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EFFECTS OF DIFFERENT CROTON (*Codiaeum variegatum* L.) GENOTYPES AND GROWTH REGULATORS ON CALLUS INDUCTION, MICRO PROPAGATION AND ANTIBACTERIAL ACTIVITIES

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ABSTRACT: The Main aim of this study was determine the effect of different croton (*Codiaeum variegatum*) genotypes (Gold Dust and Petra) and growth hormones on callus induction, micro propagation and antibacterial activities. Murashige and skoog (MS) medium with four compositions of growth hormones for callus induction, mursh M1 (2 mg/l 2, 4 Dichlorophenoxy acetic acid (2, 4-D), M2 (3 mg/l 2, 4-D), M3 (2mg/l 1-Naphthalen acetic acid (NAA), M4 (1mg/l 2, 4-D + 1 mg/l NAA) were used. For micro propagation, four different hormone balance were used, *i.e.,* M1 (1 mg/l Benzyl adenine (BA) + 25 mg/l peptone), M2 (1mg/l BA + 50mg/l peptone), M3 (3mg/l BA + 25mg/l peptone), M4 (3mg/l BA + 50mg/l peptone).For rooting induction the combinations of growth regulators were used as follows, Mr1 (1mg/lIndolbuteric acid(IBA), Mr2 (2mg/l IBA), and Mr3 (1mg/l IBA+ 1mg/l NAA). Results showed that the highest genetic response for callus induction frequencies were seen for Petra genotype (91.25%) followed by Gold Dust. Medium M3 showed the highest response for callus induction frequencies (82.4%) followed by M1.Interaction between genotypes and media each alone were highly significant in callus fresh weight character. Regarding the micro propagation, Petra genotype gave highly genetic response followed by Gold Dust, also medium (M1) gave highly response for micro propagation followed by M2. *In vitro* roots were successfully induced by (1 or 2) mg/l of IBA which gave longest and few roots, while 1mg/l NAA gave shorter and more root number. Plants with roots were moved to the green house for acclimatize in pots contain sand /farmyard manure (8:2VN). Alkaloids extracted from callus for both genotypes were detected for antibacterial activities against Gram positive and negative bacteria. Alkaloids extracted from calli for media M1 and M2 were gave a highest antibacterial activity. Finally the present results gives scientific evidence on the ideal composition of the media to product the alkaloid extracts from *C. variegatum* (Gold Dust and Petra) as medicinal plants to be used as antibacterial agents against different pathogens.

Key words: *Codiaeum variegatum*, Tissue culture, antibacterial activity, acclimatization.

INTRODUCTION

The croton (*Codiaeum variegatum*) plant belongs to the family Euphorbiaceae is one of the beautiful indoors and out door plants need extensive agriculture development. The leaves extracts of crotons are reported to have many medicinal properties including purgative, sedative antifungal and anti-cancerous activities **(Deshmukh and Borle, 1975; Kupchan *et al.,* 1976)**. The plant is also a good nature source for the production of secondary metabolites of alkaloids, tropenes and flavonoids **(Maciel *et al.,* 1998; Puebla *et al.,* 2003; Simona *et al.,* 2008)**. Croton can be propagated by various methods such as cuttings, grafting, by seeds and air layering. From shoot tip cuttings, one mother/stock plant can yield only 20 plants per

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year. Further 200 varieties of the croton plants in the world and different in leaf sizes, shapes and color patterns. The agricultural strategy is now much on to the ornamental plants production for local and exportation **(Sana *et al.*, 2012)**. The result of the slow rate of multiplication, this made it very high in demand. Although an Alkaloids are not common in Euphorbiaceae, but some croton species are prominent for their alkaloids.The most croton alkaloids are compounds identical or similar to substances found in biogenetically and related to benzylisoquinolines, such as morphinandienones and tetrahydroprotoberberine alkaloids. Glutarimide alkaloids and a new class of sesquiterpene guaiane- type alkaloids have been obtained from Croton species **(Antonio *et al.*, 2007).**

Micropropagation is a comparatively new technology and application of micropropagation method has play a role to overcome handicap the progress in the multiplication of these species with further improvements are expected. *In vitro* growth and development is very influenced by several factors like genotypes, the size and age of mother plant and explant, the growth conditions, media composition, season and various other physiological factors **(Nasib *et al.*, 2008).**

The present study aimed to discover the best hormone balance, which encourage plant regeneration as well as callus induction for production of medicinal secondary products and then produce new tissue culture protocol for this plant species, also study of interactions and effects of growth hormones and cultivars on morphological characteristics and total alkaloids production and antibacterial activities for its alkaloids products.

