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Optimizing Sterilization Condition for the Initiation of Sweet Potato (*Ipomoea batatas*) Culture *in vitro*

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ABSTRACT

This study is aimed at investigating the most efficient and cost effective sterilization condition for initiation of sweet potato culture *in-vitro*. Different concentrations of common or local household bleach (Power zone and Dettol) and non-readily available mercuric chloride as well as variation in their duration for sterilization were used to sterilize sweet potato buds to study their effectiveness in preventing microbial growth in sweet potato culture. The most common microbial contaminants observed in the sweet potato culture were fungi (*Fusarium* sp., *Aspergillus nger*, *Penicillium* sp. and *Neurospora crassa*). Mericuric chloride in combination with other treatment was found to be the most effective in suppressing microbial contaminants. Dettol in combination with other treatments was found to be ineffective in suppressing the microbial contamination. Duration of 20 min treatment was found to be the most effective in suppressing the microbial contamination but was very toxic to the explants. Treating explants for 10 min (control) was found to be ineffective in suppressing the microbial contamination but non-toxic to explants. Subjecting the sweet potato explants to sterilization for 12 and 15 min were found to be the most efficient, since there was no significant difference between them. These findings will help alleviate much of the burden associated with initiation of sweet potato cultures thereby reducing cost of plantlets production.

Key words: Detergent, explant, initiation, sweet potato, sterilant, tissue culture

INTRODUCTION

Sweet potato *Ipomea batatas* (L) Lam, is considered the seventh most important food crop in the world and is ranked fourth in developing countries (FAO, 1997). It is cultivated in more than 100 countries Horton (1987) as a valuable source of human food, animal feed and industrial raw materials (Jarret and Florkowski, 1990).

In spite of its enormous opportunities in alleviating food insecurity, sweet potato production per hectare has been on the decline in the sub-Saharan Africa (Asare, 2008). The little growth noted in sweet potato production is mainly due to increase in hectarage under the crop, rather than increase in productivity. This decline can be attributed to a number of factors such as major diseases affecting sweet potatoes (Pheneas and James, 2007). This situation is exacerbated by lack of healthy planting materials. Farmers often use cuttings sourced from old plants in propagation and this contributes to the spread of diseases.

Sweet potato has been generally cultivated by storage roots, seedlings or vine cuttings (Saiful Islam *et al*., 2002). The traditional growing of seed potatoes by planting seed roots has the disadvantage of losing yielding capacity. Cuttings sourced from old plants in propagation are often a channel for transmission of systemic infections from one generation to the other, leading to poor yields in successive seasons (Roca and Mroginski, 1991). This is also a major limitation in germplasm maintenance and exchange of materials across borders.

The multiplication rate of cuttings is also very low compared to grain crops which are propagated by true seeds. *In vitro* propagation of sweet potato helps surmount these challenges by producing disease-free planting materials in large numbers. Tissue culture techniques also provide a great opportunity to produce healthy seedlings (Ajithkumar and Seeni, 1998). This technology is faster and requires less space than that required for conventional methods of producing seedlings (Lapichino *et al*., 1991). Micro propagation is suitable for rapid and large-scale clonal multiplication of elite germplasm (De Almeida *et al*., 2007; Escalona *et al*., 1999).

Tissue culture techniques are a requisite in the quest to improve sweet potato productivity in sub-Saharan Africa (Mumtaz and Qureshi, 1989). Adoption of the technology in seedling production will improve production levels along with quality improvement. However, to optimize its application in sweet potato propagation, there is a need to identify and implement strategies that will contribute towards reducing the cost of plantlet production. This will enable resource-challenged farmers to access healthy planting materials at an affordable cost which will in turn increase sweet potato production (Savangikar, 2002).

Plant tissue culture carried out under aseptic conditions has important applications in plant biotechnology. However, microbes are common cause of contamination in tissue culture. Sterilization is one of the reliable means to control the pathogenic effect of microbes. The cost of producing plantlet through this technique can be minimized by optimizing sterilization conditions by increasing the rate of growth, thus reducing the rate of mortality (De Almeida *et al*., 2007).

Contamination by bacteria and fungi make it difficult to establish axenic culture (Cassells and Doyle, 2005). Since the use of antibiotics and fungicides are not encouraged, development of any effective sterilization protocol which would effectively eliminate fungi and bacteria without damaging the explant is a basic pre-requisite. There is the need therefore, to optimize or modify the use of existing protocols that will be more cost effective and efficient for sterilization of explants to establish a healthy culture.

