum survival percentage of explants after 25 days (73.13) percent was noted under the treatment of $HgCl_2$ (0.1%) for a period of 4.5 minutes followed by (71.91) percent by 6.0 minute and minimum survival percentage was recorded (2.85) under control treatment fallowed by (47.64) percent under $HgCl_2$ (0.1%) with 1.5 minutes. Similar result was also found by (Guranna and Hoolageri 2017) [8], (Parveen *et al.* 2019) [14], the contamination of explants significantly decreased with increase in duration treatment with Mercuric chloride as sterilants.

Table 1: Percentage of explants contaminated and survived after 10 and 25 days with Mercuric Chloride ($HgCl_2$ 0.1%) treatment period for surface sterilization of pepino fruit

Treatment	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
Control	96.22	2.85
1.5 Min.	51.66	47.64
3.0 Min.	40.62	59.23
4.5 Min.	26.35	73.13
6.0 Min.	47.86	71.91
C.D.	2.13	21.60
SE (m)	0.67	6.76
SE (d)	0.94	9.57
C.V.	2.39	26.71

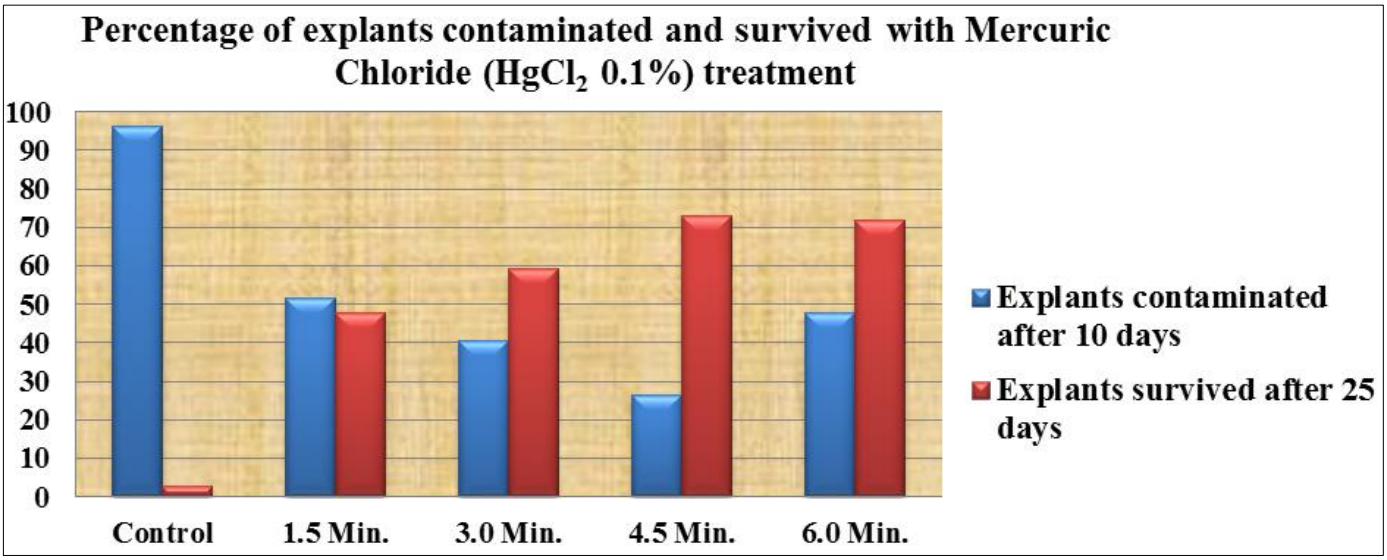

Fig 1: Percentage of explants contaminated and survived after 10 and 25 days with Mercuric Chloride ($HgCl_2$ 0.1%) treatment period for surface sterilization of pepino fruit

4.2. Percentage of explants contaminated and survived after 10 and 25 days with ethanol (70%) treatment period for surface sterilization of pepino fruit

When used the ethanol (70%) for sterilization that affect the cell of fungi and bacteria, and virus by infecting single cell of organism, the ethanol also caused its protein to coagulate, but this occurred at a much slower rate. This actually allowed the ethanol to penetrate the entire cell before it had a chance for its coagulation to block it. The entire cell is then coagulated, causing the organism to die.