MATERIALS AND METHODS

Plant Material

Two cultivars of croton *Codiaeum variegatum* L. (Petra and Gold Dust) were obtained from Fac. Agriculture, Ain Shams Univ. Collected plants were cultivated in greenhouse of genetics Dept., Fac. Agric., Zagazig Univ., under natural conditions.

Two cultivars (Fig. 1) were used in the present study, Petra cultivar (broad leaves with red veins and margin) and Gold Dust cultivar (small leaf with bright yellow dots) according to **(Deng *et al.*, 2010)**.

Surface Explant Sterilization

Explants were collected (leaf-stem), washed completely under running water for 15 min followed by sodium hypochlorite solution 60% for 20 min., then by in $HgCl_2$ (0.1%) for 10 min. Explants then were washed 3 to 5 times with sterilized distilled water.

Callus Induction

Initiation of callus using internodal segments and leaves as explants

The sterilized explants were cut into 1.0- 1.5 cm long segments each stem contain two or single node. Explants were individually transferred into 200 ml jars containing 40ml of basal MS medium with 6% (*W/V*) sucrose and different combinations of 2,4-dichlorophenoxy acetic acid (2,4-D), and Naphthalene acetic acid (NAA). MS medium **(Murashige and Skoog, 1962)** with four combinations were used as following: M1 (2 mg/ l 2, 4-D), M2 (3 mg/l 2, 4-D), M3 (2 mg/l NAA), M4 (1 mg/l 2, 4-D + 1 mg/l NAA). Agar was used as solidifier with 0.8%. The pH was adjusted to 5.7 ± 0.1. Cultures were maintained at 25 ±1°C for a 16 hr., photoperiod using Gro-lux® fluorescent bulbs. Calluses were sub cultured every 45 days to record the following data:

Callus response

The initiation time of callus induction was calculated in the two cultivars and the effects of different composition of growth regulators on callus growth were determined.

Callus nature

The morphological characters (color, surface and rigidity) of calli after 45 days of cultivation were observed and recorded.

Callus induction frequency (%)

The percentage of callus formation recorded after 45 days of cultivation following equation: Callus formation (%) = (No. of that formed callus ÷ Total number of explants) × 100.

Gold Dust **Petra**

Fig. 1. Petra and gold Dust croton cultivars

Callus fresh weight (mg)

Fresh weight of different calli cultures were recorded after 45 days of cultivation. Each treatment was made in triplicate.

Determination of Total Alkaloid Content

Collection and Preparation of samples for alkaloid extraction

Freshly harvested known mass of leaves, stems and previously induced callus of all combinations for the two cultivars collected and used for alkaloids extraction. Samples were air dried then dried in an oven dryer at 60-70^{o}C. The dried samples converted to powder by a grinder. Then save powdered samples in clean closed glass containers until preparing the alkaloids extraction.

Extraction of alkaloids

Five grams of coarse powder was weighted and packed in a cheese cloth bag. Bags placed in soxhlet apparatus for extraction using 250 ml methanol 99%. The process of extraction continues for 24 hours or till the solvent in siphon tube extract become pure or clear.

Filtration and concentration of the extracts

After the extraction process, the extracts were filtered through Whatman© filter paper No. 1then concentrated by rotary evaporator. The residues appeared a dark brown liquid and stored at 4^{o}C as a stock.

Preparation of solutions

Bromo cresol green (BCG) solution was prepared by heating 69.8mg of bromocresol green with 3 ml of 2 N NaOH and 5ml distilled water until dissolved then the solution was diluted to 1L with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1L distilled water). Atropine standard solution was made by dissolving 1mg pure atropine (Sigma Chemical, USA) in 10ml distilled water.

Preparation of standard curve

Different aliquots (0.4, 0.6, 0.8, 1 and 1.2ml) of atropine standard solution were accurately measured and transferred to different separator funnels. Then, 5ml pH4.7 phosphate buffers and 5ml BCG solution were added and the mixture was shaked with 1, 2, 3 and 4ml of chloroform. In 10 ml volumetric flask, collected the extract and then adjust the volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm using a spectrophotometer Cary 50 Bio UV-visible (Varian, Italy) associated to a software Cary win UV (Varian, Italy). Blank prepared as above but without atropine **(Shamsa *et al.*, 2008)**.

Spectrophotometric assay

Evaluated total alkaloid content was carried out with a spectrophotometric method based the

reaction with BCG **(Shamsa *et al*., 2008)**, with appropriate changes. Each extract was dissolved in 3 ml of 2 N HCl and then filtered. Transferred one ml of this solution to a separatory funnel and washed three times with 10 ml chloroform. The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then, 5ml of pH4.7 phosphate buffer was added before adding 5ml of BCG solution and shaked vigorously. Furthermore the complex formed was extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10ml volumetric flask and diluted with chloroform. The absorbance of the complex in chloroform was measured at 470 nm.

