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STERILLIZATION OF MEDIA VIA SODIUM HYPOCHLORITE (NaClO) WITHOUT AUTOCLAVING *IN-VITRO* MICRO-PROPAGATION OF GINGER (*Zingiber officinale* Rose.) CULTURE

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Abstract

Ginger which is an important tropical and sub-tropical herbaceous perennial plant, with the rhizome valued for its culinary and medicinal properties. The alternative technique for sterilization of culture medium to replace autoclaving was carried out. For sterilization of culture medium without autoclaving was provided to 50 ml Murashige and Skoog (MS) medium before medium was solidified and kept for two weeks before evaluating sterile conditions. Treated media, supplemented with sodium hypochlorite (NaClO), were compared to the control medium, autoclaved at 121 degree Celsius for 20 min. *In vitro* sterile conditions were found 20–100% from these treated media compared to 95% sterile condition from autoclaved medium. Treated media obtained 100% sterile conditions at 0.0025% active chlorine were chosen limit concentration of 5 % (v/v) sodium hypochlorite provide for culturing of ginger shoot, root and fresh biomass. Therefore, the development of this technique, using chemical sterilization of sodium hypochlorite corresponding formula was NaClO for eradicating microorganisms causing agents of contamination, to replace the autoclaving method for establishing sterile culture medium and inadequate electric power were the best opportunity for *in vitro* culture.

Keywords: Sterilizing agent, MS medium, *in vitro* micro-propagation, *Zingiber officinale*, without autoclaving

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INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), a member of the family Zingeberaceae, is an important tropical and sub-tropical herbaceous perennial plant, with the underground rhizome valued for its culinary and medicine, food, beverage and form an important raw materials in pharmaceutical industry properties [1]. Ginger production for the extraction of oleoresins and essential oils, as well as the direct use of rhizomes for culinary purposes is increasing worldwide [3]. Plant tissue culture is a useful technology for plant propagation in aseptic environment. To maintain an aseptic environment, all culture vessels, media and instruments used in handling tissues, as well as explants itself must be sterilized. The importance is to keep the air, surface and floor free of dust. All operations should be carried out in laminar airflow sterile cabinet [2].

The knowledge has been provided to agriculturists worldwide. However, most agriculturists cannot carry out plant tissue culture laboratory by themselves due to high production costs. One of the major problems is expensive equipment especially an autoclave, a sterilizing apparatus and inadequate power of electricity. Therefore, the development of techniques, using chemical sterilization of sodium hypochlorite chemical formula (NaClO) for eradicating microorganisms causing agents of contamination, to replace the autoclaving method for establishing sterile culture medium have been the best procedure for *in vitro* culture. These chemicals used for disinfectants of fungicides and bactericides such as chlorine, sodium hypochlorite, calcium hypochlorite, hydrogen peroxide were sterilized culture media without autoclave [7].

Teixeira *et al*. (2006), published data that originated a culture medium preparation protocol using very low concentrations of sodium hypochlorite as the sterilizing agent for pineapple micro propagation. Unfortunately, due to inadequate power of electricity more than 30 Litter of MS medium was discarded per a day. The purpose of this research is to examine the behavior of ginger cultured in a medium prepared and chemical sterilized via sodium hypochlorite (NaClO) without Autoclaving [6].

MATRIALS AND METHODS

All the jam jars and covers used in the preparation of the culture medium had been washed in tap water after each use, rinsed with a sterilized distilled water solution containing 0.012% active chlorine of 5% (v/v) total active chlorine household bleach and stored on shelves under normal conditions of asepsis. Although all the jam jars and covers were again rinsed in a solution of sterilized distilled water solution containing 0.025% active chlorine of 5% (v/v) of total active

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chlorine soaked for 24 hours and shelves on dry oven at 80 ^{O}C for one hour. Ginger (*Zingiber officinale*) from *in vitro* stock cultures were used as explants donors. The medium used for *in vitro* culture of ginger shoot was Murashige and Skoog [4], 100 mg/ l myo-inositol, 1 mg/ l Kin, 2 mg/l BAP and 30g/l sucrose. The medium was adjusted to pH 5.7 before solidifying with 7 g/l of agar. The treatments consisted of the following percentages (v/v) of total active chlorine equivalent to milliliter per litter and amount of active chlorine added to the culture medium for ginger plant *in vitro* micro-propagations.