Though the technique of tissue culture has been used for the propagation of sweet potato, much has not been done on the variation of various concentrations of sterilant and the time involved in the decontamination process for sweet potato explants. It was therefore, worthwhile to carry out this study with the objective of determining the most effective type and concentration of sterilant and duration for sterilizing sweet potato explants as a pre-requisite for successful micro propagation *in vitro*.

MATERIALS AND METHODS

Preparation of Murashige and Skoog (MS) Media (1 L): Measuring cylinder was used to measure 50 mL of the macronutrient stock solution into a 1 L beaker containing about 500 mL of distilled water. The beaker was placed on a hot plate magnetic stirrer. The 5 mL each of the micronutrient, chelated iron source and vitamins stocks were measured and added to the 500 mL distilled water in the beaker. Thirty gram of sucrose added to the content of the beaker.

The 1.0 mL of BAP and 0.1 mL of NAA stocks were pipetted into the beaker containing the mixture and all placed on the hot plate magnetic stirrer with the magnetic stirrer heating and stirring at the same time. About 7 g of agar was weighed, put into a beaker containing about 200 mL distilled water and placed into a microwave to dissolve the agar. The dissolved agar was then poured into the 1 L beaker on the hot plate magnetic stirrer and the final volume topped up with distilled water to about 950 mL. The pH was adjusted to 5.8 using dilute solutions of HCl or NaOH. The volume was topped up with distilled water to a final volume of 1 L (1000 mL) and stirred. The 20 mL of the medium was dispensed with hypodermic syringe into each test tube. The test tubes containing the media were packed onto racks, wrapped with aluminium foil and autoclaved at 121°C, pressure of 103.4 kPa for 15 min.

Sterilization of materials and equipment: The apparatus that was used for the inoculation was autoclaved at 121°C for 15 min to be made free of any fungal or bacterial spores. They were then kept under sterile conditions till use.

Sterilization of laminar flow hood: The hood was switched on for about 5 min. The chamber (including all surfaces) was sprayed with 70% ethanol and allowed to stay under this condition for about 2 min. The surfaces were then cleaned with sterile tissue paper soaked in 70% ethanol. The hood was allowed to be on for 30 min. Autoclaved beakers, conical flasks, petri dishes, sterile distilled water, spirit lamp (surface cleaned with 70% ethanol) and other materials that were needed for inoculation were then brought into the hood.

Source of sweet potato explant: Healthy looking sweet potato vines (Apomuden variety) were obtained from the Technology village (University of Cape Coast) in the Central Region of Ghana.

Sterilization and culturing of Sweet potato explants: Vines were cut into 2 cm segments such that each segment contained one axial bud. They were washed in soapy solution for 5 min and rinsed under running tap water for 2 min. They were then transferred to clean containers, covered and sent to the flow hood. In the flow hood, the explants were transferred to sterile beakers containing the respective sterilant for the predetermined duration. The explants were rinsed with sterile distilled water and dipped in 70% ethanol for the required time. After sterilization, the explants were rinsed three times with sterile distilled water. They were transferred into sterile petri dishes. The ends were trimmed with sterile scalpel and forceps. The scalpels and forceps were sterilized by dipping them in absolute ethanol and flamed on spirit lamp. After trimming, the explants were picked with sterile forceps and transferred to the medium such that the bud pointed upwards. The explants were pressed lightly to have it firmly fixed on the growth medium. One explant was inoculated per test tube. The control group on the other hand, were only dipped in 70% ethanol and rinsed with several changes of sterile distilled water. Each treatment, including the control, had 12 replicates. The selection of explants and placement on racks were done randomly.

The experimental layout is indicated in the Table 1-7. The sterilizing agents used were Dettol (chloroxylenol 4.8% w/v), Parazone (sodium hypochlorite 3.5% w/v), Mercuric chloride and sterilized distilled water with 70% ethanol as control (Table 1). There were six experimental set ups based on the type of sterilization agents and time to sterilize the explants.

The concentration and the time duration of the sterilizing agents used for the treatment of sweet potato explants were varied in order to identify the most effective treatment in establishing a contamination free sweet potato explants. Table 2 describes different concentrations of Dettol. Different concentration of Dettol as 1, 2, 3 and 4% were used. For Power zone the levels were 1, 2, 3 and 3.5% (Table 3). Table 4 also shows the various levels of concentration of mercuric acid. The concentration of mercuric acid used were: 0.2, 0.4, 0.6 and 1%.

