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Evaluation of sterilant effect on *in vitro* culture establishment in banana genotype grand naine (*Musa* spp.)

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Abstract
Banana is one of the most crucial fruit crop and food source for the million of people in developing countries. Now a days, efficient technique of micropropagation has gained popularity because of the uniformity, superiority, disease free and high yielding. Mainly the *in vitro* micropropagation is affected by the growth of contaminants which can cause negative effect on the *in vitro* culture establishment that can be remove by the utilization of different effective sterilization agents to remove the damaging negative effect. Keeping in this view, this study was conducted to standardized the effect of Sterilization procedure for grand naine banana genotype. The healthy explants of banana (*Musa* spp.) were collected and treated with different combination of sterilization agents at different concentrations i.e., Bavistin TM (1%), $HgCl_2$ (0.01-0.5%), NaOCl (5%) and EtOH (70%). After the proper sterilization step, the treated explants were directly inoculated on semi-solid MS media supplemented with 5 mg/LBAP + 1 mg/L IAA. The findings we recorded under the current study showed the significant differences in their activity. The optimum % culture survival and contamination with reduced necrosis was found when the Banana explants were treated with the combination of 0.1% Bavistin for 30 seconds, followed by 0.1% $HgCl_2$ for 5min, 5% NaOCl for 5min and 70% EtOH for 30 sec. This sterilization protocol will be contributive in efficient surface sterilization for *in-vitro* propagation of Grand naine genotype of Banana. Results indicated that a treatment combination no. S16 [Bavistin (1.0%) + Mercuric chloride (0.1%) + Sodium hypochlorite (5%) + Ethanol (70%)] gave the highest percentage of aseptic culture establishment *in- vitro* condition.

Keywords: Banana micropropagation, contamination, toxicity, sterilants, sterilization, *in-vitro* development

Introduction
Banana (*Musa acuminata*, 2n=3x=33, AAB) is a perennial herbaceous monocot which belongs to Musa genus of the Musaceae family and is an important fruit crop in India. Basically, Banana has gained the status of commercial and cash crop. Conventionally banana producers, with the exception of a few large national and international companies, are solely responsible for most of the global production of banana. Banana is popularly growing in more than 100 countries in sub-tropical and tropical region. Which can provide staple food for the millions of people worldwide. Banana and plantains are grown in about 140 countries globally. Banana is considered as the second largest produced fruit after citrus, which may add about 16% of the world's total fruit production. India is the largest producing country of banana and may contribute to 27% of world's total banana production. In India production of mango has been surpassed by the production of banana. Tamil Nadu is the main leading producer state of banana within India, followed by Maharashtra. In global trade, Banana proves the fifth largest agricultural commodity after cereals, sugar, coffee and cocoa. India, China, Brazil and Ecuador individually produce half of total bananas of the world. The most favourable advantage of this fruit is its availability throughout the year. Globally Banana is grown on 5.69 million hectare with the annual production of approximately 106.83 million tons with an average yield of 18.78 tons/ha while the area and production of banana in India is 0.8 million hectare and 29.72 million tons with an average yield of 37.15 tons /ha. (Vinayagamoorthi *et al.* 2019). It is widely grown in large quantities in the states such as, Tamil Nadu, Maharashtra, Gujarat, Andhra Pradesh, Karnataka, Madhya Pradesh, Bihar, Uttar Pradesh, West Bengal and Assam. It is an important foreign-exchange gainer for India. Now a days, India has become one of the largest exporters of banana worldwide. Recently Indian banana are exported to

United Arab Emirates, Saudi Arabia, Iran, Kuwait, Bahrain, Qatar, Oman, Nepal, Maldives and United states (Kishor *et al*., 2013).

For the crop improvement programmes now a days, micropropagation technique has played a major role for the production of high quality and disease-free planting suckers (Rowe and Rosales, 1996) [23]. Banana plantlets produced through the technique of *in vitro* micropropagation have been proved most reliable, faster, healthier, disease free and stronger. In this technique Murashige and Skoog is the most widely used plant culture medium. MS medium i.e., Murashige and Skoog (1962) is a salt composition that can supplies the essential macro and micronutrients for the growth and development of banana explants. To get the optimum growth, development and differentiation of cells or tissues, concentrations of inorganic nutrients must be optimized such that the medium could meet the requirements of the cells or tissues used (Ngomuo *et al.* 2014) [20]. Success of banana plant tissue culture depends upon the concentration of nutrient media and ratio of various plant growth regulators. Besides media, optimum concentration of growth regulators namely auxin, cytokinin, gibberellins and abscisic acid like kinetin, indole-3-acetic acid, benzyl amino purine etc. were used for the *in vitro* growth and regeneration of tissue culture planets of banana.