Table 1: The % (v/v) of total active chlorine or amount of active chlorine added milliliter per litter sodium hypochlorite apply to the culture medium by using ($C_1V_1 = C_2V_2$) formula

Code	5% (v/v) of Sodium hypochlorite	Active chlorine %	Sodium hypochlorite/Litter
A	Control (autoclaved MS)	0	0
B	0.005% NaClO	0.00025	0.05 ml/L
C	0.01% NaClO	0.0005	0.25 ml/L
D	0.05% NaClO	0.0025	0.5 ml/L
E	0.1% NaClO	0.005	1 ml/L
F	0.25% NaClO	0.0125	2.5 ml/L
G	0.5% NaClO	0.025	5 ml/L

The autoclaved control medium was prepared according to the conventional protocol for medium preparation [4]. The preparation of the NaClO sterilized basal media differed from the conventional procedure in that, after placing all the reagents in a culture vessel, the sterilizing agent was also added. After 10 min, the medium volume was topped up with distilled water and the pH has been adjusted to 5.7 by using 1N NaOH and 1N HCl.

The culture medium was fortified 7 g/l of agar and boiled in microwave oven for melted agar then poured in 50 ml aliquots into 300 ml culture jam jars and keeps for two weeks in media storage room. All the steps for preparing the medium, including filling the jam jars had been carried out on a laboratory bench in a non-sterile environment and lamina air flow hood a sterile environment.

The explants have been inoculated in sterile environment of laminar air flow hood. The cultured jam jars were incubated for five weeks in a culture room at a temperature of 25 ± 2 ^{O}C, with a 16-h photoperiod and 40 µmol m–2 s–1 of cool white light florescence. The experimental design, which was entirely at random, consisted of seven treatments, 20 repetitions and 1 culture jam jar with 5 explants as the experimental unit.

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Data Analysis

Sterile conditions of media were evaluated after two weeks with 20 replications before inoculation of explants. The growth parameter of ginger explants were fresh weight, shoot length, number of leaves and number of roots collected after five weeks of culturing. The completely randomized design (CRD) was used as the experimental design and data were analyzed using the analysis of variance procedure in the statistical analysis software (SAS version 9.2) and the Duncan's new multiple range test (DNMRT) was used for comparison among treatment means. For all the data analysis, probability level of less than 5% ($P < 0.05$) was considered for statistical significance.

RESULT AND DISCUSSION

The report revealed that, the efficiency of ginger plant inoculated on sodium hypochlorite corresponding chemical formula was NaClO sterilizing agents to prevent and eliminate microorganisms in MS medium to obtain sterile condition without autoclaving which has been filling on a laboratory bench in a non-sterile environment and lamina air flow hood a sterile environment were 100% cleaned after two weeks. Of course, similar results were obtained when such autoclaved and unautoclaved bottles were used for providing in and out said laminar air flow hood rooting medium [5].

Therefore, dispensing in a sterile and non- sterilize environment had not significantly difference after two weeks observation. Table 2 shows that contamination occurred only in cultures of the autoclaved control and in the cultures grown with the limit concentration active chlorine below 0.0005 provided in the medium, which was congruent with data reported in pineapple smooth cayenne variety by Teixeira *et al*. (2006).

However, all the contaminations were apparently introduced into the cultures from the explants, since they all occurred at their base. This finding suggests that the higher chlorine concentrations in the medium may have been lethal for microorganisms introduced into the culture through the explants, which would be highly advantageous, especially when introducing explants originating from field donor plants into a culture. Teixeira *et al*. (2006) also reported that microorganisms carried into the culture flasks by explants did not survive at concentrations equal to or higher than 0.001% total active chlorine in the medium. Teixeira *et al*. (2006) showed that microorganisms introduced into the flasks from the surrounding air when the flasks were filled did not survive 0.002% or higher concentrations of total active chlorine.

