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Comparative effects of different concentrations of Sterilants and their exposure time during in-vitro propagation of cocoyam (*Colocasia esculenta* (L.)schott)

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ABSTRACT: Comparative effect of different concentrations of sterilants and their exposure time during in-vitro propagation of cocoyam (*Colocasia esculanta* (L) Schott) were carried out bearing in-mind that the sustainability of cocoyam in food production has remained a mirage and its cultivation shown to be declining. Results showed that the use of higher concentrations of sterilants and exposure time has great potentials and offer meaningful results. The growth of cocoyam were more effective in higher concentrations and exposure time such as 25% and 25minitues respectively and gave higher number of clean culture from which planting materials were gotten within the shortest possible time. For food security to be assured, this method offers avenue to rapid multiplication and availability of food to the rapidly growing population. This method is also cost effective especially to the rural farmers who spend their hard-earned money for good cocoyam tubers.

KEY WORDS: Effect, different concentrations, exposure time, in-vitro, propagation, cocoyam.

INTRODUCTION

Cocoyam (*Colocasia esculenta* (L) Schott) belongs to the family Araceae. Cocoyams are important carbohydrate staple food particularly in the southern and middle belt areas of the Country (Asumugha and Mbanaso, 2002). Cocoyam rank third in importance and extent of production after yam and cassava among the root and tuber crops of economic value in Nigeria (Udealor, *et al*; 1996). Nigeria is the largest producer of cocoyam in the world, accounting for about 37% of total world output of cocoyam (FAO, 2006). Cocoyam improvement programmes are aimed at addressing the limiting factors in their production and utilization. Biotechnology has contributed to the development of improved genotypes, improved quality (nutritional values and consumer acceptability), and facilitates the breeding and selection processes (Aginam 2005). Osuji (1998) reported that research on the biotechnology of cocoyam / yams has been limited to certain areas of tissue culture. Nutritionally, cocoyam is superior to cassava and yam in the possession of higher protein, mineral and vitamin contents in addition to having a more digestible starch (Parkinson 1984, Splistoesser *et al*, 1973). Cocoyam serves as ornamental in beautifying the environment. Starch and vegetables from cocoyam serves as human food (Flach and Rumawas, 1996). Cocoyam is extensively used in south Asia as a staple food, and or dish or as a component in various sides dishes. This present study examines the effect of using different concentrations of sterilants and their exposure time during *in vitro* propagation of cocoyam. Despite this, the objective of its sustainability in food production has remained a mirage as its cultivation has been shown to be declining (Onyenweaku and Ezeh, 1987; Zuhair and Hunter, 2003). Given that attempt to improve its productivity would be a right step towards the resolution of the food crisis. This study is further justified by the need to having a better understanding of the performance of the crop sub sector as this is an issue of concern to both government and individual planners. To this end therefore, this study attempts to examine the trends in the production, area cultivated and productivity of cocoyam in Nigeria.

MATERIALS AND METHODS

Sources of Materials:

The cocoyam tubers used for this study were obtained from the cocoyam barn in the cocoyam unit located at National Root Crops Research Institutes (NRCRI) Umudike, Umuahia Abia State Nigeria .

Collection of cocoyam tubers:

The cocoyam tubers were collected from the cocoyam barn and was brought to tissue culture laboratory. These cocoyam tubers were washed thoroughly, covered and kept in a clean space for a period of 3- weeks for them to sprout. During these periods, these cocoyam tubers were spread with benlate to reduce mould and also with water to enhance the sprouting of the buds which is needed for the initiation.

Experimental design:-

This experiment was a 5x5 factional experiment fitted into completely randomized design (CRD). It was conducted to study the comparative effect of different concentrations of sterilants and their exposure time during *in-vitro* propagation of cocoyam (*Colocasia esculenta*). Let 70% ethanol and different concentrations of sodium hypochlorite be termed "a" and the exposure time be termed "b" the 70% ethauol and different concentrations of sodium hypochlorite were designated as a_1, a_2, a_3, a_4, and a_5 representing 10%, 15%, 20%, 25%, and 30% respectively. The exposure time were designated as b_1, b_2, b_3, b_4 and b_5 representing 10min, 15mins, 20mins, 25mins, and 30mins respectively.. A one way analysis of variance (ANOVA) was used and mean separation using fisher LSD to determine significant difference. (p= 0.5) with the aid of a statistical software package, called Statistical Analytical System, 1999 copyright version.

