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IDENTIFICATION OF SUITABLE SURFACE STRELIZATION METHOD FOR BAMBOO [*Dendrocalamus stocksii* (Munro.)]

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ABSTRACT

Surface sterilization is a vital step in preparation of healthy and viable explants in tissue culture because plants in the field are highly exposed to microbial contamination. Most surface contaminants, such as bacteria and fungi, can be eliminated by surface sterilization with a suitable sterilizing agent. In this study, six surface sterilizing agents were used:70% ethyl alcohol,Tween 20, Carbendezim, Streptomycin, mercuric chloride ($HgCl_2$) and sodium hypochlorite (NaOCl). Total 11 treatments were tried which consisted of different concentrations of sterilants, individually as well as in different combinations for different time durations for sterilization of *D. stocksii in vitro*. Observations on average bud break and average per cent aseptic cultivars were recorded in each treatment. The results showed that among the six sterilizing agents when used individually in this study, $HgCl_2$treatment at concentration of 0.2% for 5 minutes was most effective showing 39.235% average aseptic cultures but was less effective than treatments consisted combinations of sterilants. Among them T_9 [1%Tween 20 --> 0.2%HgCl -->70% Alcohol--> 1% Carbendezim --> 0.1% Streptomycin (5 --> 10 --> 5 --> 10 -->15) min.] and T_{11} [1% Tween 20 --> 0.1% Carbendezim --> 70% Alcohol '! 0.5% Mercurichloride --> 0.1% Streptomycin (5 --> 10 --> 0.5 --> 5 --> 5) Min.] showed maximum per cent aseptic cultivars of 89.715%, hence identified as the efficient surface sterilization method for bamboo [*Dendrocalamus stocksii* (Munro.)]

(Key words: *Dendrocalamus stocksii, in vitro*, sterilization, aseptic, sterilizing agents)

INTRODUCTION

Bamboo is a giant grass which is one of the most fascinating and versatile group of plants known to mankind. *Dendrocalamus stocksii* (Munro.) is a strong, arborescent and thorn less bamboo species. It is commonly known as Marihal, Manga, Mes, Chiva etc. (Viswanath *et al.*, 2012). It is an extremely manageable bamboo species with a great economical and ecological importance.However, large scale cultivation is hampered by non-availability of planting stocks. Propagation of bamboo by seeds is unreliable due to long and unpredictable flowering habit and also undesirable on account of large variation found in seedling propagation. Sterility of *D. stocksii* attributed to the less quantity of pollen produced, viability of pollen, percentage of anthesis, short receptivity of stigma etc.

Therefore, in order to supplement the conventional methods, an efficient *in vitro* propagation method would offer a desirable alternative for large scale multiplication of elite genotypes. Before going for micro propagation sterilization is most important step to get aseptic and healthy plants on large scale. So, the present study aimed to present an effective method for plant explants sterilization.

MATERIALS AND METHODS

Collection of explants

The present work was carried out with a species of Bamboo, *Dendrocalamus stocksii*. The bamboo explants were collected from AICRP on Agroforestry, College of Agriculture, Nagpur. The healthy explants (nodal shoot segments) were collected from mature one year old healthy culm, wiped with 70% alcohol and stored in ice box while transporting.

Processing and surface sterilization of explant

After collecting explants from plant source, the sheath enclosing the bud within was carefully removed. The explants were trimmed using stainless steel secateurs until the length of explant was about 3 to 4 cm. The explants were processed and surface sterilized by using tween 20, 70% ethanol, carbendezim, $HgCl_2$, sodium hypochlorite, streptomycin, etc. in different concentrations and for

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different time period (Table 1). Number of replicates for each treatment were 2 and each replicate consisted 5 explants. The data was collected at the end of 4th week.Cultures incubated under artificial conditions 25±2°C, 16 hrs of photoperiod and 8 hrs of dark period. Data was analysed by CRD and values were transformed by arc sin transformation as values were recorded in percentage.