The isolated buds were subjected to different sterilant conditions. The buds were subjected to conditions described in Table 2-4 at time intervals of <1, 8, 10, 12 and 15 min. For instance Table 5 shows same concentrations (1%) of Dettol was subjected to different time intervals. Table 6 show 1% concentration of Parazone for various durations while Table 7 describes 0.2% concentration of mercuric acid for various time intervals.

Inoculation of explant: The sterilized explants were transferred into petri dishes in the lamina flow hood and trimmed using the sterilized surgical blades and a pair of forceps. The MS media with different concentrations of NAA and BAP, respectively were inoculated with sweet potato

Table 1: Sterilant used, active components and their stock concentrations (12 replicate each treatment)

Label	Treatment
Z (control)	Sterilised distilled water+70% ethanol
A	Dettol (chloroxylenol 4.8% w/v)
B	Parazone (sodium hypochlorite 3.5% w/v)
C	Mercuric chloride (g)

Table 2: Sterilization with different concentrations of Dettol® for 10 min

Label	Treatment
Z(control)	Sterilised distilled water+70% ethanol
A_1	1% of 4.8% w/v chloroxylenol+4 drops of Tween 80+70% ethanol
A_2	2% of 4.8% w/v chloroxylenol+4 drops of Tween 80+70% ethanol
A_3	3% of 4.8% w/v chloroxylenol+4 drops of Tween 80+70% ethanol
A_4	4% of 4.8% w/v chloroxylenol+4 drops of Tween 80+70% ethanol

Table 3: Sterilization with different concentrations of power zone® for 10 min (Sodium hypochlorite v/v 3.5%)

Label	Treatment
Z(control)	Sterilised distilled water+70% ethanol
B_1	1% of 3.5% v/v sodium hypochlorite+4 drops of Tween 80+70% ethanol
B_2	2% of 3.5% v/v sodium hypochlorite+4 drops of Tween 80+70% ethanol
B_3	3% of 3.5% v/v sodium hypochlorite+4 drops of Tween 80+70% ethanol
B_4	3.5% of 3.5% v/v sodium hypochlorite+4 drops of Tween 80+70% ethanol

Table 4: Sterilization with different concentrations of mercuric chloride for 10 min

Label	Treatment
Z(control)	Sterilised distilled water+70% ethanol
C_1	0.2% mercuric chloride+4 drops of Tween 80+70% ethanol
C_2	0.4% mercuric chloride+4 drops of Tween 80+70% ethanol
C_3	0.6% mercuric chloride+4 drops of Tween 80+70% ethanol
C_4	1.0% mercuric chloride+4 drops of Tween 80+70% ethanol

Table 5: Treatment with Dettol at different time intervals

Label	Constant concentration	Time (min)	Replicate
Z (control)	1% of 4.8% w/v Dettol+4 drops of Tween 80+70% ethanol	<1	12
A_1	1% of 4.8% w/v Dettol+4 drops of Tween 80+70% ethanol	8	12
A_2	1% of 4.8% w/v Dettol+4 drops of Tween 80+70% ethanol	10	12
A_3	1% of 4.8% w/v Dettol+4 drops of Tween 80+70% ethanol	12	12
A_4	1% of 4.8% w/v Dettol+4 drops of Tween 80+70% ethanol	15	12
A_5	1% of 4.8% w/v Dettol+4 drops of Tween 80+70% ethanol	20	12

Table 6: Treatment with parazone at different time intervals

Label	Constant concentration	Time (min)	Replicate
Z (control)	1% of 3.5% v/v parazone+4 drops of Tween 80+70% ethanol	<1	12
B_1	1% of 3.5% v/v parazone+4 drops of Tween 80+70% ethanol	8	12
B_2	1% of 3.5% v/v parazone+4 drops of Tween 80+70% ethanol	10	12
B_3	1% of 3.5% v/v parazone+4 drops of Tween 80+70% ethanol	12	12
B_4	1% of 3.5% v/v parazone+4 drops of Tween 80+70% ethanol	15	12
B_5	1% of 3.5% v/v parazone+4 drops of Tween 80+70% ethanol	20	12

Table 7: Treatment with mercuric chloride at different time intervals

Label	Constant concentration	Time (min)	Replicate
Z (control)	0.2 g of mercuric chloride+4 drops of Tween 80+70% ethanol	<1	12
C_1	0.2 g of mercuric chloride+4 drops of Tween 80+70% ethanol	8	12
C_2	0.2 g of mercuric chloride+4 drops of Tween 80+70% ethanol	10	12
C_3	0.2 g of mercuric chloride+4 drops of Tween 80+70% ethanol	12	12
C_4	0.2 g of mercuric chloride+4 drops of Tween 80+70% ethanol	15	12
C_5	0.2 g of mercuric chloride+4 drops of Tween 80+70% ethanol	20	12

explants that were treated with different sterilizing agents. The test tubes were then tightly corked with cotton wool, sealed with Parafilm to prevent the passage of air in the test tubes and labelled according to treatment. They were arranged on racks and then sent to the growth room.