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Table 2: Number and percentage of contaminated cultures, number of shoots, shoot length (cm), number of root and mean fresh biomass weight (g) of Ginger (*Zingiber officinale)* as a function of total active chlorine (%) added to the culture medium.

Total active chlorine (%)	contaminated cultures		Number of shoot	Shoot Length (cm)	Number of root	Fresh biomass weight (g)
	No. of jars	Percents (%)				
Control (autoclaved)	20	5	5.4[b]	8.4[b]	6.9[b]	1.93[b]
0.00025	20	20	3.3[c]	5.7[c]	4.6[c]	0.65[c]
0.0005	20	20	3.6[c]	4.9[c]	4.9[c]	0.72[c]
0.0025	20	0	7.2[a]	12.1[a]	6.6[b]	3.31[a]
0.005	20	0	6.8[a]	9.7[b]	9.2[a]	3.15[a]
0.0125	20	0	5.2[b]	8.5[b]	6.8[b]	2.12[b]
0.025	20	0	3.5[c]	5.1[c]	4.3[c]	0.51[c]

*Different letters in a column indicated that a significant difference at probability level less than 5% ($P < 0.05$) according to Duncan's new multiple range test (DNMRT)

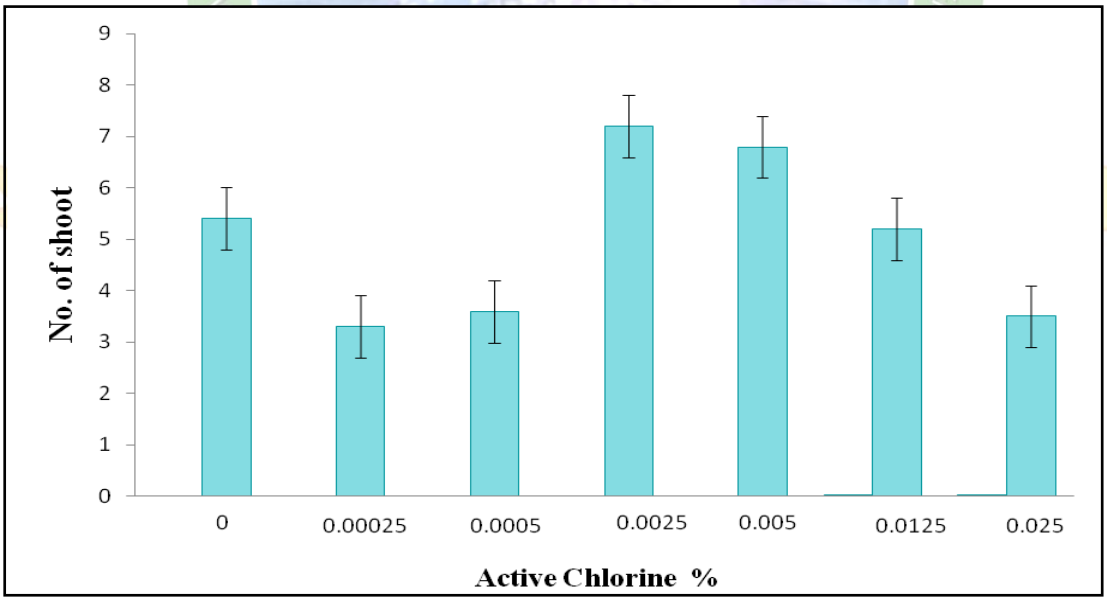

Fig.1: Comparison of shoot number with different 5% (v/v) concentration of active chlorine in percent provides to MS media.