Material and Method

Experimental protocol: The present study was conducted at the Plant Tissue culture laboratory, Department of Biotechnology, College of Agriculture, SVPUA & T, Meerut Modipuram. The healthy plant material (sword suckers) of banana cultivar (Grand naine) which belongs to the semi-dwarf Cavendish group were collected from the Horticultural Research Center, College of Horticulture, SVPUA & T, Meerut U.P.

Plant material: The healthy and disease-free explant which have shoot tips situated on the upper surface of sword suckers of banana plants covered by leaf sheath was ideal as source of explants. The isolated suckers were washed precisely under tap water to remove all the mud and other debris. Roots and other outer leaf sheath were carefully removed by the stainless-steel knife in such a way that shoot tip was not damaged. The selected explants were trimmed carefully into the following different sizes according to the length x breadth x height enclosing the shoot apex:

1. E1 = 1 x 1 x 1 cm^3.
2. E2 = E1 bisect vertically.
3. E3= 1 x 1 x 2 cm^3.
4. E4 = (E_3 Bisect vertically).
5. $E_{5=}$ (2 x 2 x 2 cm^3).
6. $E_{6=}$ (E_5 Bisect vertically).

Table 1: Evaluating the effect of different explant size on number of shoots, Average leaf number and shoot length grown in *in-vitro* cultured *Banana explants cv*. grand naine

Treatments	Number of shoots after (days)				Number of leaves after (days)				Shoot length (cm) after (days)			
	7	14	21	28	7	14	21	28	7	14	21	28
E1	1.00	1.77	3.00	3.55	0.00	1.30	2.80	3.00	1.50	3.14	4.16	5.23
E2	1.00	1.55	2.55	3.25	0.00	1.25	2.50	2.55	1.35	2.57	3.60	4.35
E3	1.00	1.25	2.50	2.55	0.00	1.20	1.85	2.45	1.30	2.25	3.45	3.80
E4	1.00	1.24	2.25	2.25	0.00	1.00	1.75	1.75	0.95	1.55	2.95	3.55
E5	1.00	1.00	2.00	2.00	0.00	1.00	1.50	2.50	0.85	1.35	2.55	3.35
E6	1.00	1.00	2.22	2.25	0.00	1.20	1.25	1.55	0.55	0.90	1.54	2.52
SE (m)	0.423				0.532				0.613			
SE (d)	0.598				0.752				0.867			
C.V.	45.95				78.74				49.60			
C.D.	N/A				N/A				N/A			

In this present study, the culture medium used was modified Murashige and Skoog (MS, 1962) basal medium for the shoot initiation and multiplication containing 30 g/l sucrose and 8 g/l of agar. The pH of the medium was optimized to 5.7 using NaOH or HCl (0.1 or 1N) prior to autoclaving. Appropriate PGRs were added to the medium before sterilization. Further the medium was autoclaved at 1.2 KPa and 121 ºC for 20 min, and then cooled at room temperature before use. Then the autoclaved media was poured into pre-sterilized culture tubes (150×25 mm) or 100 ml jam bottles as per the requirement. After 3 days media was observed if any contamination occuring.

Plant growth parameters: After certain time interval of sterilants treatment the healthy banana explants were sampled after reaching an optimum stage of shoot proliferation and regeneration. Explants of each replicate for each treatment were randomly selected for statistical growth analysis. Average number of shoots per explants, shoot length and total number of leaves per shoot were measured after 7, 14, 21 and 28 days of inoculation to investigate the effect of explants size. Banana explants were examined for evaluating the survival percentage and percent toxicity cause by the utilization of different concentrations and durations of sterilants such as Bavistin, Mercuric chloride, Sodium hypochloride and Ethanol.

Explant sterilization and sterilants: In the present investigation, two to three months old sword suckers were excised from healthy disease-free mother plant. These suckers were thoroughly washed under running tap water. Appropriate shoot tips were prepared by trimming roots and outer area leaf sheaths from the suckers. The explants were kept immersed in tap water for 5min, then they were pre-treated with 5 ml Tween 20 for 10min and a combination of 5 ml Dettol and 45 ml Savlon for 30min with constant swirling and subsequently draining off the disinfectants by washing them with running tap water 2-3 times to remove all the traces of the disinfectants. Further, the explants were then washed under running tap water for 60min.