Incubation of cultures: The cultures were arranged on shelves under white fluorescent tubes providing about 1000 lux lights in the growth room and maintained at 24±2°C for a 16 h photoperiod. Observations were made daily for microbial contamination and growth of cultures for two weeks.

RESULTS

Effect of different sterilizing agents and their different concentration on the level of microbial contamination: Different percentages of decontamination were observed among the cultures with respect to different sterilants (Fig. 1). The percentage contamination was computed at the end of two weeks of culturing. The control cultures in which explants were washed with sterile distilled water and 70% ethanol had 100% culture contamination. Dettol combined with 70% ethanol treated for 10 min, recorded 61% microbial contamination treatment, Power zone in combination with 70% ethanol treated for 10 min, recorded 24% contamination and mercuric chloride treated for 10 min, recorded 15% contamination in combination with ethanol treatment. Most contaminants were fungi which included *Fusarium* sp., *Neurospora crassa*, *Penicilium* sp. and *Aspergilus niger*.

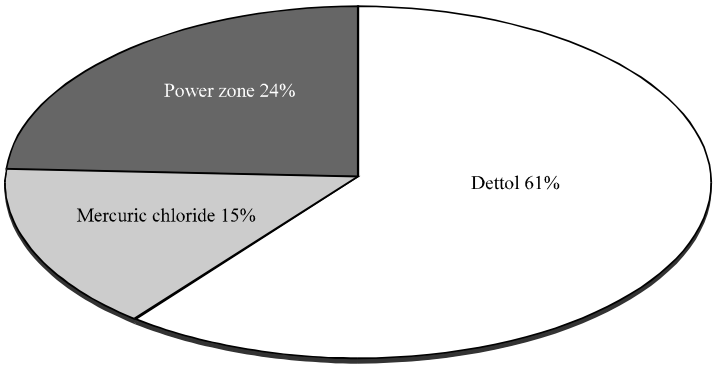

Fig. 1: Level of contamination using different sterilants on sweet potato culture two weeks after culture

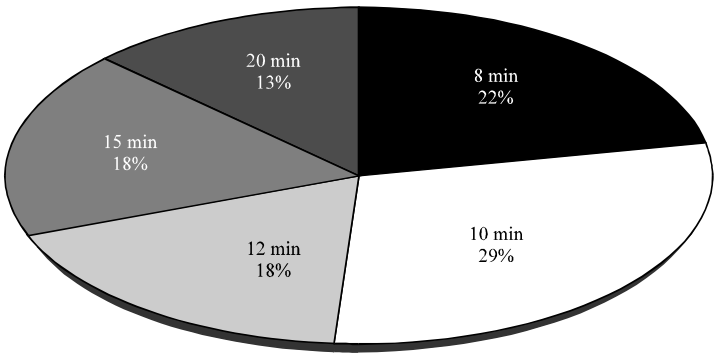

Fig. 2: Effect of different time regimes, with all the sterilants, on percentage contamination of sweet potato cultures after two weeks of inoculation

Effect of different sterilant treatment of sweet potato explants on the level of microbial contamination at different time regimes: The sweet potato cultures showed different levels of decontamination in response to different time regimes (Fig. 2). The control cultures in which sweet potato explants treatments were subjected to 10 min showed 29% microbial contamination for all the sterilants used in the treatment; 22% microbial contamination when treated for 8 min for all sterilants; 18% microbial contamination when treated for both 12 and 15 min for all sterilants and finally 13% microbial contamination when treated for 20 min for all sterilants.

Effect of different concentrations of Dettol (4.8% w/v chloroxylenol) on sterilization of sweet potato explants: Each concentration of Dettol showed different levels of decontamination. From Fig. 3, it could be observed that as the concentration of the detergent increased, the percentage of microbial contamination reduced. A concentration of 4% Dettol proved most effective, eliminating all the microbes with the exception of *Fusarium* sp. which could not be totally destroyed by the treatment. The control (0% Dettol) treatment was observed to be the least effective treatment against the microbes which recorded the highest contamination with all the four main fungal contaminants being present. On the whole, Fig. 3 shows differences when the concentration of the Dettol was varied in the decontamination process on explants.