Estimation of total alkaloids in samples

For the estimation of total alkaloids in the mother plant (leaf and stem) and callus tissues samples in the formulation, suitable aliquots of sample solutions were taken and color was developed as the method described above. Absorbance of the colored solution was recorded at 470 nm. The amount of total alkaloids in the samples were calculated using calibration curve (y= 0.0918x – 0.0026). The contents of the total alkaloids in the different samples were expressed in terms of concessive.

Antibacterial properties

Different pathogenic bacteria (Gram positive bacteria; *Bacillus subtilis, Staphylococcus aureus* and *Listeria monocytogenes* and Gram negative bacteria; *Escherichia coli, Escherichia coli g* and *Salmonella typhi Serratia marcescens*) were used to screen the antibacterial activity of four concentrations (25, 50, 100, 150 mg/ml) of each extract by disc diffusion method as performed by **(Bauer *et al.*, 1966; Somchit *et al.*, 2003)**.

Ampicillin (10 mg/ml) was used as positive control and sterile distilled water and methanol loaded on discs were used as a negative control. After incubation, the clear or zone of inhibition (ZH) around the discs was measured and the mean zone of inhibition diameters (mm) expressed as a measure of their antibacterial activity. The experiments were conducted three separate times.

Micro propagation

Nodal segments (1-1.5 cm) were used for micro propagation on MS medium with different combinations of 6-Benzyl amino purine (BAP) and peptone concentration. Eight treatments were used as following; M1 (1 mg/l BAP + 25 mg/l Peptone), M2 (1mg/l BAP + 50 mg/l Peptone), M3 (3 mg/l BAP + 25 mg/l Peptone), M4 (3 mg/l BAP+50 mg/l Peptone), M5(0.5mg/l BAP + 25 mg/l Peptone), M6 (0.5 mg/l BAP + 50 mg/l Peptone), M7 (1.5 mg/l BAP + 25 mg/l Peptone), and M8 (1.5 mg/l BAP+50 mg/l Peptone). All treatments had 2.5% sugar and 0.8% agar as a solidifying agent.

Each media formulation for each plant was inoculated by 24 explants. Data for morphological characters such as plant height, leaf length, thin, area and stem diameter were recorded after 60, 120, and 180 days and values are the means of 6 replications.

Root formation

Half MS medium with different concentrations of IAA and IBA were used as rooting induction treatments as follows; Mr1 (1 mg/l IBA), Mr2 (2 mg/l IBA), and Mr3 (1 mg/l IBA +1 mg/l NAA). All the media formulations for root induction had 2.5% sugar and 0.8% agar as a solidifying agent. Data for rooting percentage (%), root numbers and root length were recorded every week for six weeks and values are the means of 6 replications.

Acclimatization

The rooted plants were transferred to the green house for hardening under wet environment. The potting mix used was comprised of 80% sand and 20% farmyard manure. The transferred plants were monitored after every week for at least 6 weeks.

Statistical Analysis

All collected data were subjected to analysis of variance and means of treatments were compared with the least significant and highly significant difference (LSD) test at $P \leq 0.05$ and 0.01, respectively. The statistical calculations were performed with statistics software computer program version 9 (**Analytical Software, 2008**).

RESULTS AND DISCUSSION

The Effect of Hormone Balance on Callus Induction of Some Genotypes of Croton

Callus induction frequency (%)

Results presented in Tables 1 and 2 shows that Callus were induced on MS media with different concentrations of IAA and 2, 4-D after 45 days of culture. 2,4-D own up the highest yield of callus formation (92.5%) followed by the lowest yiedbor IAA (75%), respectively. All growth hormones that were used in this study are capable of inducing callus from explants. M1 and M2 were found to be the best media for induction of shoot from the explants of this plant with no significant difference among them. This is in agreement with the earlier findings of **(Lima *et al*., 2008 ; Faridah *et al*., 2012)**. Although Petra cultivar gave callus from both leaves and stem explants, the callus can't be obtained from leaves in gold Dust cultivar. These results were supported by those found by other authors. For instance, **(Landa *et al*., 1999)** did not observe callus formation in leaf explants of *Caryocar brasiliense* Camb. In the absence of 2,4-D. Otherwise, **Santiago (2003)** concluded that in leaf explants of *Piper hispidinervum* C. DC., the maximum callus production was achieved with the combination of 2,4-D and BAP. In general, for this species the growth regulator 2,4-D was necessary for the maximum callus production although, the combination of 2,4-D with NAA produces the lowest callus induction frequency.