Standardization of surface sterilization methods was carried out by treating the explants with various combinations and concentrations of different chemical sterilants viz. Bavistin, Mercuric chloride, Sodium hypochlorite and Ethanol to

optimize the minimal contamination, explant survival percentage and culture establishment as represented in (Table 2). The explants were washed three times with the autoclaved deionized water after each sterilizing treatment step. The explants were prepared carefully by removing the extended outer leaf sheaths and precisely excised without disturbing upper meristematic shoot bud using scalpel blade in 1.5-2.0 cm.

Establishment, multiplication and subculturing of *in vitro* banana explant: Optimized surface sterilized Banana explants with a meristem was directly inoculated into the jam bottle containing 20 ml of MS medium supplemented with 5 mg/L BAP and 1 mg/L IAA for multiplication and subsequent regeneration of *in vitro* developed shoots and further incubated in culture room under white fluorescent light with intensity of 4000 lux, 16 h of light and 8 h of dark photoperiod, 50-60% RH and 25±2 ºC temperature. The cultures were monitored on regular interval for growth and contamination for four weeks and data was collected. Explants were sub-cultured into fresh medium to control blackening and the browning within tissues on the explants. It was repeated for 10 days interval for about one month to minimize further blackening of the tissues.

Rooting and hardening: Healthy and appropriate shoots when grown about 3 to 5 cm in length with 3 to 6 well developed leaves, they were rescued aseptically and cultured on freshly prepared MS medium supplemented with different combinations of plant hormones specific for root induction. (IAA and IBA).

Observation recorded

Observation of data including Contamination percentage, Number of shoots per explants and average shoot length, percentage toxicity.

Statistical analysis

The data was analyzed using OPSTAT, one-way ANOVA and SPSS (computer software package version 20.0) to test mean differences among all treatments followed by Tukey's-b multiple range test and represented as mean ± SE. The value $p \leq 0.05$ was considered statistically significant in each case.

Results and Discussion

Effect of explant size on multiplication: The effect of explant size on the regeneration of the plant was established by culturing different sizes of Banana explants in a proliferation medium. The results reveals that the size of the initial explants affects the rate of proliferation. Cube like cream colour structure of explants of Grand naine Banana genotype of which 3 to 4 leaf sheaths with centrally placed shoot tip inside the explants of six sizes viz., E_1 (1 x 1 x 1 cm^3), E_2 (E_1 Bisect vertically), E_3 (1 x 1 x 2 cm^3), E_4 (E_3 Bisect vertically), E_5 (2 x 2 x 2 cm^3) and E_6 (E_5 Bisect vertically) were used to find out the suitable explant size for plant regeneration. Table 1, represents the data for number of shoot, average leaf number and shoot length after 7, 14, 21 and 28 days. The results of the size of explant effect showed no significant differences in all treatments after 7 days for number of shoot formation, total number of leaves and average shoot length. The data represents in the Table 1 showed that the Treatment E1 produced maximum number of shoots (3.55), followed by E2 in which the number of shoot was (3.25). Treatment E5 showed minimum number of shoots which is (2.0). Maximum number of leaves (3.00) and the shoot length was (5.23) was also found at E1 followed by E2 in which number of leaves obtained was (2.55) and the shoot length was (4.35). Treatment E6 produced minimum number of leaves (1.55) and shoot length (2.52). No significant differences were found in all the treatments after 7 days for number of leaves formation as well. It was observed that treatment E1 was most suitable explant size for plant regeneration.

Culture establishment: Culture establishment within banana explants is primarily dependent on sterilization protocol. The Banana explants isolated from field contains several different types of microbes or contaminants on their outer surface because plant faces several type of situation in field condition. The culture medium used in the tissue culture studies is most suitable for appropriate healthy growth, surface sterilization of collected explants is necessary. In present study, different sterilants with various concentrations with different combination had been utilized for higher culture survival and establishment (Table 2).

Preparation and surface sterilization of explant: Plant materials grown sword suckers collected from fields involves a wide range of contaminants. For *in vitro* regeneration of banana explants different concentration of disinfectant such as Bavistin, $HgCl_2$, sodium hypochlorite (NaOCl) and ethanol have been proven satisfactory in making aseptic plant tissue.