The Percentage of explants contaminated and survived after sterilization with ethanol (70%) was observed, Maximum contamination percentage of explants after 10 days (92.42) percent was noted under the control treatment followed by (57.29) and (47.75) per cent with the duration of 1.5 and 3.0 minutes respectively; while the minimum (39.55%) was noted under ethanol (70%) treating for a period of 4.5 minutes and maximum survival percentage of explants after 25 days (60.37) percent was noted under the treatment of ethanol (70%) for a period of 4.5 minutes followed by (57.76) and (51.90) per cent with the duration of 4.5 and 6.0 minutes respectively; While the minimum survival (7.08) per cent was noted under control treatment. The present finding was in conformity with observations recorded by (Aysun and Melekber 2013) [2], (Yadav *et al.* 2021) [19].

Table 2: Percentage of explants contaminated and survived after 10 and 25 days with Ethanol (70%) treatment period for surface sterilization of pepino fruit

Treatment	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
Control	92.42	7.08
1.5 Min.	57.29	42.37
3.0 Min.	47.75	51.90
4.5 Min.	39.55	60.37
6.0 Min.	41.66	57.76
C.D.	1.83	2.24
SE (m)	0.57	0.70
SE (d)	0.81	0.99
C.V.	1.78	2.77

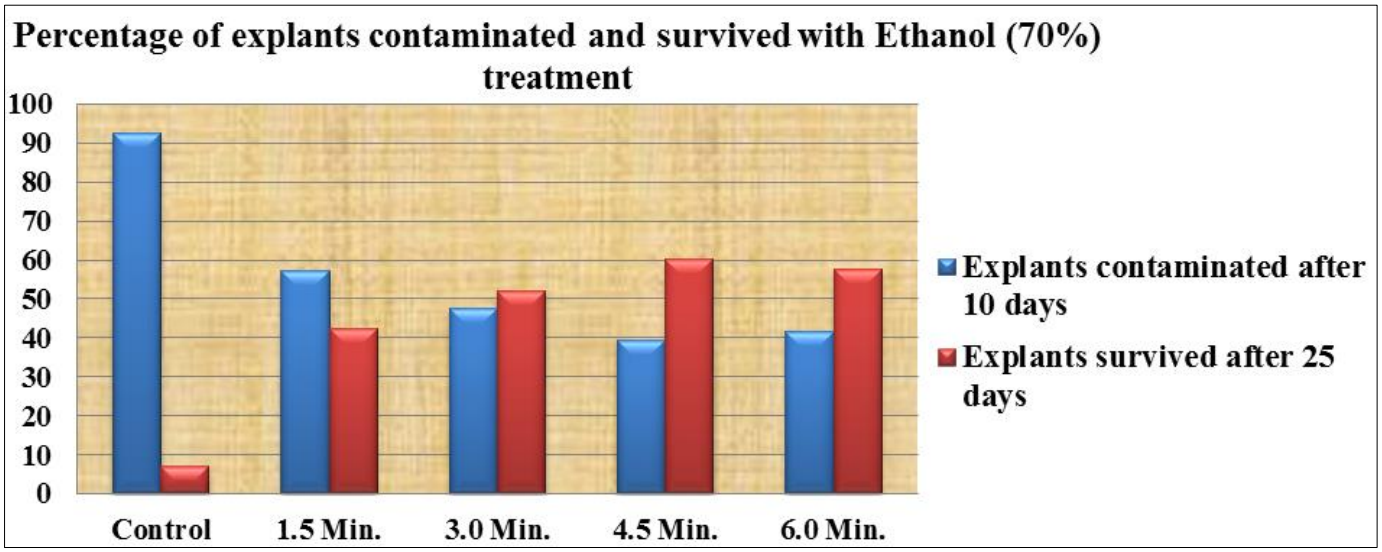

Fig 2: Percentage of explants contaminated and survived after 10 and 25 days with ethanol (70%) treatment period for surface sterilization of pepino fruit

4.3. Percentage of explants contaminated and survived after 10 and 25 days with Mercuric Chloride ($HgCl_2$ 0.1%) + Ethanol (70%) treatment period for surface sterilization of pepino fruit