Callus fresh weight (mg)

Results showed that, Petra cultivar gave the maximum fresh weight using M3 medium (9.19 mg) and M1 and M2 media were the best combinations for the Gold Dust cultivar (1.96, 1.70 mg) with no significant differences among them. M4 medium gave the lowest weight for both cultivars with 0.99 and 3.78 mg for gold Dust and Petra cultivars, respectively, as shown in Table 1 and Table 2.

Callus nature

The morphological characters of celli were observed after 45 days of cultivation. All Gold Dust induced celli show no differences in color between different media. The celli were white in color. Also all Petra induced celli from all media combinations have the same color (white greenish), except callus obtained from M4 shown to have white color. All calli obtained in this study were smooth and friable (Fig. 2).

The Effect of Hormone Balance on Total Alkaloids Content of Some Genotypes of Croton

Results showed that total alkaloids content in leaves of Petra cultivar (3.49 mg/g) were gave the highest significant content compared to stem of the same plant (1.82 mg/g). Also, total alkaloids content that obtained from the Petra cultivar was lower than total alkaloids content obtained from the mother plant (8.48 mg for leaves and 3.47 mg for stem). The same result were represented in Gold Dust cultivar, total alkaloids content from mother plant (7.92 mg for leaves and 6.16 mg for stem) was higher than total alkaloids content from callus (4.95 mg). In general, the total alkaloids content in leaves were highest compared to stem alkaloids content and Gold Dust cultivar was the best genotype in total alkaloids content (Tables 3 and 4). Furthermore, media caused significant differences of total alkaloids content and the best content was obtained from M1 and M2 media. This variation may be contributed for using of 2,4-D as stimulator hormone as showed in Table 5.

The Effect of Hormone Balance on Antibacterial Activities of Alkaloid Extracts

The antibacterial activity of alkaloid extracts from various compositions of media (M1, M2, M3 and M4) for cultivar Gold Dust show a wide variation between four media on alkaloid content production. The zone of inhibition experiment is shown in Table 6. The alkaloid extract from media (M1) was the highest extract produced inhibition zone with all of Gram positive and negative bacteria (Fig. 3). The largest zone of inhibition was observed against Gram positive bacteria *B. subtilis, S. aureus* and *L. monocytogenes* was (19, 18 and 23 mm) respectively, at concentration 150mg/ml. The value of main ± standard division (X ± SD) was also calculated and it was found that *L. monocytogenes* gave the highest value which was

Table 1. Means of squares (MS) and heritability in broad sense for callus criteria *i.e.* callus induction frequency and callus weight of two cultivars under study of croton plant

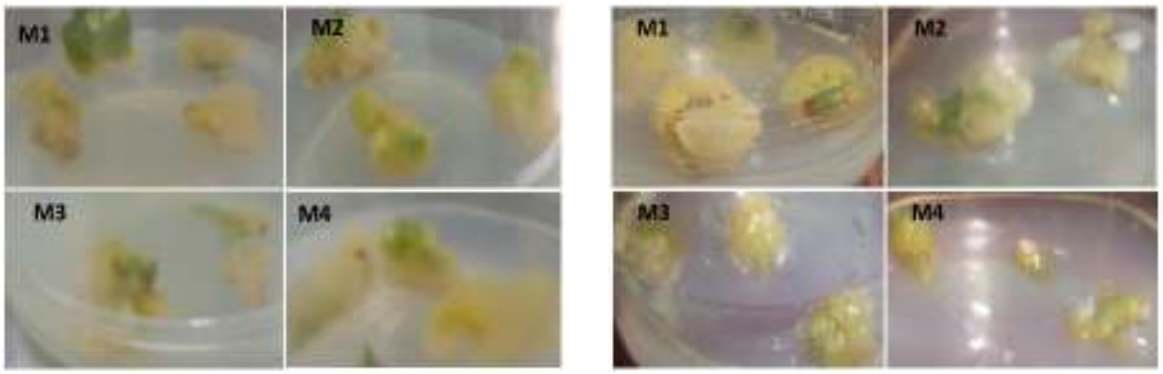
SOV	df	MS	
		Callus induction frequency (%)	Callus weight/mg
Reps	2	2.4**	0.04637**
Treats	7	2.8**	3.486**
Genotypes	1	8.1**	16.0297**
Media	3	3.3**	1.279**
G×M	3	0.66	1.449**
Error	14	0.37	0.0942
h^2		94.6%	

SOV=source of variation, df=degree of freedom, MS=mean sums of squere, * = Significant at P <0.05, ** = Significant at P < 0.01, Values are means of three replicates.

