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Studies on the effect of different surface sterilization agents under *in vitro* culture of papaya (*Carica papaya* L.) variety Red Lady

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Abstract
Present investigation studied on the effect of different surface sterilization agents under *in vitro* culture of papaya (*Carica papaya* L.) variety Red Lady was carried out at the Tissue Culture Laboratory Department of Fruit Science, Sher-e Kashmir University of Agriculture Sciences and Technology, Jammu during the year 2020-2022. Effect of three different surface sterilization agents' i.e., mercuric chloride, Sodium hypochlorite and ethanol were tested on the contamination-free establishment of papaya (*Carica papaya* L.) variety Red Lady under *in vitro* conditions. All the sterilization agents performed better results when used individually for different time intervals. Before sterilization explants were treated with bavistin (0.2%) for 20 minutes. Maximum Survival (70%) and least death (10%) percentage of explant were observed in mercuric chloride (0.1%) for 2 minutes after 3 weeks of culturing. Critical observation was recorded as the duration increased contamination percentage decrease. On the other hand Mercuric chloride for 3 minutes showed less contamination percent (15%) as compared to the Mercuric chloride for 2 minutes (20%). Significantly decrease in contamination (35%) and increase in death ratio (55%) with 10 percent survival was observed in those explants which were treated with sodium hypochlorite (5%) for 15 minutes.

Keywords: Sterilization, *in vitro* red lady, papaya

Introduction
Papaya (*Carica papaya* L.) is short lived herbaceous plant belonging to Caricaceae family has originated from Tropical America and was introduced in India 16th century. Papaya is fourth most traded tropical fruit after Banana, Mango and Pineapple. It is cultivated for its delicious fruit having high nutritive value such as a good source of protein, fat, fiber, carbohydrates, minerals, calcium, phosphorus, iron, vitamin C, citric acid and malice acid (green fruits) and volatile compounds. Globally, its popularity increasing day by day, this can be judged from the phenomenal increase in the production of papaya during the recent years. Owing to its wide climatic and soil adaptation, papaya gave high returns and has tremendous potential in India. In India papaya growing states are Andhra Pradesh, Assam, Odisha, Maharashtra, West Bengal, Gujarat, Karnataka, Madhya Pradesh, Uttar Pradesh and Rajasthan. Papaya is propagated through seed shows variable performance. Conventional method of propagation like cutting or grafting has not been found successful in papaya. However, in order to meet the ever increasing demand of papaya plants, it is important to develop a different method which can help us to fulfill with the present demand. The propagation through seed is time consuming and season dependent. The cost of seed is very high and secondly, in papaya serious decline has been reported due to complex interaction of disease. The plants produced through seeds are not true to type, in this regard clonal propagation help to increase the economic way of producing new uniform true-to-parental type planting materials of known superior lines. And also produce disease free plants. In order to achieve all these objectives several workers have developed tissue culture protocols in papaya plantlets (Hossain *et al*., 1993 and Rohman *et al.*, 2007) [2, 7]. However, it is important to standardize the Micropropagation technique under local conditions because the performance of tissue culture plants dependent upon the number of factors which is intimately connected with the physiological state of the donor and the explants.

Material and Method

Present investigation was carried out at the Tissue Culture Laboratory Department of Fruit Science, Sher-e Kashmir University of Agriculture Sciences and Technology, Jammu during the year 2020-2022. For obtaining the explant material, the nodal segments were excised from the papaya plants by removing the leaves. The explant material was washed thoroughly in running tap water for 30 minutes and then treated with 10 per cent detergent solution (Teepol, BDH) for 10 minutes. All the traces of detergent were removed by washing in double glass distilled water. Further, sterilization procedures were carried out under aseptic conditions in a laminar-air flow cabinet. The explants were subjected to surface sterilization using mercuric chloride (0.1%), Sodium hypochlorite and ethanol. The explants were then rinsed several times with sterile distilled water and then culture on MS basal medium. For developing efficient surface sterilization procedure for micropropagation establishment of contamination free platelets of papaya a trial was conducted using different chemicals as given below

Table 1: For developing efficient surface sterilization procedure for micropropagation establishment of contamination free platelets of papaya a trial was conducted using different chemicals as given below

S. No	Sterilant	Concentration (%)	Duration
1	Mercuric chloride	0.1%	2 minutes
2	Mercuric chloride	0.1%	3 minutes
3	Mercuric chloride	0.1%	4 minutes
4	Sodium hypochlorite	5%	5 minutes
5.	Sodium hypochlorite	5%	10 minutes
6.	Sodium hypochlorite	5%	15 minutes
7.	Sodium hypochlorite + Ethanol	5% + 70	5 minutes + 30 seconds
8.	Sodium hypochlorite + Ethanol	5% + 70%	10 minutes + 30 seconds
9.	Mercuric chloride (0.1%) + Ethanol (70%)	0.1% + 70%	2 minutes + 30 seconds
10.	Mercuric chloride (0.1%) + Ethanol (70%)	0.1% + 70%	3 minutes + 30 seconds