RESULTS AND DISCUSSION

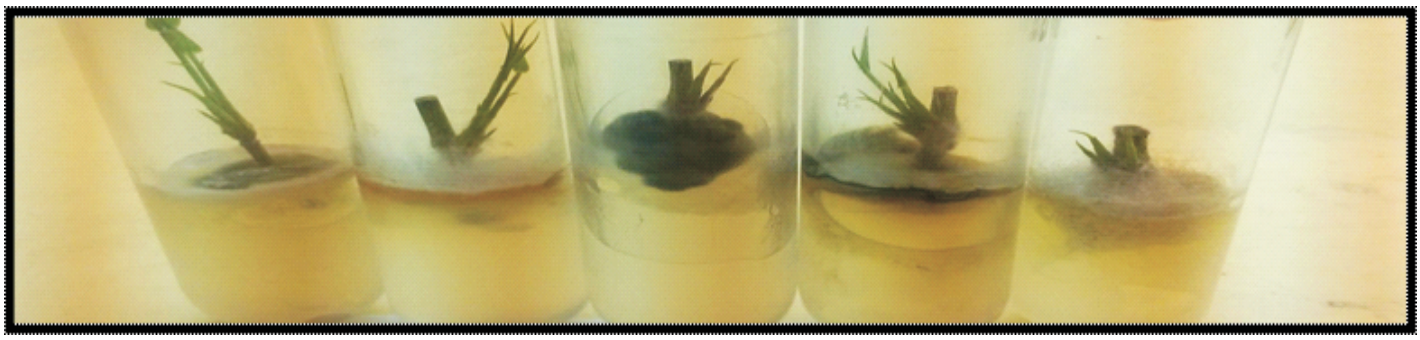
Surface sterilization

Different sterilizing agents in various concentrations for different time period were used for surface sterilization of nodal explants. The explants were surface sterilized with different 11 treatments (Table 1). It was observed from data that, on an average 2.136 days required for budbreak during sterilization in all treatments. The treatments T_1, T_2, T_3, T_4, T_5, T_8,T_9, T_{11} required 2 days on average while remaining T_6, T_7,T_{10} takes 2.5 days on average for bud breaking. There was no any significant difference observed in number of days to bud breaking. All sterilizing agents when used individually, it was observed that at high concentration of $HgCl_2$ negatively affect bud break.The aseptic cultures were ranged from 0.284 to 89.715 per cent. The treatments T_9 [1% Tween 20 for 5 minutes --> 0.2% $HgCl_2$ for 10 minutes --> 70% alcohol for 5 minutes --> 1% carbendezim for 10 minutes --> 0.1%streptomycin for 15 minutes] and T_{11} [1%Tween 20 for 5 minutes --> 0.1% carbendezim for 10 minutes --> 70%alcohol for 0.5 minutes --> 0.5% $HgCl_2$ for 5 minutes --> 0.1%streptomycin for 5 minutes] resulted in highest percentage of aseptic cultures. 0.2% $HgCl_2$ found most effective agent for sterilization. When all sterilizing agents were used individually for treatments showed less effect as compared to different combinations of sterilizing agents which was more effective. Similar findings were also reported by Sanjaya *et. al.* (2005) but concentration of $HgCl_2$was 0.075%.

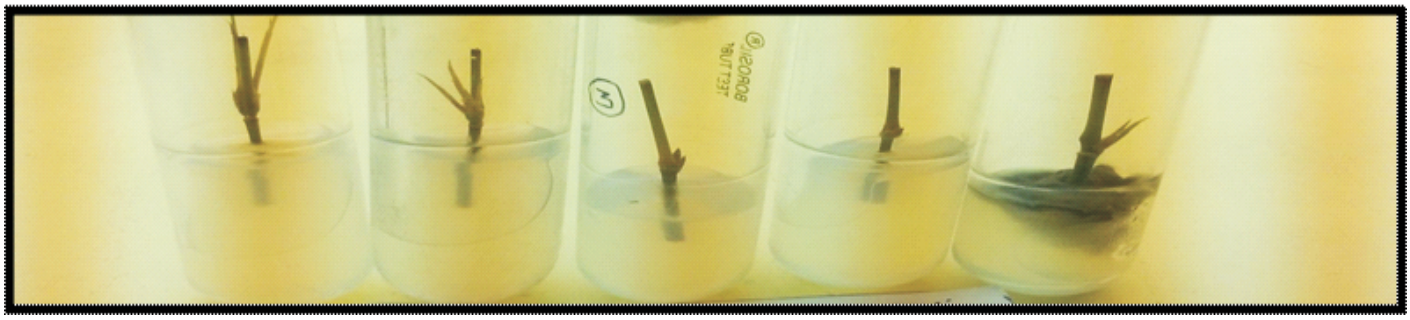
Kapruwan *et al.* (2014) reported that 0.2% $HgCl_2$ was effective.Wei *et al.* (2015) also reported that shorter exposure time to $HgCl_2$ (for 5 minutes) was effective with lower microbial infection and higher survival rate.Yeptomi*et al.*used treatment (Geepol for 5 min.+ Carbendezim for 25 min. + 70% ethanol for 15 min.) similar to T_{11} [1%Tween20 for 5 minutes ---> 0.1% carbendezim for 10 minutes ---> 70% alcohol for 0.5 minutes ---> 0.5% $HgCl_2$ for 5 minutes ---> 0.1% streptomycin for 5 minutes] for surface sterilization in ginger.