Table 2. Means of callus criteria *i.e.* callus induction frequency and callus weight of two cultivars under study of croton plant

Treatment	Callus induction frequency (%)		Mean	Callus weight/mg		Mean
	Gold Dust	Petra		Gold Dust	Petra	
M1	85	100	92.5	1.96	4.07	3.015
M2	85	95	90	1.7	8.34	5.02
M3	75	90	82.5	1	9.19	5.09
M4	70	80	75	0.99	3.78	2.38
Mean	78.75	91.25		1.412	6.345	
LSD 0.05	0.53		-	0.5362		-
LSD 0.01	0.74		-	0.744		-

LSD = Least significant deference, Values are means of three replicates.



Petra Gold Dust

Fig. 2. The effect of four media; M1, M2, M3 and M4 on callus morphology for the two cultivars

Table 3. Means of squares (MS) and heritability in broad sense for total alkaloids content (mg/100 g) from callus and mother plant of two cultivars under study of croton plant

SOV	df	MS			
		Callus		Mother plant	
		Gold Dust	Petra	Gold Dust	Petra
Reps	2	0.0025	0.275**	0.015	0.013
Genotypes	1	0.122**	0.292**	0.523**	4.148**
error	2	0.1292	.015	0.004	0.026
h^2			94.6%		

SOV = Source of variation, df = Degree of freedom, Ms= Mean sum of squere, * = Significant at P <0.05, ** = Significant at P < 0.01, Values are means of three replicates.

Table 4. Average means for total alkaloids content from mother plant (leaves and stem) of two cultivars under study of croton plant

Treatment / Mother plant	Leaf	Stem	Mean
Gold Dust	7.925	6.16	7.042
Petra	8.489	3.47	5.979
Mean	8.207	4.815	6.511
LSD 0.01	0.131	0.178	-
LSD 0.05	0.056	0.077	-

LSD=least significant deference, Values are means of three replicates.

Table 5. Average means for total alkaloids content from callus under different media of two cultivars under study of croton plant

Treatment	Callus				
	Petra		Mean	Gold Dust	
	Leaf	Stem		Leaf	Stem
M1	3.85	1.64	2.74	-	3.53
M2	3.49	1.82	2.65	-	4.59
M3	2.92	1.27	2.09	-	3.65
M4	2.12	1.15	1.63	-	3.17
Mean	3.09	1.47		-	3.73
LSD 0.01	0.306		-	0.168	
				0.255	
LSD 0.05	0.22		-	0.255	

LSD = Least significant deference, Values are means of three replicates.

(12 ± 4.7) compared to the other Gram positive bacteria. On the other hand, the M1 and M2 recorded highest values 20 ± 2.6 and 18 ± 3.0, respectively. The same concentration with Gram negative bacteria was for *E. coli* (20mm), *E. coli g* and *S. Typhi* (21mm) and with *S. marcescens* (14mm). The *E. coli, E. coli g* and *S. typhi* recorded highest values of (X ± SD) which were equal in mean but standard division ranged from 3.5 to 3.9. While M1 and M2 at 150 mg/ml recorded highest (X±SD) values 19 ± 3.3 and 17±2.3, respectively.

Minimum zone of inhibition ranged from (9 and 11 mm) in all Gram positive and negative bacteria at 25 mg/ml. Medium (M1) was the best between the other media followed by medium (M2) as an antibacterial activity compared to M3 and M4. All of media show the inhibition zone and all of pathogen bacteria have sensitive. On the other hand, alkaloid extracts from different media for Petra cultivar was a totally low as antibacterial activity about cultivar Gold Dust as shown in Table 7. The largest zone of inhibition at 150mg/ml showed also in alkaloid extract from media (M1) with Gram positive bacteria *S. aureus* which recorded (15mm), and ranged from (10 to 13 mm) with all the other Gram positive and negative bacteria (Fig. 4). The highest value of (X±SD) was (10 ± 2.1) with *B. subtilis* compared to the other Gram positive bacteria at 150 mg/ml. Also M1 and M2 media recorded highest values 13 ± 1.1 and 12 ± 0.5, respectively. However, Gram negative bacteria *E. coli* record highest X ± SD (9 ± 1.6). Media M1 and M2 were recorded highest (X ± SD) values (11 ± 1.2 and 11 ± 0.8), respectively at concentration of 150 mg/ml. For compare between two cultivars from where the minimum of inhibition which ranged in Petra cultivar from (7 and 8 mm) with all tested bacteria. No inhibition zone was recorded only in Petra cultivar with media (M4) at all concentrations with Gram positive and Gram negative bacteria. In general, the tested gram positive and Gram negative bacteria have sensitive to the selected extracts of both cultivars.