Establishment of suitable concentration and duration of sterilant for banana tissue culture: In this study, the explant of Grand naine banana genotype were tested with different combinations of sterilants to optimize the best concentration and combination of sterilant and the study showed the optimized result with minimum contamination was achieved when the explants are treated with the combination of 0.1% $HgCl_2$ for 5 min followed by NaOCl 5%, solution for 5 min and 70% Ethanol for 30 sec. By gradual increase in concentration and duration of both chemicals has obtained the remarkable decrease in contamination of the explants. Results represented in Table 2, for the effect of various sterilization combination on banana explant. For the *in vitro* micro propagation technique of Banana plantlets, the surface sterilization is very necessary aspect. Explants are generally collected from field grown plants, so that the plant material is highly contaminated by micro-organism. (Mendes *et al*., 1999 and Muhammad *et al*., 2004) [17] reported that Sodium hypochlorite is the most potent and commonly used disinfectant for surface sterilization of banana explants. (Banerji and Sharma, 1988 and Habiba *et al*., 2002) [5, 13] have replaced sodium hypochlorite with low concentration of mercuric chloride. (Van den Houwe, 1998; Nandwan *et al*., 2000) [19] stated that sometimes explants have to be treated with fungicides and antibiotics to minimize the percentage of contamination in *in vitro* cultures. (Silva *et al*., 1998; Rahman *et al*., 2004 and Jalil *et al*., 2003) [24, 22, 14] used Ethanol for disinfection purposes. (Muhammad *et al*., 2004) [17] had sterilized the explants for 15 minutes with 50% commercial bleach (Clorox 5.75% NaOCl) to which few drops of Tween-20 were added.

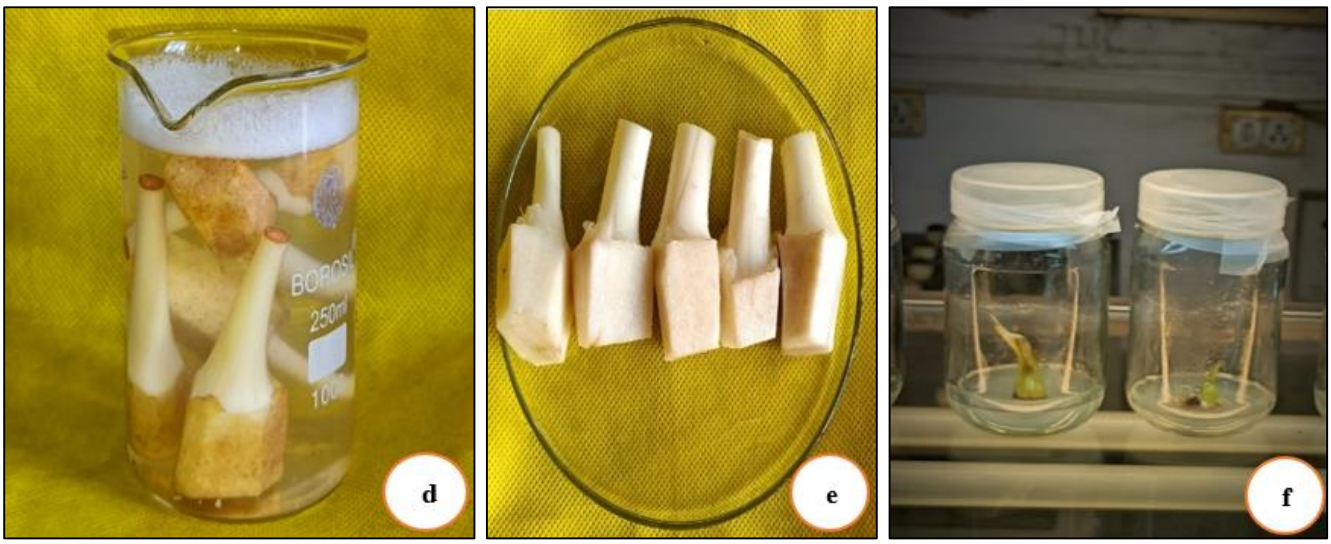
Table 2: Effect of different Surface Sterilants/disinfectants on culture survival percentage, percentage toxicity, phenolic browning in banana genotype Grand naine after 4 weeks of explant inoculation on culture media