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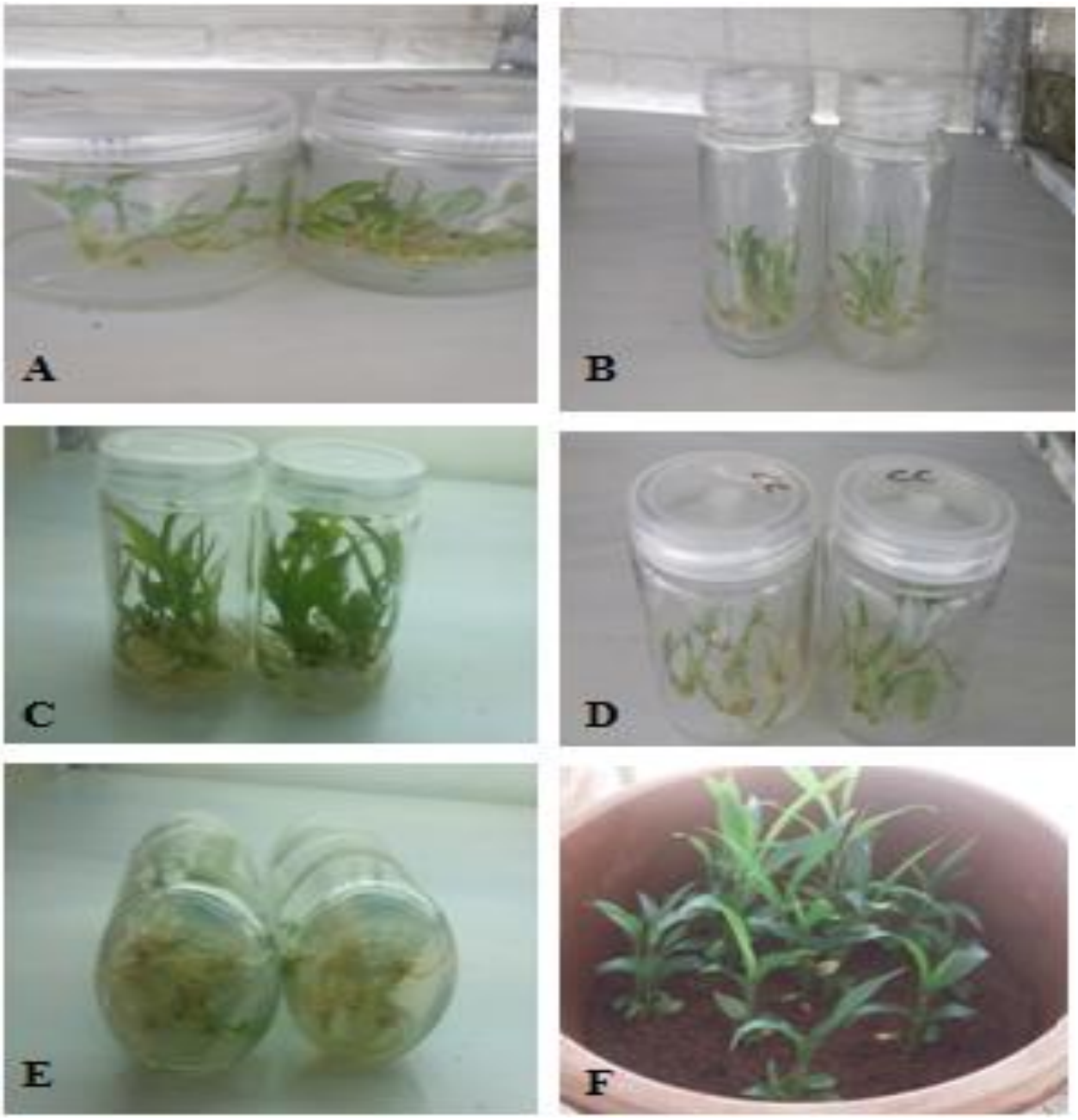

Fig.2: Growth of a new shoot from ginger cultured for five weeks showed (A) control autoclaved basal MS (B) applying 0.00025% active chlorine, (C) applying 0.0025% active chlorine, (D) applying 0.025% active chlorine, (E) rooting by applying 0.0025% active chlorine and (F) acclimatizing in said green house raise from 0.0025% active chlorine

CONCLUSION

Among the difference dose of active chlorine sterilizing agents that provided 100% sterile conditions of MS medium at the concentration of 0.005% active chlorine of 5% (v/v) of total active chlorine filling to a litter of MS medium were effective for eradicate microorganisms

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causal agents of *in vitro* contamination and provided completely sterile condition of solid MS medium without autoclaving to initiated ginger shoots and roots. Moreover these sterilizing agents added in MS medium can be used for culturing ginger shoots and the growth of shoots were comparable to those obtained from autoclave medium.

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