From this result, the two cultivars Gold Dust and Petra showed antibacterial activities. The antibacterial activity in cultivars due to high content from total alkaloid produced on media (M1) and (M2) compared with media (M3) and (M4). Regardless the aesthetic purposes of *C. variegatum*, it is also known to have medicinal uses, these include as a purgative and a sedative also has antifungal and anticancerous activities **(Christopher and Offeibea, 2014).**

Cytotoxicity of methanol extracts and alkaloids content of *C. variegatum* Petra were assessed against hepatocellular carcinoma, human Caucasian breast adenocarcinoma, colon cell line and lung carcinon and proved to be active with activity range of 17.3% - 98% **(Hassan *et al.,* 2013)**. While in Malaysian cultivars, tumour-promotor activity was observed in human lymphoblastoid cell line harbouring the Epstein-Barr virus (EBV) genome, Notice from the regular users of this plant **(Norhanom and Yadav, 1995).**

The present study gives the confirmation for the use of the alkaloide extracts from *C. variegatum* Gold Dust and Petra as antibacterial agents against different pathogens bacteria according to **(Jackie *et al.,* 2016).** They could be used as protective agents in disinfection and in process for drug or food supplement development. Also could be saving and effective in traditional medicines which lead to access to health care. The world health organization **(WHO, 2002)** reported at period from 2002 –2005 about traditional medicine strategy. Various studies have demonstrated that medicines extracted from medicinal plants can be developed as safe, effective and low cost as an alternative to the present medicines against bacterial infections **(Vermani and Garg, 2002)**.

The Effect of Hormone Balance on Microprpagion of Some Genotypes of Croton

Different concentrations of BA (6-Benzylamino purine), were used to assess its effect on the axillary shoot formation. The plant growth differs with changed in BA concentration. There was an increment in axillary shoot formation until the BA concentration reached to (1 or 3) mg/l. This might be due to the fact that the necessary concentration of each type of growth hormone differs greatly according to the plant being cultured **(George, 1993)**. Results clearly suggest that maximum shoot induction can be achieved by the use of 0.5 mg/l of BA. Peptone in different concentrations was tested to assess

Table 6. Antibacterial activity of alkaloid extracts for Gold Dust cultivar from different tissue culture media

Microorganisms	Zone of inhibition diameter (mm)																
	Concentration of alkaloid extract																
	25 mg/ml				50 mg/ml				100 mg/ml				150 mg/ml				
	Media				Media				Media				Media				X±SD
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	
Gram positive bacteria																	
B. subtilis	9	7	7	7	13	13	12	7	15	14	14	10	19	15	16	12	11±3.7
S. aureus	9	8	7	7	11	11	9	9	14	14	13	11	18	18	16	12	11±3.5
L. monocytogenes	11	11	8	7	14	12	10	8	19	17	11	9	23	21	12	11	12±4.7
X±SD	9±1.1	8±2.1	7±.5	7±0	12±1.5	12±1	10±1.5	8±1	16±2.6	15±1.7	12±1.5	10±1	20±2.6	18±3	14±2.3	11±.7	
Gram negative bacteria																	
E. coli	11	10	8	7	13	13	10	9	16	14	13	11	20	18	14	13	12±3.5
***E. coli* g**	9	9	8	8	12	11	10	10	15	15	13	12	21	19	15	15	12±3.8
S. Typhi	11	9	7	7	12	12	10	10	15	15	13	13	21	19	16	15	12±3.9
S. marcescens	9	7	7	7	10	8	8	8	12	9	9	9	14	14	10	9	9±2.2
X±SD	10±1.1	8±1.2	7±.5	7±.5	11±1.2	11±2.1	9±1	9±.9	14±1.7	13±2.8	12±2	11±1.7	19±3.3	17±2.3	13±4	13±2.8	

Table 7. Antibacterial activity of alkaloid extracts for Petra cultivar from different tissue culture media

Microorganisms	Zone of Inhibition Diameter (mm)																
	Concentration of alkaloid extract																
	25 mg/ml				50 mg/ml				100 mg/ml				150 mg/ml				
	Media				Media				Media				Media				X±SD
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	
Gram positive bacteria																	
B. subtilis	8	8	7	7	10	9	8	8	12	12	11	9	13	13	12	9	10±2.1
S. aureus	7	7	7	-	10	9	9	-	13	11	10	8	15	12	11	9	9±2.3
L. monocytogenes	7	7	7	-	11	10	8	7	12	11	10	8	13	12	11	9	9±2.1
X±SD	7±.7	7±.7	7±0	7±0	10±.7	9±.7	8±.7	7±.7	12±.7	11±.5	10±.5	8±.5	13±1.1	12±.5	11±.5	9±0	
Gram negative bacteria																	
E. coli	8	7	7	-	10	9	8	-	11	11	9	8	13	11	10	9	8±1.7
***E. coli* g**	8	7	7	7	9	9	8	8	11	10	10	9	12	12	11	9	9±1.6
S. Typhi	7	7	7	7	8	8	8	8	9	9	9	9	11	11	11	9	9±1.4
S. marcescens	7	7	7	-	9	8	8	-	10	9	9	-	10	10	9	-	8±1.1
X±SD	7±.5	7±0	7±0	7±0	9±.8	8±.5	8±0	8±0	10±.9	10±.9	9±.5	8±.5	11±1.2	11±.8	10±.9	9±0	