Sterilants	Concentration (%)	Duration	Culture survival percentage	Percent toxicity in culture	Shoot induction and growth pattern
S1 [Ethanol]	70	30 sec	30.0±0.00a	58.0±0.00a	Medium, non-uniform
S2 [Ethanol]	70	1min	50.0±0.00b	60.0±0.00b	Medium, uniform, contaminated
S3 [Mercuric chloride]	0.1	5min	72.5±0.57cd	32.8±1.12a	No shoot induction
S4 [Mercuric chloride]	0.1	6min	65.2±1.15d	38.5±0.57b	Medium, uniform, contaminated
S5 [Mercuric chloride]	0.1	8min	36.5±0.33c	44.3±1.00ab	Medium, non-uniform
S6 [Mercuric chloride]	0.2	5min	32.0±1.12ab	66.3±1.14cd	Healthy, uniform
S7 [Mercuric chloride]	0.2	6min	22.2±0.22b	62.9±0.53d	Healthy, uniform
S8 [Mercuric chloride]	0.2	8min	18.6±0.52a	56.8±0.11c	Medium, uniform, contaminated
S9 [Bavistin]	1.0	30min	35.2±0.52a	45±1.14b	Healthy, uniform
S10 [Bavistin]	1.0	60min	40.2±1.14b	28.2±0.15a	Medium, uniform, contaminated
S11 [Mercuric chloride (0.1%) + Ethanol (70%)]	0.1+70	5min+30sec	38.3±0.52c	40.2±0.55ab	Healthy, uniform
S12 [Mercuric chloride (0.1%) + Ethanol (70%)]	0.1+70	6min+30sec	25.2±0.32a	74.6±0.57b	Medium, uniform, contaminated
S13 [Mercuric chloride (0.1%) + Ethanol (70%)]	0.1+70	8min+30sec	28.3±0.14b	76.2±0.22a	Medium, non-uniform
S14 [Sodium Hypochlorite (5%)]	5	5min	64.2±1.52b	46.2±0.54a	Healthy, uniform
S15 [Sodium hypochlorite (5%)]	5	10min	45.4±1.52a	46.5±0.57b	Medium, non-uniform
S16 [Bavistin (1.0%) + Mercuric chloride (0.1%) + Sodium hypochlorite (5%) + Ethanol (70%)	1.0+0.1+5+70	30min+5min+ 5 min+30sec	90.4±0.57c	14.6±0.52a	Healthy, uniform, vigorous shoots

Data represents mean ± SE of three replicates per treatment in three repeated experiments. Means within the same column followed the different letters are significantly different according to DMRT at 5% level.