The affectivity of mercuric chloride ($HgCl_2$ 0.1%) + Ethanol (70%) as surface sterilization agent depends on the duration of mercuric chloride ($HgCl_2$ 0.1%) + Ethanol (70%); mercuric chloride ($HgCl_2$ 0.1%) + ethanol (70%) destroys the cell wall/membrane of virus fungi and bacteria by denaturing their proteins and dissolving their lipids and pass the bacterial membrane/wall (effective against most bacteria, fungi and some viruses). Maximum contamination percentage of explants (96.10) per cent was noted under control with the treatment of mercuric chloride (0.1%) + ethanol (70%) followed by (52.23) and (50.43) per cent with the duration of 1.5 and 3.0 minutes respectively; while the minimum (31.55) per cent was noted under mercuric chloride (0.1%) + ethanol (70%) treating for a period of 4.5 minutes and maximum survival percentage of explants after 25 days (68.07) per cent was noted under the treatment of mercuric chloride (0.1%) + ethanol (70%) for a period of 4.5 minutes; while the minimum survival percentage (3.33) per cent was noted under control treatment. So, it was observed that contamination and survival percentage of explants was anew vice versa to the duration of the treatment with mercuric Chloride ($HgCl_2$ 0.1%) + ethanol (70%) The present findings are also in accordance to the observations recorded by (Jan *et al*. 2013) [12], (Guranna and Hoolageri 2017) [8] (Palei *et al*. 2017) [13], (Yadav *et al*. 2021) [19].

Table 3: Percentage of explants contaminated and survived after 10 and 25 days with Mercuric Chloride ($HgCl_2$ (0.1%) + Ethanol (70%) treatment period for surface sterilization of pepino fruit

Treatment	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
Control	96.10	3.33
1.5 Min.	52.23	47.75
3.0 Min.	50.43	49.46
4.5 Min.	31.55	68.07
6.0 Min.	42.68	56.98
C.D.	1.84	2.15
SE (m)	0.57	0.67
SE (d)	0.81	0.93
C.V.	1.83	2.58

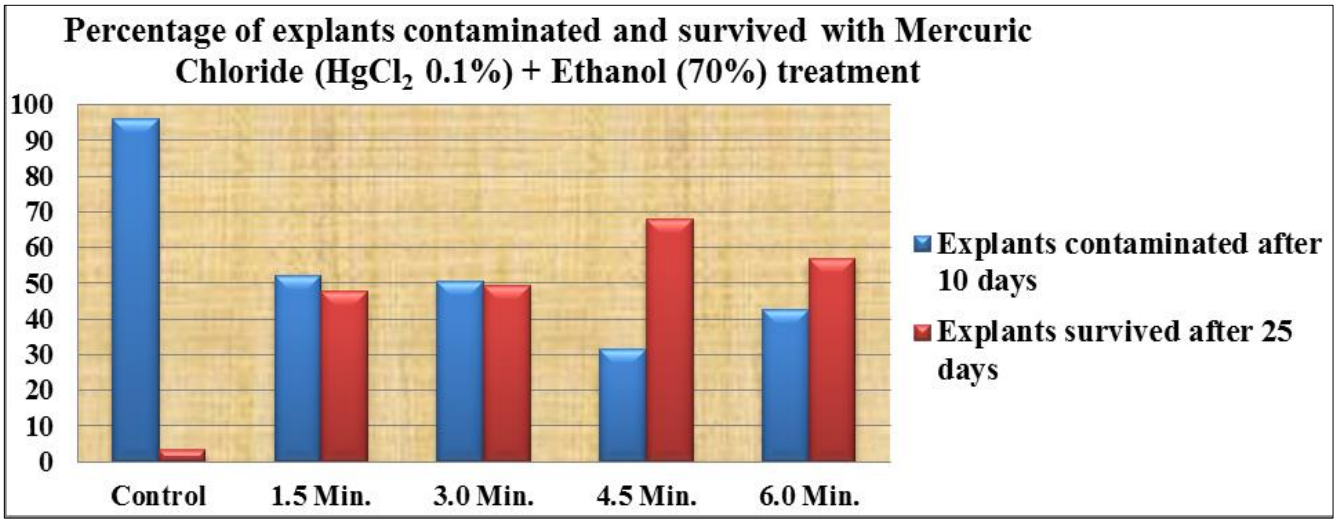

Fig 3: Percentage of explants contaminated and survived after 10 and 25 days with Mercuric Chloride ($HgCl_2$ 0.1%) + Ethanol (70%) treatment period for surface sterilization of pepino fruit

4.4. Percentage of explants contaminated and survived after 10 and 25 days with Sodium hypochlorite (NaOCl) (70%) treatment period for surface sterilization of pepino fruit

Sodium hypochlorite, commonly known as bleach, is most frequently used as a sterilization agent. It is a sterilization agent that is effective for the disinfection of viruses, bacteria, fungi, and mycobacterium. Surface sterilization explants of pepino through sodium hypochlorite (NaOCl) (70%) treatments was significantly increased with increasing of duration from 1.5 to 6.0 minutes. Maximum contamination percentage of explants (95.60) per cent was noted under the treatment of control; while the minimum (38.53) percent was noted under sodium hypochlorite (NaOCl) (70%) treating for a period of 4.5 minutes and maximum survival percentage of explants (61.46) per cent was noted under the treatment of sodium hypochlorite (NaOCl) (70%) for a period of 4.5 minutes and minimum survival percentage was recorded (3.81) under control. The observations are in close proximity to the findings of (Ines *et al.* 2013) [11], (Jan *et al.* 2013) [12], (Wolella 2017) [18].