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RESULTS

The results of the comparative effect on different concentrations of sterilants and different exposure time during in-vitro propagation of cocoyam are here by reported in tables 1 to 5. Table 1 shows the result of different concentrations of sterilants at 10 minutes exposure time during in-vitro propagation of cocoyam. a_1, b_1, had the highest contamination and was significantly different (p= 0.05) from the other concentrations. Treatment a_3b_1, a_4b_1 and a_5b_1 recorded no contamination during 3 days of treatment of the cocoyam. Treatment a_1b_1 had the lowest effect on the number of uncontaminated culture. Number of uncontaminated cultures were highest in a_3b_1, a_4b_1 and a_5b_1 treatments and they were not significantly different (p=0.05).

During 6 days of treatment, the number of contaminated culture was highest and significantly different (p=0.05) in a_1b_1 and a_5b_1 treatments. a_4b_1 treatment gave the lowest number of contamination and was significantly different (p=0.05). Number of uncontaminated culture was significantly highest (p=0.05) with a_4b_1 treatment and lowest in a_1b_1 and a_5b_1 treatments. 9 days application of the treatment showed that a_1b_1, a_3b_1 and a_5b_1 gave the highest number of contamination while a_4b_1, treatment was least. Number of uncontaminated cultures was highest in a_4b_1. No contamination was recorded with a_1b_1, a_3b_2 and a_5b_1 treatments. 14 days of application showed that all the treatments recorded highest number of contamination and there was no significant difference (p>0.05).

In table 2, a_1b_2, a_2b_2, a_3b_2 and a_4b_2 showed no contamination during 3 days of application while a_2b_2 treatment recorded the highest (5.0) number of contamination of the cocoyam culture. The number of contaminated cultures were highest in the treatment except in treatment a_5b_2. The result showed no significant difference (p>0.05) in the number of uncontaminated cultures except with a_5 b_2 treatment. During 6 days application, contaminated culture was highest with a_5b_2 and was significantly different (p=0.05). There was no significant difference in the rate of contamination with a_1b_2 and a_4b_2, a_2b_2 and a_3b_2. Mean values of contaminated culture ranged from 5.0-15.0. Number of uncontaminated culture was highest in both a_2b_2 and a_3b_2 while a_5b_2 was significantly lowest (p=0.05). Mean values of uncontaminated culture ranged from 10.0-20.0. Application of treatment for 9 days recorded highest contamination with a_5b_2. There was no significant different in number of contamination with a_1b_2, a_3b_2 and a_4b_2. Uncontaminated cultures was highest with a_2b_2 treatment. During 14 days, the results showed that all cultures were contaminated with the treatments and number of uncontaminated cultures was nil. There was no significant different in the number of contamination with the treatments.

In table 3, 3 days of application of treatments showed no contamination of culture. During 6 days of application, contaminated culture was highest in a_2b_3 and a_4b_3 treatments. There was no significant difference (p>0.05) in number of contamination a_1b_3 and a_3b_3, a_2b_3 and a_4b_3. Lowest contamination was recorded with a_5b_3 treatment. Number of uncontaminated culture was highest with a_5b_3 and lowest with a_4b_3 and a_2b_3. During 9 days of application the result showed that number of contamination increased and number of uncontamination decreased. There was no significant difference in number of contaminated and uncontaminated cultures among the treatments except with a_5b_3. Increase in contamination of the culture increased during 14 days of application, though number of uncontaminated culture was slightly recorded with a_2b_3 and a_4b_3 contaminations. There was no significant difference among the two treatments.

Table 4, showed that all concentrations of the treatments showed no contamination of the cultures during 3 days of application and they all recorded high number of uncontamination of the culture. There was no significant difference (p>0.05) in the number of uncontaminated culture among the treatments. During 6, 9 and 14 days showed increase in number of contamination and decrease in number of uncontaminated cultures. The rate of contamination was most pronounced in 14 days with a_1b_4 and a_5b_4 treatments.

Table 5, showed that 3 days of application recorded no contamination of the culture except with a_1b_5 treatment. Number of uncontaminated culture was highest and showed no significant difference (p>0.05) among the treatments, except with a_1b_5. During 6 days of application, contaminated cultures was highest with a_1b_5 and was significantly different (p=0.05). There was no significant different in the rate of contamination with a_4b_5 and a_5b_5, a_2b_5 and a_3b_5. The mean value of contaminated cultures ranged from 10.0 -20.0. Number of uncontaminated cultures was highest in both a2b_5 and a_3b_5 while a_5b_5 was significantly lowest (p=0.05). Mean value of uncontaminated cultures ranged from 5.0-15.0. 9 days application recorded highest contamination with a_1b_5.

