Effect of Chitosan and Chlorocholine Chloride on the Minituberization of Cocoyam (*Xanthosoma sagittifolium* L. Schott)

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Abstract**—*This study was carried out in order to evaluate the effect of chitosan (1, 2 and 3gL^{-1}) and chlorocholine chloride (5, 10 and 15mgL^{-1}) on the minituberization of cocoyam (Xanthosoma sagittifolium). Results showed that both phytohormones reduced the growth cycle of cocoyam from 6 to 5months with the best results obtained at the concentrations 2gL^{-1} and 15mgL^{-1} for chitosan (CTH) and chlorocholine chloride (CCC) respectively. The average number of leaves was greater in CTH at 2gL^{-1} (3.80±1.40) and 10mgL^{-1}(3.60±0.70) for CCC. The optimal height of the plant for CTH was obtained at 1gL^{-1} (17.87±5.47 cm) and 15mgL^{-1} (21.15±1.99 cm) for CCC. The average leaves surface was greater at 1gL^{-1} (36.39±17.02 cm^2) for CTH and 15mgL^{-1} (25.87±5.62 cm^2) for CCC. The number of minitubers harvested as well as the percentage of tuberization was maximum with 29minitubers at 2gL^{-1} and 23 for 15mgL^{-1}.The size of the minitubers was best at 3gL^{-1}(2.60±0.26 cm) for CTH and 10mgL^{-1} (0.56±0.27 cm) for CCC. The mass of the minitubers increased with best results obtained at 3gL^{-1}(2.80±0.84g) and at 10mgL^{-1} (0.20±0.36g) for CTH and CCC respectively.*

Keywords**— chitosan, chlorocholine chloride, minitubers, minituberization,Xanthosoma sagittifolium.**

I. INTRODUCTION

Cocoyam (*Xanthosoma sagittifolium)* is among the world's six most important root and tuber crops [1].It is pantropical and has been domesticated in most communities in Oceania, Africa, and Asia [2]providing sustenance for over 400 million people [3,4].Africa is the major producer with West and Central Africa, notably, Nigeria, Ghana, and Cameroon contributing to over 60% of the total African production [5].Thus, the importance of cocoyam to regional food security cannot be overstated. In spite of this growing importance, the production of cocoyam has been stagnant for many years. This is mainly due to (1): the low productivity of planting material [6],(2): the low availability of traditional planting material (corm cuttings) and (3): viral and fungal infections [7].In Cameroon, the main pathogen of cocoyam is*Pythium myriotylum*, which causes root rot and is responsible for up to 90% loss in yield in some plantations[8].Meristem culture technique is used to produce plants free of viruses and fungi especially in vegetative propagated plants [9].Plant tissue culture techniques have becomeapowerful tool for propagation of cocoyam to overcomemany problems facingtraditional methods of propagation. Different explants were used to produce disease free planting materials [10].

Many authors have shown that it is possible to produce tubers in *in vitro* conditions and that the tubers can be considered as seeds through the technique of microtuberization (*in vitro*) or minituberization (*in vivo*) [11].It has been showed that the addition of silver nitrate in the medium of microtuberization permits to inhibit the activity of ethylene what favorizes the good unrolling of tuberization in Irish potatoes [12].In agriculture, chitosan is used primarily as a natural seed treatment and plant growth enhancer, and as an ecologically friendly biopesticide substance that boosts the innate ability of plants to defend themselves against fungal infections [13].Agricultural applications of chitosan can reduce environmental stress due to drought and soil deficiencies, strengthen seed vitality, improve stand quality, increase yields, and reduce fruit decay of vegetables [14].Soluble chitosan helps acclimatization *in vivo*, and increase yield and seed quality of minitubers of treatments of different concentrations with best minituber number and yield in controlled conditions also increased as shown in the work done on potato by [15].Chlorocholine chloride (CCC) is a biosynthesized inhibitor widely used in tissue culture media to promote microtuber formation [16].Although CCC stimulates tuber initiation by recalcitrant genotypes, it can inhibit microtuber growth in *Solanum tuberosum* cultivars that form tubers readily in its absence [17].

This research was aimed at studying the effect of chitosan and chlocholine chloride on the minituberization of cocoyam (*Xanthosoma sagittifolium* L. Schott). More specifically; to produce vitro plants of white cultivars of *Xanthosoma sagittifolium,* introduce minituberization in *Xanthosoma sagittifolium* through the supply of chitosan and chlorocholine chloride.