Table 4: Percentage of explants contaminated and survived after 10 and 25 days with Sodium hypochlorite (NaOCl) (70%) treatment period for surface sterilization of pepino fruit

Treatment	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
Control	95.60	3.81
1.5 Min.	55.82	43.84
3.0 Min.	50.71	49.18
4.5 Min.	38.53	61.46
6.0 Min.	43.18	56.49
C.D.	5.03	4.76
SE (m)	1.57	1.49
SE (d)	2.23	2.11
C.V.	4.81	6.01

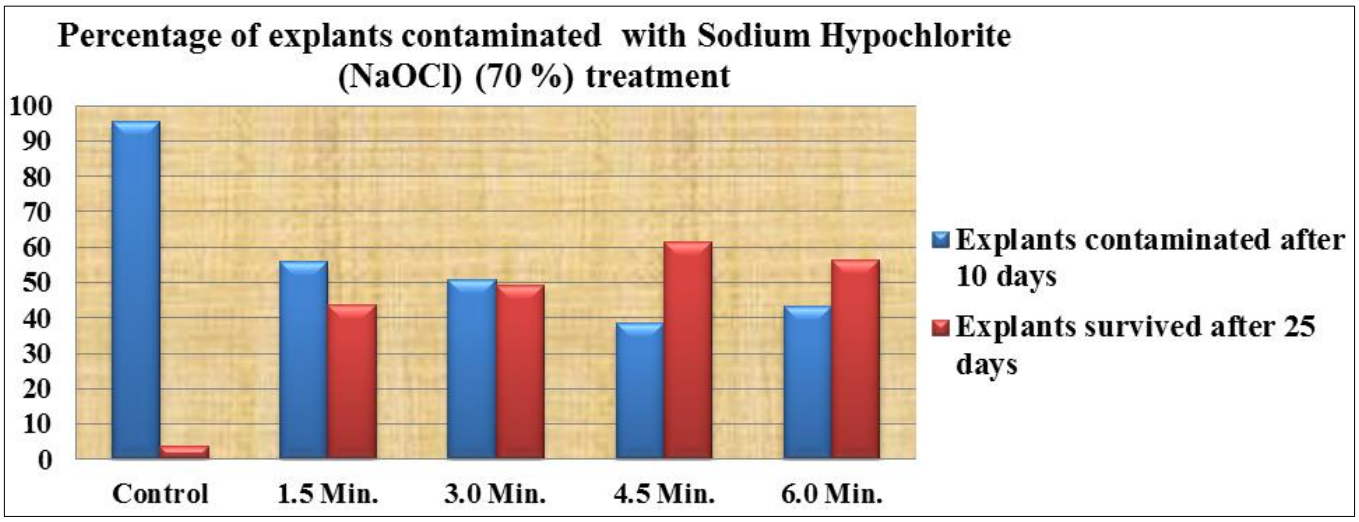

Fig 4: Percentage of explants contaminated and survived after 10 and 25 days with Sodium hypochlorite (NaOCl) (70%) treatment period for surface sterilization of pepino fruit

4.5. Percentage of explants contaminated and survived after 10 and 25 days with Mercuric chloride ($HgCl_2$ 0.1%) + Fungicide (Bavistin) (0.15%) percent for pepino fruit

Mercuric chloride is antimicrobial, with action against both fungi and bacteria, but frequently also kills the explants. At low concentrations (up to 0.1%) it is the most effective disinfective agent for explants with soil-borne and the epiphytic fungi and bacteria. So this was the reason to prefer use of mercuric Chloride ($HgCl_2$) as (0.1%) for surface sterilization of explants and bavistin is a broad spectrum systemic fungicide effective against a wide range of pathogenic fungi and is highly specific in its control of important plant pathogens during tissue culture.