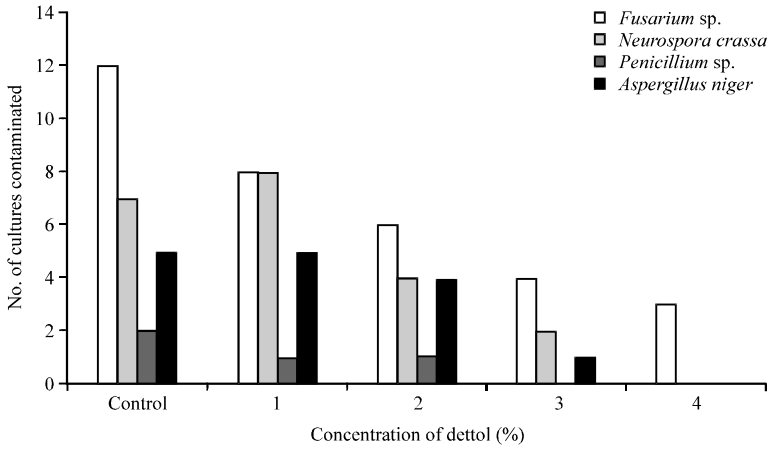

Fig. 3: Effectiveness of different concentrations of Dettol (4.8% w/v cloroxylenol) on sterilization of sweet potato explants

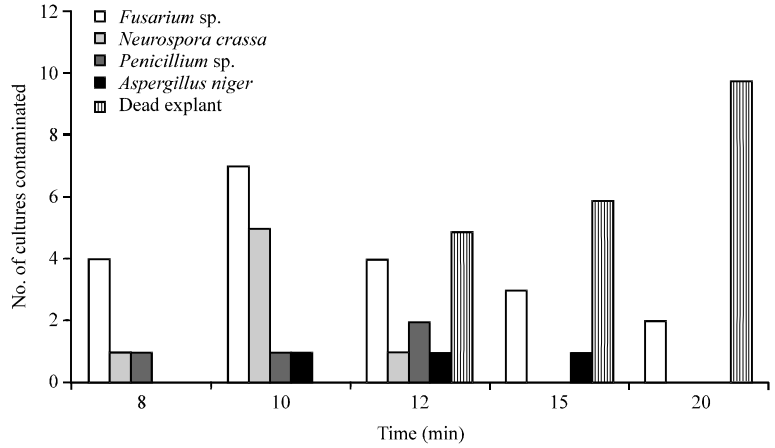

Fig. 4: Effect of 1% Dettol sterilization at 2 weeks time period of after culture

Effect of 1% Dettol (Chloroxylenol) sterilization at different durations at two weeks of inoculation: The sweet potato explants were subjected to 1% Dettol for different time periods (8, 10, 12, 15 and 20 min). As can be observed from Fig. 4, contamination generally decreased as the time of exposure increased after 10 min but 8 min appeared to be better than 10 min. Duration of 12, 15 and 20 min recorded death of 5, 6 and 10 cultures, respectively (Fig. 4).

Effect of different concentrations of power zone (Sodium hypochlorite) the sterilization of sweet potato explants: The effectiveness of different concentrations of sodium hypochlorite on sterilization of sweet potato explants is depicted in Fig. 5. Each treatment showed different levels of decontamination. The concentrations used were in a range of 0% (control), 1, 2, 3 and 3.5% of the Power zone. *Neurospora crassa* was the dominant species, contaminating the cultures even when a higher concentration of the hypochlorite was used. *Fusarium* sp., *Penicillium* sp. and *Aspergillus niger* however, recorded decreasing rates of contamination as the concentration increased.

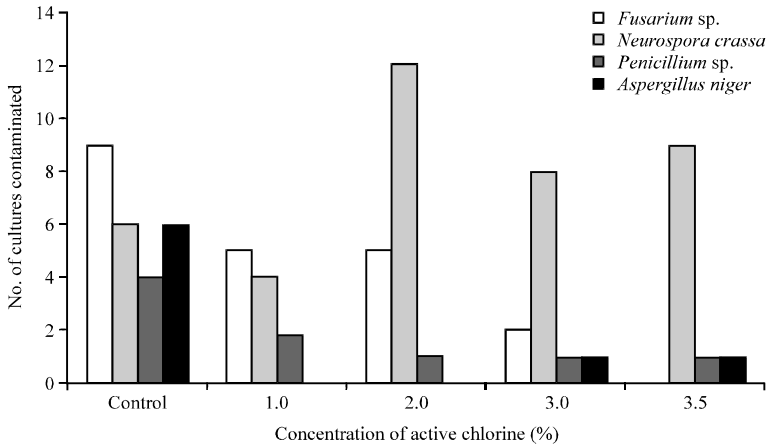

Fig. 5: Effect of different concentration of power zone (Sodium hypochlorite) on the sterilization of sweet potato explant at two weeks after culture