II. MATERIAL AND METHOD

2.1. Cocoyam plantlets culture

The plant material constitute of cocoyam *vitro* plants obtained through *in vitro* culture of the apex of white cultivar of *Xanthosoma sagittifolium* harvested from the farm. The explants issued from plants were disinfected according to the method of [18]modified. After one month of in vitro culture, young cocoyam plants with a root system and well developed leaves, agar was washed away with tap water, and then transferred in plastic pots containing a mixture of black soil, sawdust and sand in the ratio 2:1:1 readably sterilized in an oven (REPLEX mark) at 170°C for 48 hours. The union (pots + plantlets) were placed in the culture room. The union was then watered with tap water in the morning before sunrise and in the evening after sunset. The plants were head dressed with a transparent lid to keep a high humidity. After 14 days, the lids were taken off. The union was again left for 14 days before being transferred out of the culture room away from precipitations and sun rays. After others 14 days of acclimatization in ambient temperature, the plantlets were thus ready for the different treatments. The numbers of leaves were counted, height of plants measured with a ruler and the leaf surface was gotten by measuring the length and width and their averages were later calculated.

2.2. Effect of chitosan and Chlorocholine chloride on the growth of cocoyam

2.2.1. Morphological analysis

2.2.1.1. Treatment of plantlets and induction of minitubers

The induction of minitubers was realized on the action of chitosan (CH) and chlorocholine chloride (CCC) Thus, different treatments were realized. For each treatment, 45 plantlets were used divided in to 3 plots of 15 plantlets each. 10 mL of the mineral solution (constituted of macro and micro elements presents in the Murashige and Skoog solution) [19]were supplied to the plantlets every 10 days. The treatments of plantlets placed in plots with 5ml of different concentrations Chitosan (1 g/L, 2 g/L and 3 g/L) and chlorocholine chloride (5 mg/L, 10 mg/L and 15 mg/L) was apply after every 20 days. On day 100, the harvest took place due to total yellowing of the leaves of the treated plantlets.

The harvested minitubers were weighed and their height measured according to the different treatments and compared to the control.

2.2.1.2. Statistical analysis

During induction of minituberization, the height of plants was measured until harvest. The minitubers harvested was weight and their number was also determined. The percentage of tuberization was also determined for each treatment. All the statistical analysis were done using excel for the treatment and realization of curves and histograms. Student-Newman Keul's and Duncan's test with the least significant difference of 5 % were used for the comparative analyses of the results with the help of SPSS 16.0.

III. RESULTS AND DISCUSSIONS

3.1. Morphological analysis of cocoyam plantlet during minituberization

3.1.1. Average number of leaves of plants per treatment with time

The average number of leaves in the presence of CH, increased in all treatments from D_0 and then decrease till D_{100} with the highest obtain with 2g on D_{40} of 3.80±1.40b and the lowest at 3g on D_{100} of 1.90±0.57c. There exist no significant difference between the control 1g but it exists between the control, 2g and 3g. (Table.1). Also with CCC there exist a significant between the control, 5mg and 15mg on D_0, D_{40} and D_{80} as well as 10mg from D_{20} to D_{100}.The highest was obtained with 10mg on D_{20} of 3.60±0.70b and the lowest with 5mg on D_{100} of 2.10±1.29a (Table.1).

3.1.2. Average height of *X. sagittifolium* plants per treatment with time

There exist a significant difference between the control, 2g (D_{40}-D_{100}) and 3g (D_{80}-D_{100}) but no significant difference between the control and 1g (Table.1). The maximum average height for plants treated with CTH was obtained with 1g on D_{20} of 17.87±5.20ab cm and the minimum with the control on D_{80} of 8.69±3.76ab cm. In the presence of

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CCC, the average height of the plants decreased from D_0 to D_{100} in all treatments. There is a significant difference between the control and all treatments on D_0, D_{40} and D_{100} with a maximum height obtained with 15mg on D_{20} of 21.15±1.99a cm and a minimum with 5mg on D_{60} of 8.43±2.89a cm (Table.1).