Table 1. Response of surface sterilization on percentage of aseptic cultures

Treatments	Treatment details	Average days to bud break	Average per cent of aseptic cultures
1.	Control (DDW washing) (20 min.)	2	0.284
2.	2% Tween 20 (15 min.)	2	0.284
3.	2% Carbendezim(5 min.)	2	0.284
4.	0.2% $HgCl_2$ (5 min.)	2	39.235
5.	70% Alcohol (5 min.)	2	0.284
6.	1% Tween 20 ---> 0.1% Carbendezim --->1% sodium hypochloride ---> 0.1% streptomycin (5 ---> 10 ---> 5 ---> 15 min.)	2.5	63.439
7.	1% Tween 20 ---> 1% Carbendezim ---> 1% Sodium hypochlorite ---> 0.1% Streptomycin (5 ---> 15 ---> 15 ---> 30) min.	2.5	47.882
8.	1% Tween 20 ---> 0.1% Carbendezim ---> 1% Sodium hypochlorite ---> 0.1% Streptomycin (5 ---> 20 ---> 20 ---> 45) min.	2	56.78
9.	1% Tween 20 ---> 0.2% $HgCl_2$ ---> 70% Alcohol --->1% Carbendezim ---> 0.1% Streptomycin (5 ---> 10 ---> 5 ---> 10---> 15) min.	2	89.715
10.	1% Tween 20 ---> 0.1% Carbendezim ---> 70% Alcohol --->1% Sodium hypochlorite ---> 0.1% Streptomycin (5 --->15 ---> 0.5 ---> 5 ---> 10) min.	2.5	45
11.	1% Tween 20 ---> 0.1% Carbendezim ---> 70% Alcohol ---> 0.5% Mercuric chloride ---> 0.1% Streptomycin (5 --->10 ---> 0.5 ---> 5 ---> 5) min.	2	89.715
	GM	**2.136**	**39.354**
	S E (m) ±	**-**	**0.87**
	CD	**-**	**2.708**
	CV	**-**	**3.129**

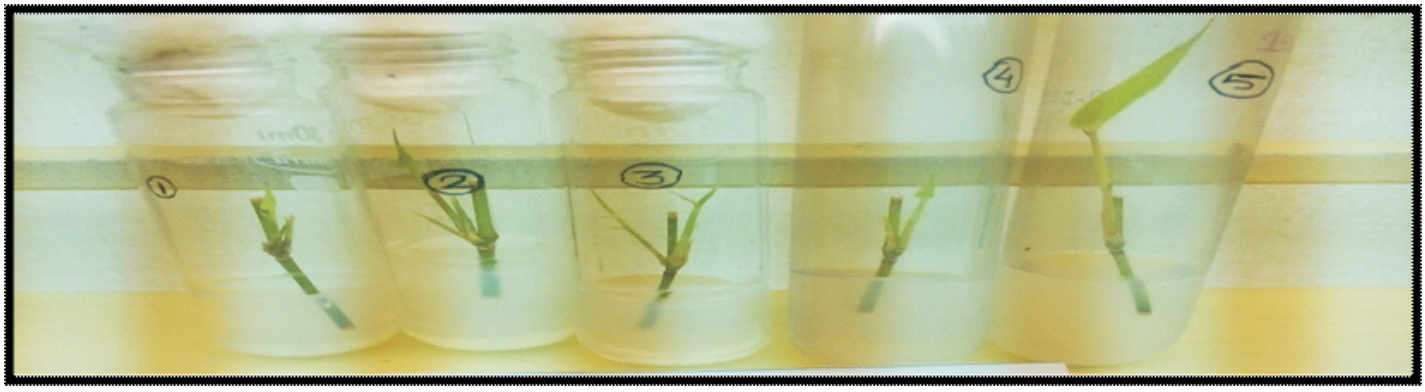


Contaminated cultivars



$HgCl_2$ Treated cultivars

T9 [1% Tween20 ---> 0.2% HgCl ---> 70% Alcohol ---> 1% Carbendezim ---> 0.1% Streptomycin (5 ---> 10 ---> 5 ---> 10 --->15)min.]



T_{11} [1% Tween 20 ---> 0.1% Carbendezim ---> 70% Alcohol ---> 0.5% Mercuric chloride ---> 0.1% Streptomycin (5 ---> 10 ---> 0.5 ---> 5 ---> 5)Min.]

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