Different steps of banana micropropagation *cv.* Grand naine

Explant selection **Washing of explant** **Explant Preparation**



Sterilization **Ideal size of explant** **Aseptic inoculation**

Fig 1: Shoot establishment and multiplication

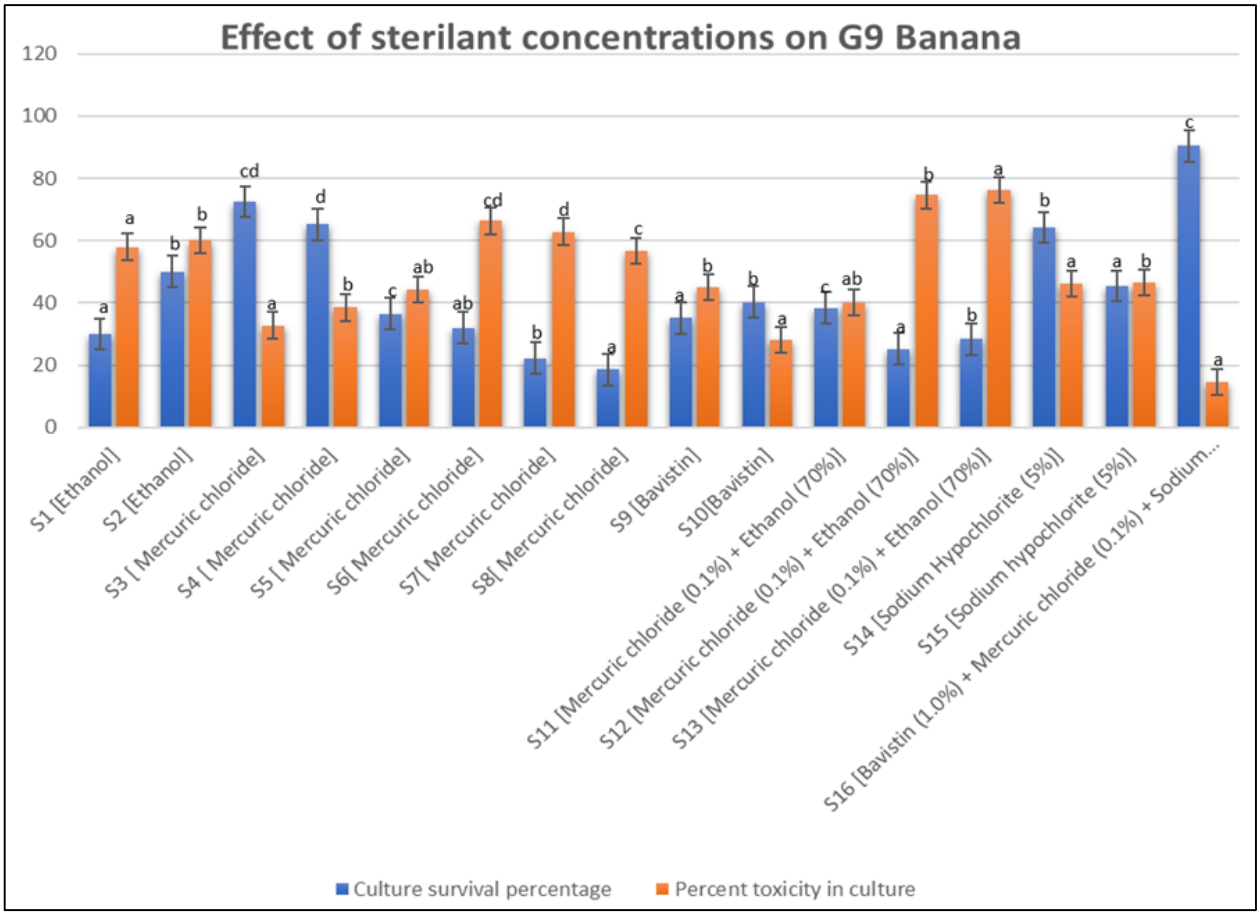


Fig 2: The effect of different concentrations of sterilizing agents on surface sterilization of explants after four weeks of incubation of cultures of Banana *cv.* Grand naine

This study focuses on the standardization of best sterilant combination and concentration and the time duration of exposure was the major factors so that the explants must be alive and free from any microbes and contaminating particles. The efficiency and efficacy of disinfection is the most important step for any micropropagation technique. Initial treatments choices the banana suckers were sterilized with Ethanol and two step sterilization procedure were also investigated. Ethanol is most potent chemical which is highly used as the strong phytotoxic sterilizing agent. Several researchers reported that alcohols have the higher bactericidal activity as compared to other bacteriostatic agents against

bacterial vegetative cells but do not destroy spore cells (Bloomfield, 1978) [7]. In this study, various concentrations of ethanol had been used to investigate its effect on explant sterilization and their survival that recorded to be ranged from 33 to 50% (Table 2). The explants treated with sterilant (S2) 70% ethanol for 1min showed a significant growth with higher culture survival percentage at 50%. (p<0.05) with healthy and uniform shoot growth as compared to other Ethanol treatments but shoot induction was slower.

The Banana explants when treated with various concentrations of $HgCl_2$ had recorded culture survival percent within a range of 18.6 to 72.5% and found to be statistically different (p < 0.05) as shown in Table 2. The explants treated with (S3), 0.1% $HgCl_2$ for 5 min showed a significant (p < 0.05) increase in culture survival percent upto (72.5%) with healthy and uniform shoot growth in comparison to other treatments. The treatment of 0.1% Mercury chloride for 5 min showed minimum contamination and highest culture establishment as stated by (Onuoha *et al.* 2011) [21]. Increase in concentration of HgCl 2% resulted in deleterious effect on explants survival, explants turned into brownish black and failed to develop and proliferate during incubation period as a result of tissue necrosis. It may me the results of bleaching action of two chloride atoms and ions that combinedly with proteins and cause necrosis to plant tissues. The results of this study are in agreement with earlier researcher reports of (Johnson *et al*., 2011 and Wesely *et al*., 2011) [15].