3.1.3. Average leaf surface of *X. sagittifolium* plants with time

The average leaf surface of the plants in the presence CH, decreased from D_0 to D_{20} in all treatments including the control with the lowest value of 12.07±12.31a cm^2 obtained on D_{40} and the maximum average leaves surface with 1g on D_0 and D_0 and D_{40} of 36.39±17.02d cm^2 and 31.26±16.37c cm^2 respectively (Table.1). Also with CCC, the average number of leaf surface increased from D0 to D40 with a maximum of 25.87±5.62 cm^2 at 15 mg/L on D_{40}. After D_{40}, the average leaf surface decreased and the lowest value of 10.65±5.57a cm^2 was obtained at 5 mg/L on D_{80}.There exist a significant difference between the control and 5mg from D_{60}-D_{100} and with 10mg and 15mg from D_0-D_{80} (Table.1).

Table.1: Effect of chitosan and chlorocholine chloride on the growth of cocoyam plants

Parameters	Times (days)	Treatments						
		control	CTH (g/L)			CCC (mg/L)		
			1	2	3	5	10	15
Average Height of plants (HP) (cm)	D0	15.08±2.96a	17.87±5.47abc	15.59±4.81a	16.41±5.73a	15.33±3.29ab	20.44±4.38b	21.06±2.07b
	D20	14.69±3.21a	17.87±5,20ab	15.94±4.70a	16.82±6.97a	15.38±3.31a	20.52±4.35a	21.15±1.99a
	D40	12.70±3.71a	17.83±5.06b	16.75±4.65ab	16.22±6.96a	12.90±6.00ab	20.34±4.22a	20.85±1.74ab
	D60	9.29±4.69a	16.45±4.56b	15.64±2.68b	16.48±6.47a	8.43±2.89a	15.12±4.88a	14.49±4.44a
	D80	8.69±3.76ab	16.89±5.01c	14.65±3.88c	14.98±7.07a	7.71±3.44ab	14.53±2.82ab	12.47±3.97ab
	D100	9.76±3.21ab	14.92±3.67c	15.13±3.59c	15.67±5.54a	8.75±3.22a	12.06±6.15a	13.21±2.67a
Average Number of leaves (NL)	D0	3.20±1.14b	3.00±0.82b	2.90±1.10ab	2.20±0.63ab	2.60±0.52a	3.10±0.57b	3.00±0.47c
	D20	3.10±0.88a	3.20±1.55a	3.60±1.35a	2.70±1.25ab	3.30±0.48a	3.60±0.70b	3.00±0.67b
	D40	2.90±0.88ab	3.00±1.33ab	3.80±1.40b	2.60±0.97ab	3.00±0.82a	2.50±1.27b	3.00±0.94b
	D60	2.80±1.14a	2.05±0.85a	2.80±1.32a	2.20±0.92b	2.40±0.70a	2.40±1.08b	2.40±1.06b
	D80	2.80±0.94b	2.30±0.68ab	2.20±0.79ab	2.00±0.47c	2.50±1.354a	2.40±1.08c	2.80±1.03ab
	D100	2.70±0.68a	2.20±0.79a	2.30±0.48a	1.90±0.57c	2.10±1.29a	2.30±1.06abc	2.60±1.17bc
Average Leaves surface (SF) (cm^2)	D0	15.87±6.63a	36.39±17.02d	29.79±7.41cd	26.11±8.57bc	16.04±5.55a	20.98±5.79ab	24.31±5.05abc
	D20	15.20±6.92a	29.42±11.78b	25.91±8.29b	24.56±11.50b	14.98±5.30a	22.04±7.12ab	23.96±5.11b
	D40	12.07±12.31a	31.26±16.37c	28.40±10.71bc	26.90±10.62bc	12.72±5.42a	20.91±6.47ab	25.87±5.62bc
	D60	14.71±11.89ab	26.81±15.46c	24.19±8.59bc	26.39±10.64c	11.55±3.28a	21.79±6.59bc	18.67±7.81abc
	D80	12.57±6.37ab	26.82±15.60c	21.53±10.03bc	22.01±11.97bc	10.65±5.57a	22.23±10.43bc	17.6±7.72abc
	D100	15.54±6.19ab	20.72±5.16c	21.79±8.69c	21.92±8.77c	12.37±5.28a	19.17±9.85ab	18.74±4.41ab

3.2. Minitubers harvested according to the treatment of chitosan and chlorocholine chloride

From results, an increase in the number and weight of the minitubers was obtained with best results from CTH treatments compared to CCC treatments. The maximum results of 29 minitubers were obtained at 2 g/L with CTH and 23minitubers with CCC was obtained at 15 mg/L. The lowest values obtained were 9 minitubers at 3 g/L and 4 mg/L at 5 mg/L (Fig.1.).