There was no significant difference in numbers of contamination with a_2b_5 and a_4b_5, a_3b_5 and a_5b_5. Uncontaminated culture was highest in a_3b_5 and a_5b_5 treatments. During 14 days the result showed that treatments a_1b_5, a_2b_5 and a_3b_5 recorded the highest number of contamination. Uncontaminated cultures were highest in a_4b_5 and a_5b_5 treatments.

DISCUSSION:-

The results of this investigation showed clearly that in-vitro application of different concentrations of sterilants and exposure time in combination, favored the in-vitro performance of cocoyam. Although not all the concentrations performed well in the different exposure times, but those that performed well were mainly those of higher concentrations and exposure time. Similar trends were observed by Ammirato, (2004) who reported that in *D*, *bnlbifera* and *D. alata*, cytokinin at moderate concentrations enhanced shoot development that helps to produce more number of shoots, buds, and leaves. From the result it was noted that low concentration series between 10% -15% did not produce shoots and buds, rather the addition of moderate concentrations between 20% - 30%

Table 1: Effect of Different Concentrations of Sterilants at 10 Minutes Exposure Time

Treatment	3 days		6 days		9 days		14 days	
	No of co-ntaminated cultures	No of un-contamin-ated cultures	No of co-ntaminated cultures	No of un-contamin-ated cultures	No of contamin-ated cultures	No of un-contamin-ated cultures	No of contamin-ated cultures	No of un-contamin-ated cultures
a_1b_1	15.0^a	10^c	20^a	5.0^c	25.0^a	ml	25.0^a	nil
a_2b_1	5.0^b	20^b	10^b	15.0^b	20.0^b	5.0^b	25.0^a	nil
a_3b_1	nil	25^a	10^b	15.0^b	25.0^a	nil	25.0^a	nil
a_4b_1	nil	25^a	5.0^c	20.0^a	15.0^c	10.0^a	25.0^a	nil
a_5b_1	nil	25^a	20^a	5.0^c	25.0^a	nil	25.0^a	nil
LSD (5%)	1.15	1.82	1.82	1.82	1.82	1.15	1.82	

Mean values down the columns with different superscript are significantly different (p? 0.05).

Table 2: Effect of Different Concentrations of Sterilants at I5 Minutes Exposure Time

Treatment	3 days		6 days		9 days		14 days	
	No of co-ntaminated cultures	No of un-contamin-ated cultures	No of co-ntaminated cultures	No of un-contamin-ated cultures	No of contamin-ated cultures	No of un-contamin-ated cultures	No of contamin-ated cultures	No of un-contamin-ted cultures
a_1b_2	nil	25.0^a	10.0^b	15.0^c	20.0^b	5.0^b	25.0^a	nil
a_2b_2	nil	25.0^a	5.0^c	20.0^a	15.0^c	10.0^a	25.0^a	nil
a_3b_2	nil	25.0^a	5.0^c	20.0^a	20.0^b	5.0^b	25.0^a	nil
a_4b_2	nil	25.0^a	10.0^b	15.0^c	20.0^b	5.0^b	25.0^a	nil
a_5b_2	5.0	20.0^b	15.0^a	10.0^b	25.0^a	nil	25.0^a	nil
LSD (5%)	0.81	1.82	1.82	1.82	1.82	1.63	1.82	

Mean values down the columns with the same superscript are not significantly different (p>0.05).

Table 3: Effect of Different Concentrations of Sterilants at 20 Minutes Exposure Time.

Treatment	3 days		6 days		9 days		14 days	
	No of co-ntaminated cultures	No of un-contamin-ated cultures	No of co-ntaminated cultures	No of un-contamin-ated cultures	No of un-contami-anated cultures	No of un-contamin-ated cultures	No of un-contamin-ated cultures	No of un-contamin-ated cultures
a_1b_3	nil	25.0^a	10.0^b	15.0^b	20.0^a	5.0^b	25.0^a	nil
a_2b_3	nil	25.0^a	15.0^a	10.0^c	20.0^a	5.0^b	20.0^b	5.0^a
a_3b_3	nil	25.0^a	10.0^b	15.0^b	20.0^a	5.0^b	25.0^a	nil
a_4b_3	nil	25.0^a	15.0^a	10.0^c	20.0^a	5.0^b	20.0^b	5.0^a
a_5b_3	nil	25.0^a	5.0^c	20.0^a	15.0^b	10.0^a	25.0^a	nil
LSD (5%)	—	1.82	1.82	1.82	1.82	1.82	1.82	1.15

Means down the columns with the same superscript are not significantly different (p>0.05).