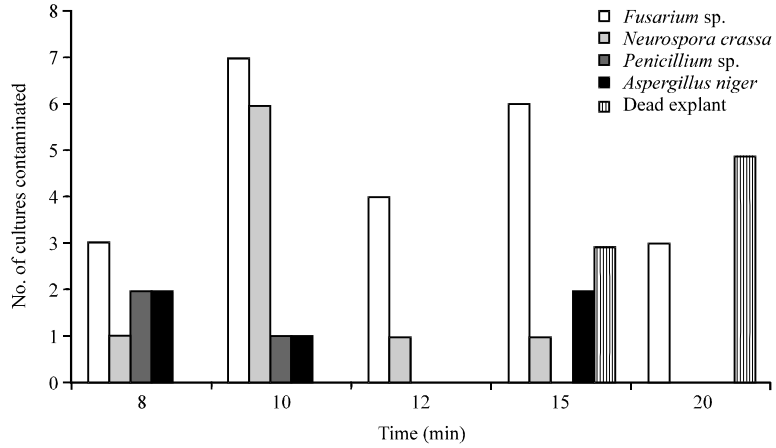

Fig. 6: Effectiviness of Power zone (Sodium hypochlorite) as a sterilization agent on sweet potato explants at different durations

Effect of sterilization with sodium hypochlorite at different durations: Explants were subjected to 1% sodium hypochlorite for different durations. In general, percentage contamination decreased as the time of exposure to the hypochlorite increased (Fig. 6). Duration of 12 min was observed to be the optimum since it was able to completely eliminate two of the microbial contaminants (*Penicillium* sp. and *Aspergillus* sp.) and without any death of explant recorded. Duration of 15 and 20 min resulted in death of 3 and 5 explants, respectively. Rate of death generally increased as the time of exposure increased beyond 12 min.

Effect of different concentrations of mercuric chloride on sterilizing sweet potato explants: Each treatment (0.2, 0.4, 0.6 and 1.0%) of mercuric chloride showed different levels of decontamination. Figure 7 shows a reduction in percentage of microbial contamination as the concentration of the sterilant increased after 2 weeks of inoculation. The control (0%) treatment recorded the highest percentage of microbial contamination followed by an increase in

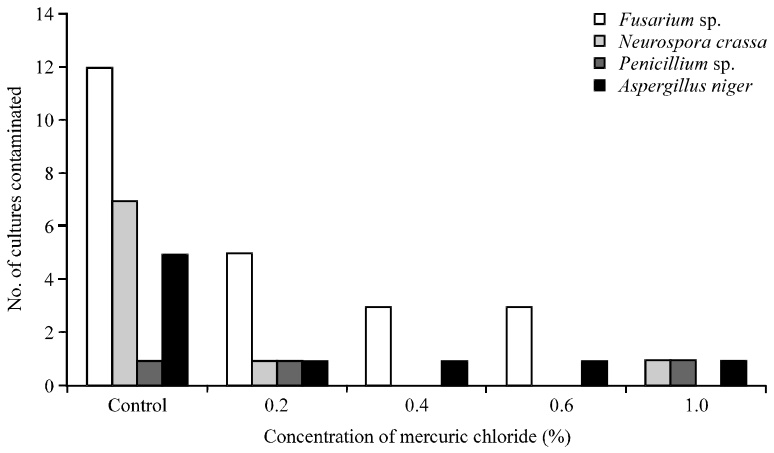

Fig. 7: Effect of different concentrations of mercuric chloride sterilization on sweet potato explant at two weeks after culture

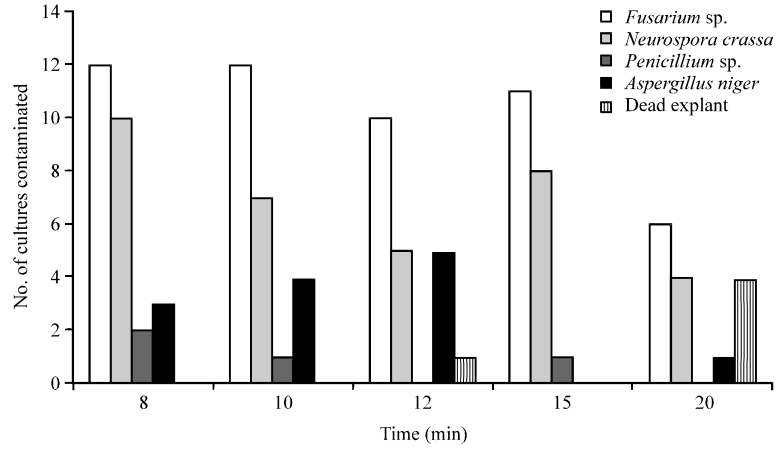

Fig. 8: Effect of 0.2% mercuric chloride sterilization on sweet potato explants at different durations two weeks after culture