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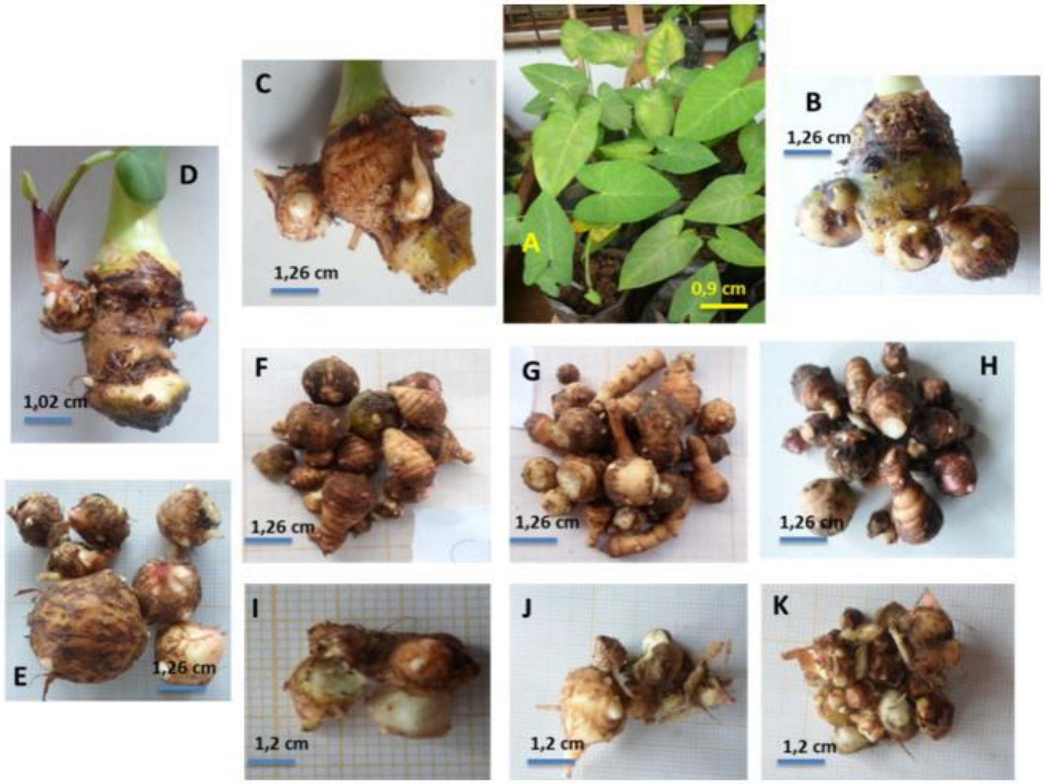

Fig.1: Minitubers harvested: Young plants ready for harvest (A): Plant treated with CTH(B):Plant treated with CCC(C): Control(D): Minitubers obtained from the; control(E), 1g CTH(F), 2g CTH(G), 3g CTH(H), 5mg CCC(I), 10mg CCC(J) and 15g CCC(K).