(Yadav *et al.* 2017) [26] investigated the aseptic inoculation and culture establishment of banana *cv*. Grand naine with three different surface sterilization agents i.e., mercuric chloride, bavistin and ethanol. They examined minimum contamination with higher survival percentages at 0.1% $HgCl_2$ at different time intervals in banana *cv*. Grand Naine. In the present study 0.1% $HgCl_2$ in combination with 70% ethanol (S11) gave significant results during sterilization of Banana explants i.e., 38.3±0.52 culture survival percentage.

Sodium hypochlorite (NaOCl) is a compound which is most widely used for the water purification as disinfectants also used in various concentrations to show the effect on explant sterilization. The culture survival for banana explant was found to be ranged from 45.4 to 64.2% under studied concentrations of NaOCl. The explants sterilized with NaOCl at the concentration 5% for 5 min (S14) showed better survival with uniform growth.

The present study reveals that among the different treatments of sterilizing agent tried and their efficacy were tested out of which the best sterilants proved for the surface sterilization of the explant of banana genotype Grand naine were (S16). A significantly (p<0.05) higher culture survival percent (90%) was achieved having vigorous shoot growth with minimal phytotoxicity when treated with combination of 0.1% Bavistin for 30 min followed by 0.1% $HgCl_2$ for 5 min followed by 5% NaOCl for 5 min and 70% EtOH for 30 seconds with subsequent three washing with de-ionized water after each treatment. While, none of the sterilant alone for different time exposure did not showed highest aseptic establishment within banana explant cultures (Goswami and Handique, 2013) [12]. Similar observation was recorded by (Namrata K and Pragati Mishra 2016) [18]. However, treatment with single sterilant resulted in good shoot proliferation but subsequently failed to control microbial contamination. In our study, on the basis of observation made and results obtained it can be concluded that the highest culture survival with minimum toxicity was achieved when the explants are treated with the Combination of sterilizing agents viz., 0.1% $HgCl_2$ for 5 min followed by 5% NaOCl for 5 min and 70% EtOH for 30 seconds *in vitro* micropropagation of banana (*Musa* spp.).

Conclusion

In- vitro propagation technique using shoot tip cultures is a necessary approach in dealing with the problems encountered in conventional method of propagation. This technique will ensure sustainable production of banana planting materials and can provide the healthy fruits. Most of the organised cultures, especially the shoot tips maintain the strict genotypic and phenotypic stability under tissue culture technique (Bennici, 2004) [6]. A large number of uniform, healthy and disease-free plants can be produced by this technique from a single plantlet or even a small plant tissue (explants) showing good genetic potential (Timmins *et al*., 2006). Taking all these factors in to account, this experiment was accomplish to develop a standardized protocol to produce disease free and genuine quality planting material required to satisfy the requirement of the consumer. In this study, we were tried to check the effect of different sterilant treatment on Grand naine banana genotype, it is evident from the Table 2 that the maximum culture establishment 90% with minimal toxicity was found when the explant were treated with the combination of sterilants [Bavistin (1.0%) + Mercuric chloride (0.1%) + Sodium hypochlorite (5%) + Ethanol (70%)] gave the highest percentage of aseptic culture establishment *in vitro* condition.

References

1. Abdullah K, Khan IA, Siddiqui SH, Ahmed M, Siddiqui KA. *In vitro* culture of indigenous and exotic banana clones for maximum multiplication. Pak. J Bot. 1997;29:143-150.
2. Ahmed S, Sharma A, Singh AK, Wali VK, Kumari P. *In vitro* multiplication of banana (*Musa* sp.) *cv*. Grand Naine. Afr. J Biotechnology. 2014;13:2696-2703.
3. Alango K, Sunil T, Hajare, Akilu B. Studies on *in vitro* Micropropagation in Banana Int. J Curr. Microbiol. App. Sci. 2018;7(7):3366-3375.
4. Amin MR, Rahman S, Mamun ANM. *In vitro* micro propagation of banana (*Musa* spp.). Bangladesh J Agril. Res. 2009;34:645-659.
5. Banerji KK. Kinetics of Oxidation of Some α-Hydroxy Acids by Sodium N-Bromobenzenesulfonamide, 1988.
6. Bennici A, Anzidei M, Vendramin GG. Genetic stability and uniformity of Foeniculum vulgare Mill. regenerated plants through organogenesis and somatic embryogenesis. Plant science. 2004;166(1):221-227.
7. Bloomfield. The antibacterial properties of sodium dichloroisocyanurate and sodium hypochlorite formulations, 1978.
8. Chidananda S, Sashikala B, Sandeep R, Rajalaxmi B. High Frequency *in vitro* Cloning of Banana (*Musa acuminata*) *cv*. Grande Naine. International Journal of Agriculture, Environment and Biotechnology, 2015.
9. Colgecen H, Koca U, Toker G. Influence of different sterilization methods on callus initiation and production of pigmented callus in *Arnebia densiflora* L. Tur. J Biol. 2011;35:513-520.
10. Gajendra K, Singh Y, Mishra1 SP, Hemant K. Potential Use of Banana and Its By-products: A Review