Result

The results of surface sterilization of nodal segments explant of papaya cv. Red Lady are presented in table 1. It is clearly seen from the table that contamination, death of culture and culture establishment was remarkably influenced by surface sterilants. Before sterilization explants were treated with bavistin (0.2%) for 20 minutes. Out of various treatment mercuric chloride (0.1%) for 2 minutes gave maximum culture establishment (70%) and minimum death ratio (10%) followed by mercuric chloride (0.1%) for 3 minutes gave (65%) culture establishment and (20%) percent death ratio after 3 weeks of culturing. On the other hand as the duration increased contamination percentage decrease as shown in figure 1. Mercuric chloride for 3 minutes showed less contamination percent (15%) as compared to the Mercuric chloride for 2 minutes (20%). Considerable decrease in contamination (35%) and increase in death ratio (55%) with 10 percent survival was observed in those explants which were treated with sodium hypochlorite (5%) for 15 minutes followed by sodium hypochlorite (5%) for 10 minutes.

Table 2: Standardization of surface sterilization method of papaya explants var. Red Lady.
Medium: MS
Incubation: 3 weeks

S. No.	Sterilants	Duration	Contamination (%)	Death of culture (%)	Culture establishment (%)
1.	Mercuric chloride (0.1%)	2 minutes	20 (26.55)	10 (18.42)	70 (56.97)
2.	Mercuric chloride (0.1%)	3 minutes	15 (22.58)	20 (26.55)	65 (54.37)
3.	Mercuric chloride (0.1%)	4 minutes	10 (18.42)	25 (29.91)	65 (54.37)
4.	Sodium hypochlorite (5%)	5 minutes	40 (39.21)	15 (22.58)	45 (42.07)
5.	Sodium hypochlorite (5%)	10 minutes	35 (36.17)	50 (44.98)	15 (22.58)
6.	Sodium hypochlorite (5%)	15 minutes	35 (36.17)	55 (47.89)	10 (18.42)
7.	Sodium hypochlorite (5%) + Ethanol (70%)	5 minutes + 30 seconds	30 (33.19)	30 (33.19)	40 (41.80)
8.	Sodium hypochlorite (5%) + Ethanol (70%)	10 minutes + 30 seconds	25 (29.91)	40 (39.21)	35 (36.14)
9.	Mercuric chloride (0.1%) + Ethanol (70%)	2 minutes + 30 seconds	15 (22.58)	30 (33.19)	55 (48.22)
10.	Mercuric chloride (0.1%) + Ethanol (70%)	3 minutes + 30 seconds	10 (18.42)	40 (39.21)	50 (44.98)
	S.E(m) ±		1.79	1.56	6.11
	CD (0.05)		5.34	4.64	18.17

Figures in brackets are transformed value

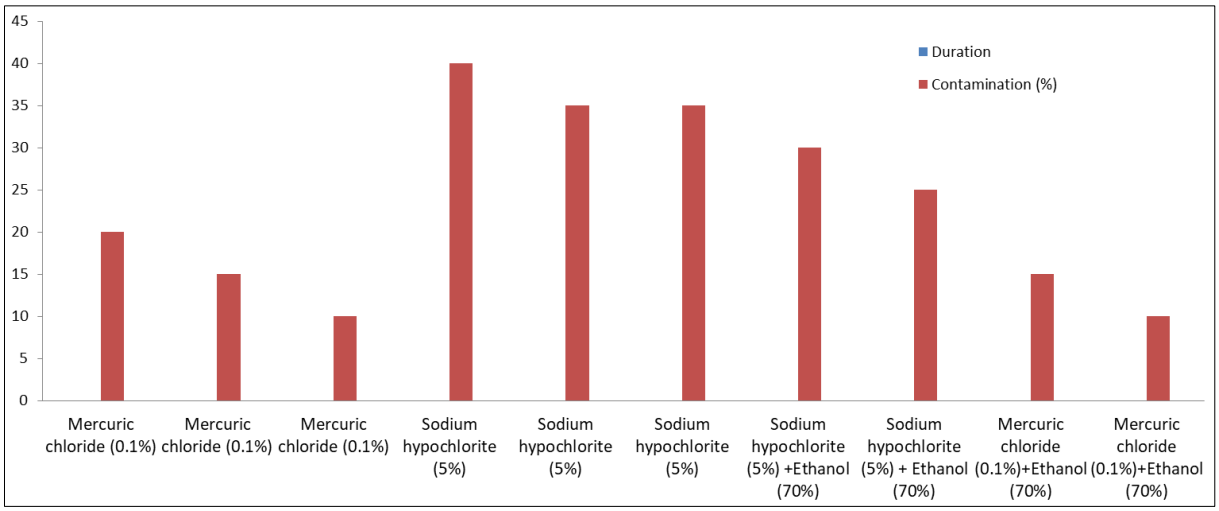

Fig 1: Effect of surface sterilization on contamination on explant of papaya var. Red Lady