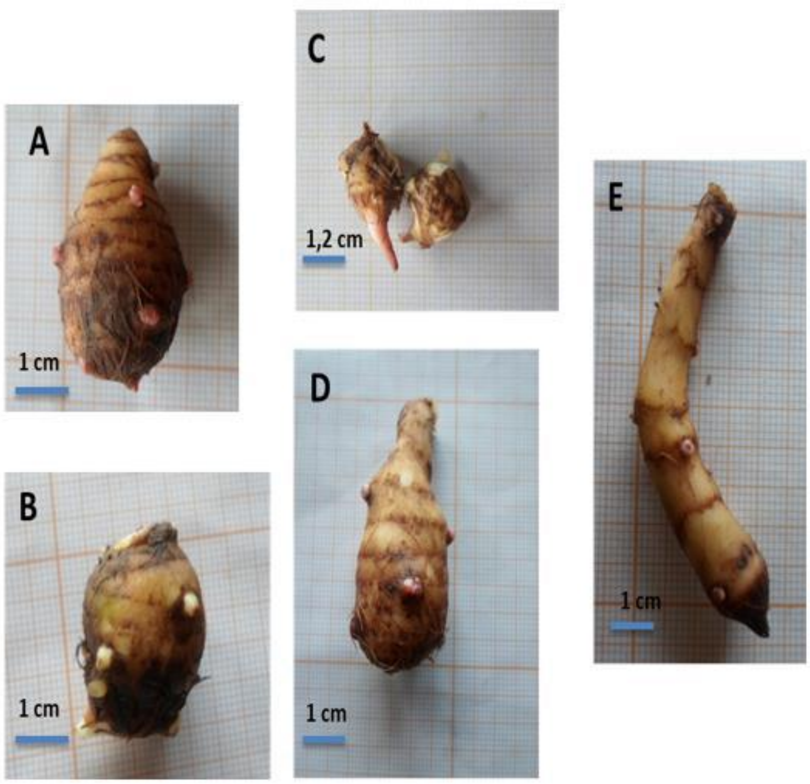

Fig.2: Shape of minitubers harvested: Oval (A), Round (B), Button (C), Pear (D) and crescent (E)

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3.2.1. Number of minitubers

The number of minitubers in the presence of CTH, increased with a maximum of 29 minitubers at 2 g/L and the lowest number of minitubers of 9 obtained with 3 g/L compared to the control of 18 minitubers with a great significance of 5% existing between all the treatments compared to the control (Fig.3). In the presence of CCC, the number of minitubers obtained increased with the concentration and a maximum of 23 minitubers at 15 mg/L and the lowest number of minitubers of 4 obtained with 5 mg/L with a great significant difference 5% between all the treatments and the control (Fig.4).

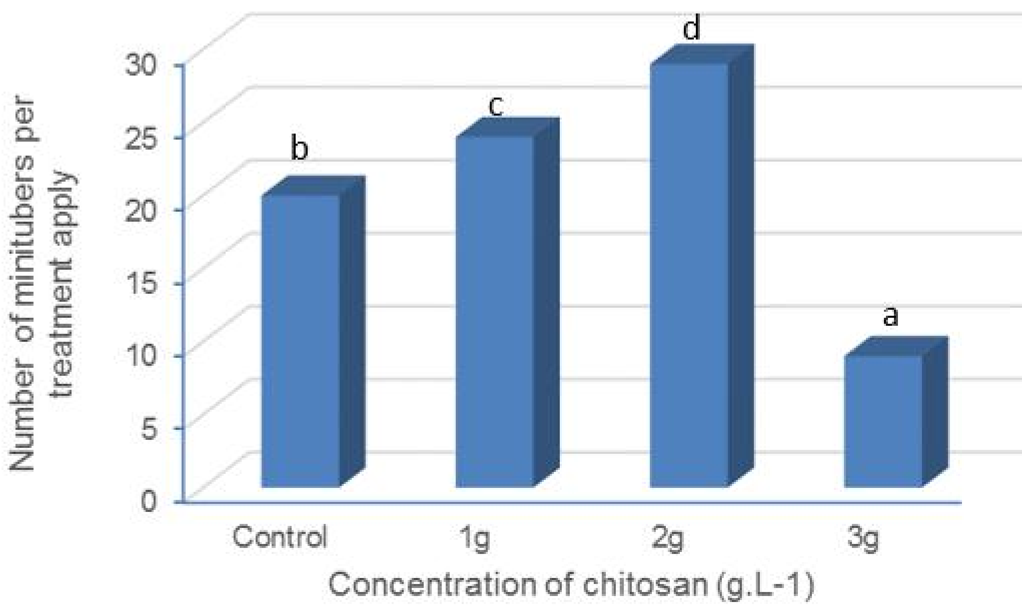

Fig.3: Number of minitubers per treatment of chitosan applied.