Table 4: Effect of Different Concentrations of Sterilants at 25 Minutes Exposure Time.

Treatment	3 days		6 days		9 days		14 days	
	No of co-ntaminate-d cultures	No of un-contamin-ated cultures	No of co-ntaminated cultures	No of un-contami-nated cultures	No of contamin-ated cultures	No of un-contamin-ated cultures	No of contamin-ated cultures	No of un-contamin-ated cultures
a_1b_4	nil	25.0^a	15.0^a	10.0^c	20.0^a	5.0^c	25.0^a	nil
a_2b_4	nil	25.0^a	15.0^a	10.0^c	15.0^b	10.0^b	20.0^b	5.0^a
a_3b_4	nil	25.0^a	10.0^b	15.0^b	15.0^b	10.0^b	20.0^b	5.0^a
a_4b_4	nil	25.0^a	5.0^c	20.0^a	10.0^c	15.0^a	20.0^b	5.0^a
a_5b_4	nil	25.0^a	10.0^b	15.0^b	15.0^b	10.0^b	25.0^a	nil
LSD (5%)	__	1.82	1.82	1.82	1.82	1.82	1.82	1.41

Means down the columns with the same superscript are not significantly different (p>0.05).

TABLE 5: Effect of Different Concentrations of Sterilants at 30 Minutes Exposure Time.

Treatment	3 days		6 days		9 days		14 days	
	No of co-ntaminate-d cultures	No of un-contamin-ated cultures	No of co-ntaminated cultures	No of un-contami-nated cultures	No of contamin-ated cultures	No of un-contamin-ated cultures	No of contamin-ated cultures	No of un-contamin-ated cultures
a_1b_5	10.0^a	15.0^b	20.0^a	5.0^c	25.0^a	Nil	25.0^a	nil
a_2b_5	nil	25.0^a	10.0^c	15.0^a	20.0^b	5.0^b	25.0^a	nil
a_3b_5	nil	25.0^a	10.0^c	15.0^a	15.0^c	10.0^a	25.0^a	nil
a_4b_5	nil	25.0^a	15.0^b	10.0^b	20.0^b	5.0^b	20.0^b	5.0^a
a_5b_5	nil	25.0^a	15.0^b	10.0^b	15.0^c	10.0^a	20.0^b	5.0^a
LSD (5%)	1.81	1.82	1.82	1.82	1.82	1.82	___	1.41

Mean down the columns with the same superscript are not significantly different (p>0.05).

and prolonged exposure time stimulated multiple shoot and bud production (tables 3 and 4). Chaturved: (1977) obtained from cultures of *D. Floribunda*, an average of 5-6 shoots and buds, in 20 days by culturing single node cuttings which is in agreement with this results. (Table4). The control, 0% concentration gave the tallest height. This is in agreement with Lakshmi et al; (2006) when they observed that the growth and morphogenetic responses of in-vitro cultures depend among other factors, on the correct constituents and balances of growth regulators. The phenomena of in-vitro micro tuberization were observed in Ms medium with 30% concentration and 10min exposure time. This phenomenon was also observed by Mantell, (2002) when he cultured *D. rotunda* with media containing $5mgl^{-1}$ 1AA and in the presence of $0.5mgl^{-1}$-kinetin.

From the result, it was noted that those with higher concentrations and exposure time gave the highest number of clean cultures which led to the production of large number of shoots, buds nodes and leaves. This was in agreement with the finding of Skoog and Miller (1957) which showed that at higher concentrations of phytohormone a hard undifferentiated callus was obtained consisting of small thick walled cells.

CONCLUSION

The use of higher concentrations and exposure time for cocoyam cultures has great potential and can offer meaningful results. However, the growth of cocoyam (*Colocasia esculenta*) were more effective in higher concentrations and exposure time such as a_4 (25%) and b_4 25(mins) respectively than in lower concentrations and exposure time and gave high number of clean cultures in which planting materials were gotten within the shortest possible time. For food security to be assured, this method offers avenue to rapid multiplication and getting planting materials within the shortest possible time and also increase the availability of food to the rapidly growing population.

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