Maximum contamination percentage of explants (91.89) per cent was recorded after under the treatment control; while the minimum (21.76) percent contamination was noted under mercuric chloride ($HgCl_2$ 0.1%) + fungicide (bavistin) treating for a period of 4.5 minutes. Maximum survival percentage of explants (77.36) per cent was noted under the treatment of mercuric chloride ($HgCl_2$ 0.1%) + fungicide (bavistin) for a period of 4.5 minutes; minimum survival percentage was recorded (7.34) under control. Similar trend also observed by (Guranna and Hoolageri 2017) [8], Kay Thi Oo *et al.* (2018), (Parveen *et al.* 2019) [14].

Table 5: Percentage of explants contaminated and survived after 10 and 25 days with Mercuric chloride ($HgCl_2$ 0.1%) + fungicide (Bavistin) (0.15%) percent for pepino fruit

Treatment	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
Control	91.89	7.34
1.5 Min.	38.38	60.74
3.0 Min.	36.97	62.15
4.5 Min.	21.76	77.36
6.0 Min.	26.86	72.22
C.D.	4.51	4.92
SE (m)	1.41	1.54
SE (d)	1.99	2.18
C.V.	5.66	4.77

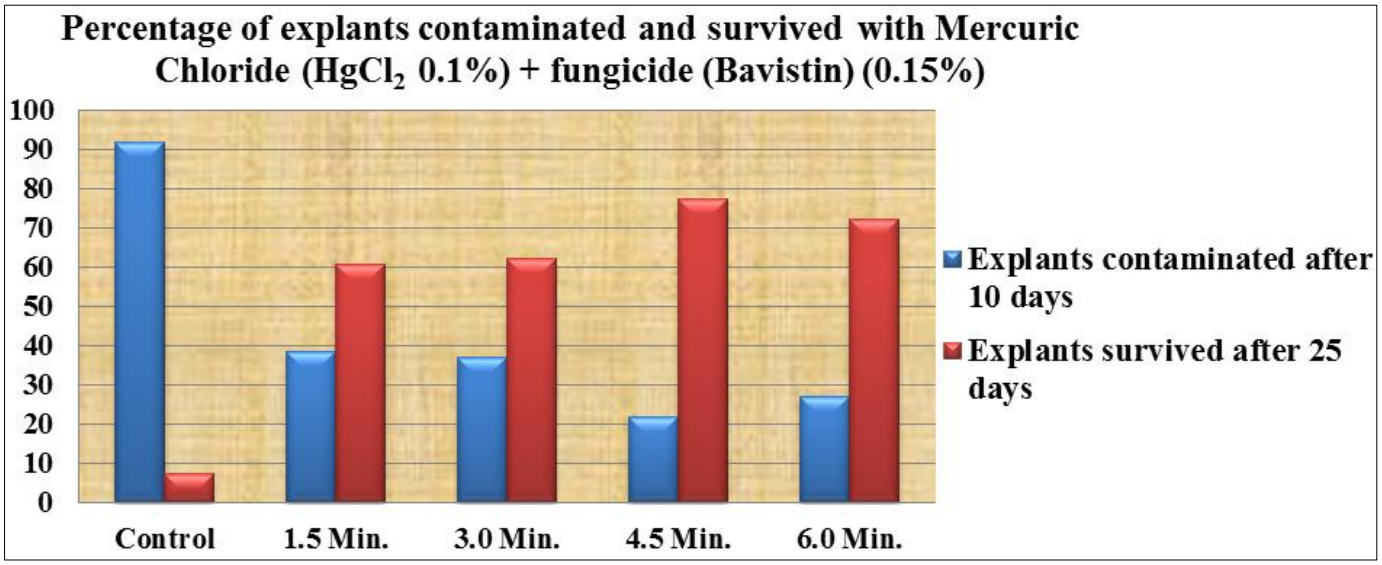

Fig 5: Percentage of explants contaminated and survived after 10 and 25 days with Mercuric chloride ($HgCl_2$ 0.1%) + fungicide (Bavistin) (0.15%) percent for pepino fruit

5. Conclusion

On the basis of the experimental findings, it can be concluded that the Mercuric Chloride ($HgCl_2$ 0.1%) + fungicide (Bavistin) (0.15%) was found best for minimum contamination at 10 days after and maximum survival percentage at 25 days interval with duration of 4.5 minutes, and can be used for surface sterilization of explants during *in-vitro* culture of pepino (*Solanum muricatum* Ait.) Cv. Valentia.

6. Acknowledgment

This work was carried out in collaboration among all authors. Author YP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of manuscript. Author SP managed the analyses of the study. Authors AK, V, PC and GS read and approved the final manuscript.

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