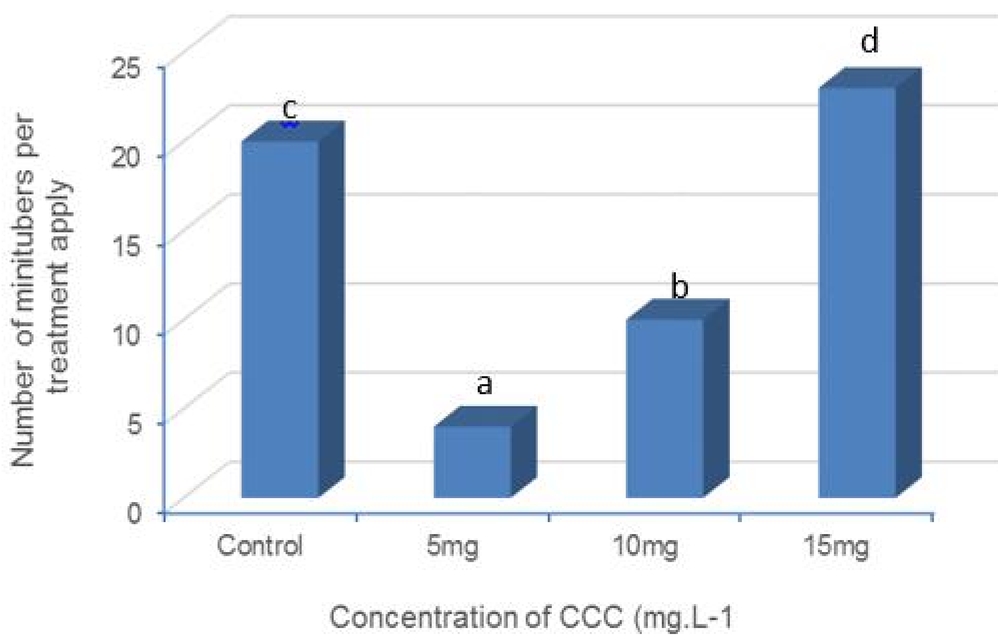

Fig.4: Number of minitubers per treatment of chlorocholine chloride applied.

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3.2.2. Percentage of tuberization

The percentage of tuberization in the presence of CTH, increased with a maximum of 92.31 % at 2 g/L and the lowest percentage of tuberization of 38.46 % obtained at 3 g/L compared to that of the control of 61.54 % and a great significant difference of 5% between all the treatments and the control (Fig.5). In the presence of CCC, the percentage of tuberization increased with the concentration with a maximum of 61.54 % at 15 mg/L, same obtained with the control hence no significant difference between 15mg/L and the control. The lowest percentage of tuberization of 15.39 % was obtained at 5 mg/L (Fig.6).