International Journal of Current Microbiology and Applied Sciences ISSN. 2018;7:2319-7706.
11. Goswami NK, Handique PJ. *In vitro* Sterilization Protocol for Micropropagation of Musa (AAA group) 'Amritsagar Musa (AAB group) 'Malbhog' and Musa (AAB group) Chenichampa Banana, 2011.
12. Goswami NK, Handique PJ. *In vitro* sterilization protocol for micropropagation of Musa (AAA group) 'Amritsagar' Musa (AAB group) 'Malbhog' and Musa (AAB group) 'Chenichampa' Banana. Indian Journal of Applied Research. 2013;3(6):51-54.
13. Habiba U, Reza S, Saha ML, Khan MR, Hadiuzzaman S. Endogenous bacterial contamination during *in vitro* culture of table banana: Identification and prevention. Plant Tissue Cult. 2002;12(2):117-124.
14. Jalil M, Khalid N, Othman RY. Plant regeneration from embryo genic suspension cultures of acuminata *cv*. PISANG MAS. Plant Cell, Tissue & Organ Culture. 2003;75:209-214.
15. Johnson M, Wesely EG, Kavitha MS, Uma V. Antibacterial activity of leaves and intermodal callus extracts of *Mentha arvensis* L. Asian Pacific J Trop. Med. 2011;4:196-200.
16. Kumar R, Mishra P. Highly Efficient Micropropagation of Grand Naine Banana IJSRD-International Journal for Scientific Research and Development. 2016;3:2321-0613.
17. Muhammad A, Hussain I, Naqvi SMS, Rashid H. Banana plantlet production through tissue culture. Pak. J Bot. 2004;36:617-620.
18. Namrata Kumari N, Misra P. Mass *in vitro* micro propagation of banana (*Musa* sp.) Department of Molecular and Cellular Engineering, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, 2016.
19. Nandwani D, Zehr U, Zehr BE, Barwale RB. Mass propagation and *Ex vitro* survival of banana *cv*. 'Basrai' through tissue culture. Gartenbauwissenschaft. 2000;65(6):237-240.
20. Ngomuo M, Mneney E, Ndakidemi PA. The *in vitro* propagation techniques for producing banana using shoot tip cultures. American Journal of Plant Sciences, 2014.
21. Onuoha C, Chinonye J, Chibuikem IN. *In vitro* Prevention of Browning in Plantain Culture. Department of Plant Science and Biotechnology, Journal of Biological Sciences. 2011;11(1):13-17.
22. Rahman MZ, Nasiruddin KM, Al-Amin M, Islam MN. *In vitro* response and shoot multiplication of banana with BAP and NAA. Asian J Plant Sci. 2004;3:406-409.
23. Rowe P, Rosales F. A Triploid Parental Line for breeding disease-resistant cooking Bananas for East Africa. International Symposium on Banana: I International Conference on Banana and Plantain for Africa. 1996;540:169-175.
24. Silva AD, Matssumoto K, Bakry F, Souza RBB. Plant regeneration from long term callus culture of AAA-group dessert banana. Pesq. Agropec. Brasilia. 1998;33:1291-1296.
25. Timmins NE, Scherberich A, Früh JA, Heberer M, Martin I, Jakob M. Three-dimensional cell culture and tissue engineering in a T-CUP (tissue culture under perfusion). Tissue engineering. 2007;13(8):2021-2028.
26. Yadav AK, Prasad Y, Prakash S, Chand P, Singh BSG, Singh G. Effects of surface sterilization agents on *in vitro* plant growth in banana cultivar "Grand Naine". Int. J Chem. Stud. 2017;5(4):1744-1747.
27. Van H, Nandwani *et al*., *In vitro* Sterilization Protocol for Micropropagation of Musa (AAA group) 'Amritsagar' Musa (AAB group) 'Malbhog' and Musa (AAB group) 'Chenichampa' Banana, 2000.