concentration from 0.2, 0.4, 0.6 and 1.0% in that order. The predominant contaminants were *Fusarium* sp. and *Aspergillus* sp. which occurred in all the concentrations but with decreasing rates as the concentration increased. Mercuric chloride appeared very effective in completely eliminating *Penicillium* sp. and *Neurospora crassa* at concentration 0.4, 0.6 and 1.0%.

Sterilization of explants with mercuric chloride at different time intervals: The sweet potato explants were exposed to 0.2% mercuric chloride for different durations. Each treatment showed different levels of decontamination (Fig. 8). The percent microbial contamination was high, with *Fusarium* sp. being the predominant contaminant, followed by *Neurospora crassa*, *Aspergillus* sp. and *Penicillium* sp. and explants mortality was observed in 12 and 20 min treatments. The optimum duration for sterilizing sweet potato explants with 0.2% mercuric chloride was 20 min since it recorded the highest number of survived explants as compared to the other durations. The least effective was between 8-10 min since they recorded the highest numbers of cultures contaminated.

DISCUSSION

The contaminants observed after the decontamination process were all fungal contaminants such as *Fusarium oxysporium, Neurospora crassa, Penicillium* sp. and *Aspergillus niger*. These results are in agreement with Enjalric *et al.* (1988) who reported fungi as a constant problem and which can compromise development of all *in vitro* techniques.

However, it could be observed that the highest percent of microbial contaminations occurred in the control cultures (sterilized distilled water and 70% ethanol only) when the various sterilants and their respective concentrations were evaluated. This attest to the publications by many authors including Cassels (1996), on how important sterilization could be in micropropagation. However, among the microbial contaminants observed, *Fusarium* sp. appeared to be the dominant in all the cultures and *Penicillium* sp. the least. This suggests that the decontamination process was effective against *Penicillium* sp. as compared to *Fusarium* sp.which proved to be the least effective.

In Fig. 1, it could be observed that Dettol was the least effective in the decontamination process as against Mercuric chloride with values 61 and 15% contamination, respectively. Thus, the non-readily available sterilant (Mercuric chloride) was found to be adequate for sterilizing sweet potato explants for a duration of 10 min for culture initiation. Earlier studies have however, discouraged the use of mercuric chloride in sterilizing explant because of its phytotoxicity and mammalian toxicity (NIH, 2002). Therefore, its alternative, the readily available sterilant Power zone with active ingredient Sodium hypochlorite that proved to be most effective as compared with Dettol will be more preferable.

The sweet potato cultures showed different levels of decontamination in response to different time regimes (Fig. 2). The 10 min (control) of surface sterilization was found to be more effective by most authors including Kobza and Vachunova (1991). Kobza and Vachunova (1991) reported that active chlorine at 10% concentration and $HgCl_2$ at 0.1% for 10 min were the best sterilants for *Dracena* explants and most other plant species. However, results from Fig. 2 showed the highest percentage of microbial contamination (29%) when explants were subjected to 10 min surface sterilization contradict the findings of Kobza and Vachunova (1991). Another observation was that 20 min surface sterilization of explants for all sterilant recorded the least percentage of microbial contamination (13%). Thus, 20 min of surface sterilization of sweet potato explant was found to be adequate in suppressing microbial contamination. The result is in agreement with the classic report by Sivparsad and Gubba (2012) who developed an efficient plant regeneration protocol for sweet potato by manipulating the hormones whiles maintaining a constant sterilization procedure using 5% (v/v) sodium hypochlorite for 20 min.

From the result in Fig. 3, it could be observed that each treatment showed different levels of decontamination when the concentrations of detergents were varied. It was also observed that as the concentration of the sterilant increased, microbial contamination reduced. Omamor *et al.* (2007) reported that increase in the concentration/or exposure time will probably reduce fungal contaminants but can consequently lead to death of the culture materials. This was evident in the result when the concentration of Dettol was varied. There were no deaths of explants recorded because the concentrations were not increased to the extreme. A concentration of 4% of the dettol proved to be effective in eliminating all the microbes. *Fusarium* sp. was dominant throughout but was recorded as the least contaminant when sweet potato explants were subjected to a concentration of 4% Dettol treatment. The control (0% Dettol) treatment was observed to be inadequate in suppressing microbial contamination. This result proves how important sterilization is, in micropropagation as recommended by many researchers including Enjalric *et al.* (1988).