Discussion

Explants were collected from the mother block of papaya. The field growing plants contained fungi and bacteria. So, it is necessary to sterilized explants before culturing in MS basal media. Before sterilization explants were treated with bavistin (0.2%) for 20 minutes. In this trial mercuric chloride, sodium hypochlorite and ethanol used as sterilants, which were more effective against bacteria and fungi. The results of this experiment revealed that explants which were treated with $HgCl_2$ (0.1%) for 2 minutes gave minimum death rate and maximum culture establishment. The results obtained under this study supported by Anandam *et al.*, 2011, Rayavalad *et al*. (2019) and Malik *et al*. (2020) [5] who worked on papaya. The explants which were treated with $HgCl_2$ (0.1%) for 3 minutes showed less contamination as compared to $HgCl_2$ (0.1%) for 2 minutes. This mainly depends upon the tissue type and nature of explant (Padhi and Singh 2017) [6]. Shen *et al*. (1990) [9], Kaur *et al*. (2005) [3] and Kumar *et al*. (2020) [4] found the same results while doing working on micropropagation of Chinese gooseberry, strawberry and papaya. Shukla *et al*. (2019) [10] observed that sterilization agent performed better results, when they were used singly for different time intervals. Mercuric chloride is highly antimicrobial and kills both fungi and bacteria. Sultan *et al*., 2006 reported that 5% Sodium hypochlorite does not perform better as sterilization even on increasing time and concentration in the medicinal plants. However mercuric chloride showed best result as compare to sodium hypochlorite because of have bleaching action of two chloride ions that combined strongly with proteins and causes tissue necrosis.

Conclusion

In this way concludes that singly Mercuric chloride (0.1%) for 2 minutes was found to be best to generate maximum contaminated free plants in papaya var. Red Lady.

References

1. Ananadan R, Thirugnanakumar D, Sudhakar D, Balasubramanian P. *In vitro* organogenesis and plantlets regeneration of (*Carica papaya* L.). Journal of Agricultural Technology. 2011;7(5):1339-1348.
2. Hossain M, Rahman SM, Islam R, Joarder OL. *In vitro* clonal propagation of papaya through culture of lateral buds from mature field grown plants. Plant tissue culture. 1993;3(1):47-50.
3. Kaur R, Gautam H, Sharma DR. A low cost strategy for micropropagation of strawberry (*Fragaria x ananassa* Duch.) Cv. Chandler. Acta Horticulturae. 2005;696:129-133.
4. Kumar M, Rajbhar YP, Kumar V, Yadav L, Kumar J, Singh VP, *et al*. Studies on the effect of different surface sterilization agents under *in-vitro* culture of Strawberry (*Fragaria* × *ananassa* Duch.) variety Chandler. Journal of Pharmacognosy and Phytochemistry. 2020;9(6):1833-1835
5. Malik N, Sengar RS, Yadav MK, Singh SK, Singh G, Kumar M. Effect of different plant growth regulators on *in vitro* callus induction in *Carica papaya* cv. Pusa Nanha. International Journal of Current Microbiology and Applied Sciences. 2020;8(7):1217-1225.
6. Padhi M, Singh SP. Surface sterilization for reducing microbial contamination in *in vitro* propagation of lasora (*Cordia myxa* Roxb.) using nodal segments. International Journal of Microbiology and Applied Sciences. 2017;6(8):836-842.
7. Rohman MM, Islam NM, Alam SM, Ahmad MR, Paul TK. Lateral bud culture of papaya (*Carica papaya* L.) for clonal propagation. Biotechnology. 2007;6:339-343.
8. Ryavalad S, Malabasari TA, Shantappa T, Uppar DS, Biradar BD, Mantur SM. Micropropagtion studies in papaya (*Carica Papaya* L.) cv. Surya. International Journal of Current Microbiology and Applied Sciences. 2019;8(7):2362-2367.
9. Shen XA, Wan JZ, Luo WY. Propagation *in vitro* of Chinese gooseberry (*Actinidia chinensis*) through the development of axillary buds. Scientia Horticulture. 1990;42:45-54
10. Shukla S, Singh A, Kumar A, Rajbhar Y, Kumar M, Singh SP. Effect of surface sterilization agents under in vitro culture of banana (*Musa paradisiaca* L.) variety Udhayam. Journal of Pharmacognosy and Phytochemistry. 2019;8(1):843-846.