Gram positive

Gram nigative

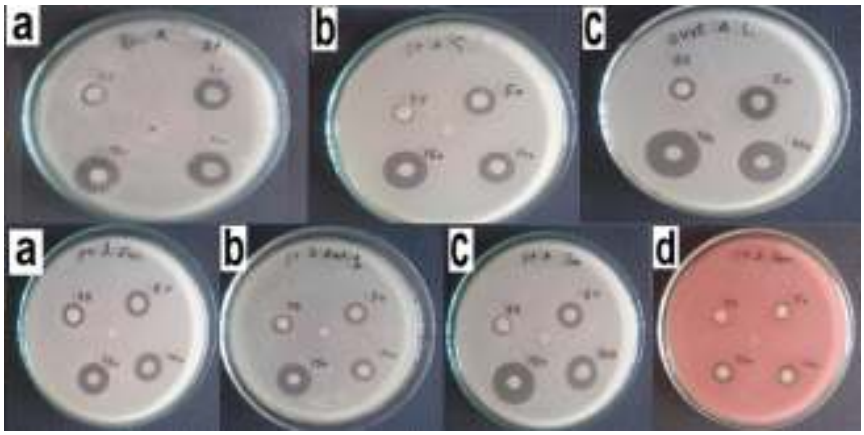


Fig. 3. Zone of inhibition (ZH) by different alkaloid extracts for Gold Dust croton cultivar produced from different media against Gram positive bacteria. a) *B. subtilis* b) *S. aureus* c) L. monocytogenes and Gram nigative bacteria. a) *E. coli* b) *E. coli* g c) *S. Typhi*. d) *S. marcescens*

Gram positive

Gram nigative

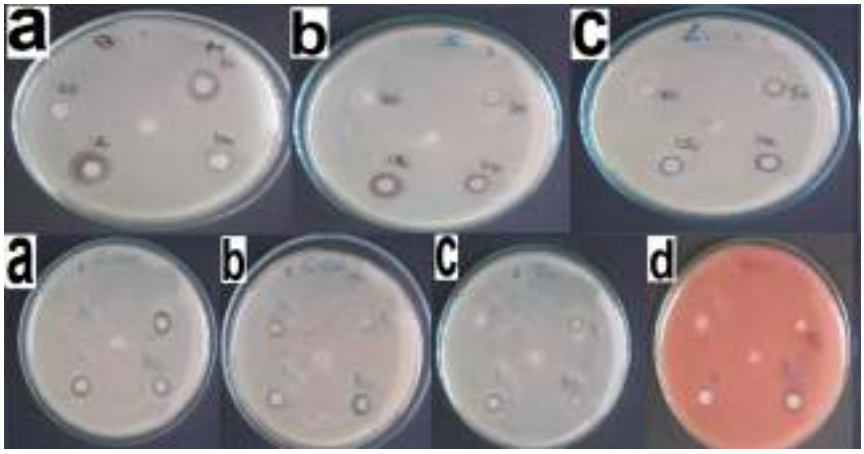
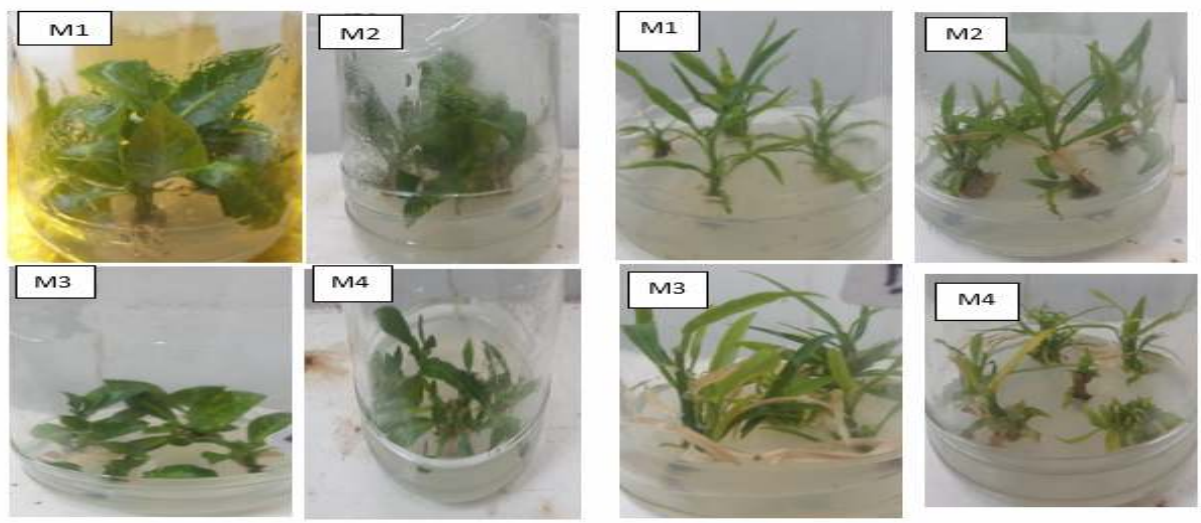