Observations made in Fig. 4 shows some level of inconsistencies when the concentration of Power zone (active chlorine) was varied in the surface sterilization of explants. These inconsistencies in the bar chart contradicts the findings of Omamor *et al*. (2007) which might be due to chances. Sodium hypochlorite is one sterilant that is mostly used by many researchers and has been recommended by many authors including Ogero *et al*. (2012), Odutayo *et al*. (2007) and Sivparsad and Gubba (2012). However, results in Fig. 4 showed high rate of contamination by *Neurospora crassa* even though the concentrations were increased. Thus, the commercially prepared bleach Power zone, with an active ingredient of sodium hypochlorite was found to be inadequate in suppressing the rate of *Neurospora crassa*. The other microbial contaminants *Fusarium* sp, *Penicillium* sp. and *Aspergillus* sp. were suppressed as the concentration increased and this was in agreement with the findings of Omamor *et al*. (2007). The probable cause for the high levels of *Neurospora crassa* might have been secondarily introduced. This might have happened during the inoculation process or fungal spores in the growth room. The 1% concentration of Power zone proved to be the most effective in suppressing the rate of contamination.

Figure 5 demonstrates the effect of sterilization by varying the concentration of mercuric chloride on sweet potato explants. Each treatment showed different levels of decontamination in response to microbial contamination. Figure 5 also demonstrates a reduction in the rate of microbial contamination as the concentration of sterilant (mercuric chloride) increased. The result is in agreement with Omamor *et al*. (2007) findings. The control (0%) treatment recorded the highest microbial contamination followed by an increase in concentration to 0.2, 0.4, 0.6 and 1.0% in that order. The dominant contaminants recorded were *Fusarium* sp. and *Aspergillus* sp. in all the variation of the concentrate but at a decreasing or a constant rate on the level of contamination. The 1.0% of the concentration of mercuric chloride recorded the least degree of microbial contamination. Thus 1.0% of mercuric chloride was adequate for surface sterilization of sweet potato explant at 10 min for culture initiation. However, Berger *et al*. (1994) developed disinfection protocol for rhizomes whereby rhizomes soaked in 1.0% $HgCl_2$ resulted in 90% free of microorganism. This was evident in the results on the efficacy of 1.0% mercuric chloride treatment.

Among the various sterilants, Dettol (chloroxylenol) treatment recorded the highest explant mortality and lower microbial contamination for the various durations. Although Fig. 6-8 show some levels of inconsistencies in the variation of the time, it also follows the concept between increasing time for treating explants and death of explant which are directly related. Earlier studies have shown that increase in concentration or time is very effective in reducing the rate of contamination, but to the extreme becomes toxic or harmful to the explant consequently resulting in death of explant. Razdan (1993) also said the type of sterilant used, concentration and time depends on the nature of explant and species. This could also be attributed to one of the reasons why the Fig. 6-8 showed some levels of inconsistencies on the decontamination process varying the time. Another observation made was that mercuric chloride recorded the least rate of death of explant as the time increased and higher rate of microbial contamination.

CONCLUSION

From the results the following conclusions were made:

- *Fusarium* sp., *Neurospora crassa*, *Penicillium* sp. and *Aspergillus niger* were the only contaminants observed in the cultures. Among these fungal contaminants, *Fusarium* sp. proved to be the predominant in all the cultures with *Penicillium* sp.being the least

- Increasing the time, the percentage of contamination reduced but increases the percentage of death of explants. Thus increasing time is more harmful than increasing the concentration
- The contamination percentage in Dettol treatment was high while mercuric chloride was the most effective in suppressing the rate of microbial contaminants
- There was a significant difference in applying the different time regimes but there wasn't any significant difference between 12, 15 and 20 min and also 8 and 10 min
- The most effective concentration for sterilizing sweet potato explants using
  - Power zone (3.5% w/v sodium hypochlorite) is 1%
  - Dettol (4.8% chloroxylenol) is 4%
  - Mercuric chloride is 1.0%
- The most effective time (duration) for sterilizing sweet potato explants using:
  - Power zone (3.5% w/v sodium hypochlorite) is 12 min
  - Dettol (4.8% chloroxylenol) is 8 min
  - Mercuric chloride is 20 min

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