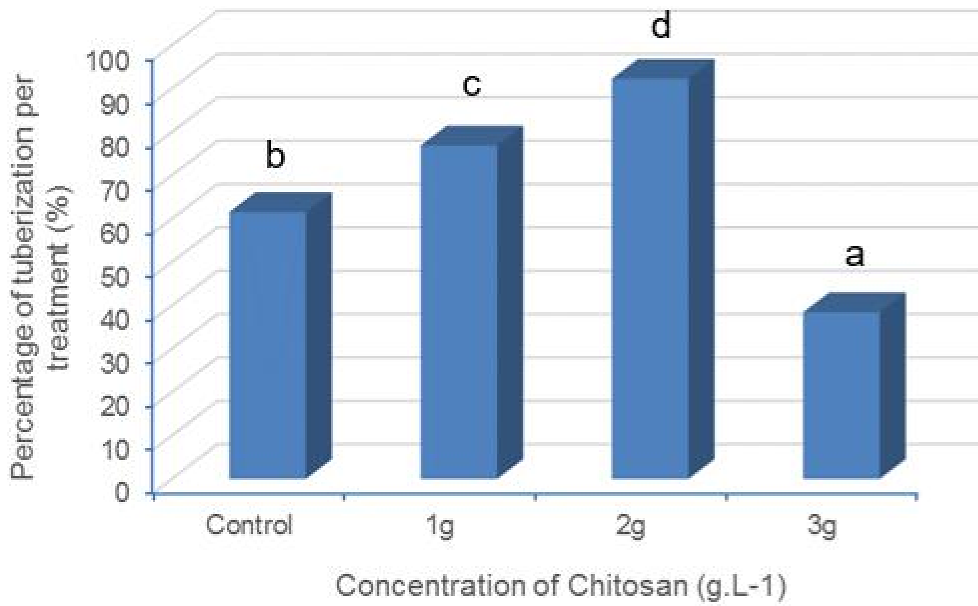

Fig.5: Percentage of tuberization per treatment of chitosan applies

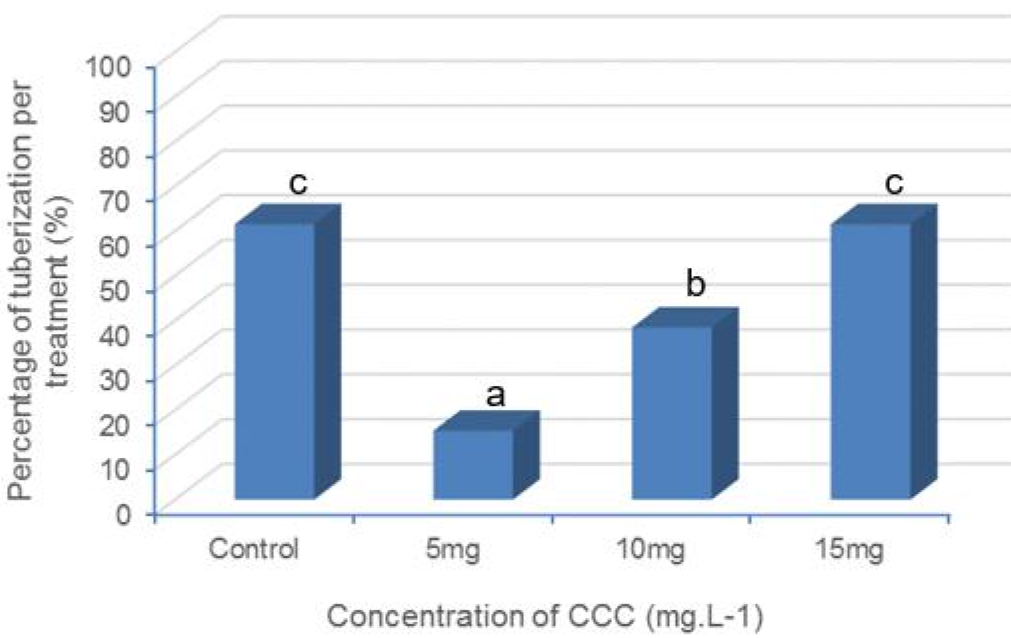

Fig.6: Percentage of tuberization per treatment of chlorocholine chloride apply

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3.2.3. Size of minitubers per treatment

There exist great significant differences between all CTH treatments with a maximum size obtained at 3g of 2.6±0.26cm and a minimum of 1.43±0.82cm compared to the control of 0.76±0.48cm (Table.2). There also exist a great significant difference between all treatments of CCC with a maximum size obtained at 10mg of 0.56±0.27cm and a minimum at 5mg of 0.24±0.24cm compared to the control (Table.2).

3.2.4. Mass of the minitubers per treatment

For the average mass of minitubers harvested from plants treated with CTH showed a significant difference compared to the control with a maximum mass obtained at 3g of 2.8±0.84g and a minimum at 1g of 1.47±0.42g (Table.2). There also exist significant differences between all treatments of CCC compared to the control with a maximum value obtained at 10mg of 0.20±0.36g and a minimum at 5mg of 0.06±0.03g.

Table.2: effect of chitosan and chlorocholine chloride on the size and the mass of minitubers harvested.

Parameters	Treatments						
		CTH ($g.L^{-1}$)			CCC ($mg.L^{-1}$)		
	Control	1	2	3	5	10	15
Average size of minituber (cm)	$0{,}76\pm0{,}48^{d}$	$1{,}43\pm0{,}82^{e}$	$1{,}96\pm0{,}41^{f}$	$2{,}6\pm0{,}26^{g}$	$0{,}24\pm0{,}24^{a}$	$0{,}56\pm0{,}27^{c}$	$0{,}50\pm0{,}22^{b}$
Average mass of minituber (g)	$0{,}51\pm0{,}95^{d}$	$1{,}47\pm0{,}42^{e}$	$1{,}80\pm0{,}56^{f}$	$2{,}8\pm0{,}84^{g}$	$0{,}06\pm0{,}03^{a}$	$0{,}20\pm0{,}36^{c}$	$0{,}12\pm0{,}13^{b}$

Data sharing the same letter in the same line are significantly different at 5% level (Duncan's multiple range tests)

IV. DISCUSSION

The morphological analysis shows that plants treated with chitosan compared to plants treated with CCC permitted to obtain best performances on the quality and quantity of minitubers with relation to those obtained from traditional cuttings. This may be due to the fact that the plantlets are from the origin, exempted from all contaminations which are contrary to traditional cuttings which carry pathogen microorganisms, susceptible of limiting the production of minitubers. This result is in concordance with the work of [20],who showed that the quality of the plant materials influence the production of yam (*Discorea alata* L.). Their works stipulates that, to obtain minitubers from plantlets, it takes twenty weeks which is in concordance with our work where by the harvest of minitubers took place 171days after planting.

From this work, the average number of leaves, average height of the plants and average leaf surface, increased with the concentration of Chitosan, but decreased with time, this is in agreement with the works of [21],who realized the application of Chitosan solution ranging from 4-250 ppm significantly enhanced the vegetative growth, yield and quality of okra. [22]also remarked that chitosan increased the growth and yield of coffee and had a highly positive correlation with chlorophyll and carotenoid accumulations in the leaves and additionally, may increase mineral uptake and stimulate the coffee growth rate. It was confirmed that chitosan is a second source composed not only of carbon and nitrogen but, some other elements in the chain which are essential minerals for the growth of the plant[23].They also noticed that chitosan may also be attributed to the promoting effects on nutrient uptake and nutritional status: nitrogen, potassium and phosphorus especially result in higher plant growth. Work on soybean in 2010, also noted that even though the mineral composition of the soil that was mixed with chitosan before and after cultivation of soybean, was unchanged [24]. He explained it was because the content of nitrogen, potassium and phosphate significantly increased with the application of chitosan.

Results also shows that the average number of leaves of plants treated with CCC decreased while average height and leaf surface increased with the concentration and time. These results are in agreement with those of [25] on gaur cultivars who reported that exogenous application of CCC significantly increased the leaf surface area per plant contrary to this report, some reports says that exogenous application of CCC, significantly reduced the total leaf area in plants like soybean cultivar [26]and *Brassica juncea*[27]On the other hand, the beginning of the decrease in number of leaves, height and leaf surface of the plants also marks the beginning of minituberization of cocoyam.

The number of tubers increased with the concentration in the plants treated with chitosan of concentration 1 g/L and 2 g/L. The best tuberization with chitosan was obtained with 2 g/L (92.31 %) and 15 mg/L (61.54 %) for CCC. These results are in line with that obtained by [28] on *Solanum tuberosum* L. which showed most effective improved acclimatization of plantlets in the greenhouse as expressed by significance in the number of minitubers and yield of potatoes with 500 mg/L. This can also be explained by the report of chitosan known as a growth promoter in various crops such as soybean sprouts [29].With CCC, the number of minitubers as well as the percentage of tuberization increased with continuous increase of the concentration, this is similar to the results obtained by [30]on potatoes, who realized increasing the rate of CCC increased the number and average weight of microtubers recorded at 500 mg/L CCC. The weight and height of the minitubers also increased with the concentration which is in line with the results obtained by [31]on potato, they found that the maximum number of microtubers per plants with 500 mg/L CCC and the weight of microtubers, decreased with the rate of CCC concentration. The highest tuber weight was recorded in the absence of CCC while the minimum at 500 mg/L (145.7mg) which is in agreement with those of [32]but disagree with those of [33]with relation to the weight. The height of the minitubers treated with CCC are smaller compared to the control and plants treated with chitosan. These results were obtained because according to [29],CCC produces a reduction in the height without malformation by reducing cell elongation and also by lowering cell division.

V. CONCLUSION

The general objective of this work was to study the effect of chitosan and chlorocholine chloride on the minituberization of cocoyam (*Xanthosoma sagittifolium* L. Schott). The results obtained shows that the plantlets which were treated with different concentrations of chitosan (1 g/L, 2 g/L and 3 g/L) are more productive with best results compared to plants supplied with different concentrations of CCC (5 mg/L, 10 mg/L and 15 mg/L). The morphological studies shows that the best results were obtained from plants treated with chitosan of concentration 2 g/L. It produced the greatest average number of leaves, height of plants, number of minitubers and weight of minitubers whereas in CCC, the best result was obtained with 15 mg/L.

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