Fig. 4. Zone of inhibition (ZH) by different alkaloid extracts for Petra croton cultivar produced from different media against Gram positive bacteria a) *B. subtilis* b) *S. aureus* c) *L. monocytogenes* and Gram nigative bacteria a) *E. coli* b) *E. coli* g c) *S. Typhi* d) S. *marcescens*

its effect on axillary shoot induction and multiplication. The peptone was able to enhanced the axillary shoot formation greatly, in combination of (25 or 50) mg/l. Thus the results clearly suggest that the use of peptone markedly enhanced the axillary shoots formation in croton. This is in agreement with the earlier findings of **(Nasib *et al*., 2008)** and supported by other authors **(Sana *et al*., 2012; Resende *et al*., 2015).**

The two cultivars could be propagated on only four media which were M1 (1 mg/LBA +25 mg pept), M2 (1 mg/ LBA +50 mg pept), M3 (3 mg/LBA +25 mg pept) and M4 (3 mg/LBA +50 mg pept) Fig. 5.

Results indicated that *in vivo* there were significant deference between the two genotypes and mother plants in open field conditions. The best cultivars in morphological characteristics (plant height, leaf thickness, leaf length, leaf breadth, stem diameter and leaf area) was Petra cultivar and the most effective media in this regard was 1 mg/l BA + 25 mg/l Pept in the three stages after 60, 120 and 180 days from culture.



Petra cultivars **Gold Dust cultivars**

Fig. 5. The effect of four media; M1, M2, M3 and M4 on shoot multiplication for the two cultivars

Plant height, stem diameter, leaf breadth, leaf length, leaf area and leaf thin after 60 days

Results showed that Petra and Gold Dust cultivars gave the maximum fresh growth using M1 medium (5.5 mg), (4.25 mg), (20 mg), (370.49 mg), (7.2 mg) and (.85 mg), while differences between M2 and M3 were not significant. M4 medium gave the lowest growth for both cultivars with (3.5 mg), (3.2 mg), (11.5 mg), (155.2 mg), (5.2 mg) and (0.75 mg) for Gold Dust and Petra cultivars respectively as shown in Figs. 6, 7, 8, 9, 10 and 11.

Plant height, stem diameter, leaf breadth, leaf length, leaf area and leaf thin after 120 days

Results showed that Petra and Gold Dust cultivars gave the maximum fresh growth using M1 medium (9.75 mg), (4.3mg), (30 mg), (718.31 mg), (9.05 mg) and (0.95 mg), differences between M2 and M3 were not significant. M4 medium gave the lowest growth for both cultivars with (6.3 mg), (3.15 mg), (15 mg), (240.2 mg), (5.9 mg), (0.77 mg) for Gold Dust and Petra cultivars, respectively as shown in Figs. 6, 7, 8, 9, 10 and 11.

Plant height, stem diameter, leaf breadth, leaf length, leaf area and leaf thin after 180 days

Results showed that Petra and Gold Dust cultivars gave the maximum fresh growth using M1 medium (12.95 mg), (4.4 mg), (31 mg), (983.21 mg), (11.1 mg) and (0.98 mg), but M2 and M3 were with no significant differences between them. M4 medium gave the lowest growth for both cultivars with (8.7 mg), (3.1 mg), (13.5 mg), (277 mg), (7.7 mg), (0.77 mg) for Gold Dust and Petra cultivars respectively as shown in Figs. 6, 7, 8, 9, 10 and11.

Shooting Percentage (%)

Results in (Fig. 12) show that the best cultivars was Petra (91.25) and the best media was M1: 1.0 mg/l BA (92.5).the maximum response (91%) was obtained from all studied media in Petra cultivar,while Gold Dust cultivar was the lowest that gave response valued, 78.75%. It was found that the maximum percentage of multiple shoots formation (92%) was achieved after 8 weeks on (MS) with (1.0 mg/l BA +25 mg/l Peptone).

Root Formation

Different concentrations of IBA and IAA were used for the induction of roots. As indicated in Tables 8 and 9. IBA in the concentration of

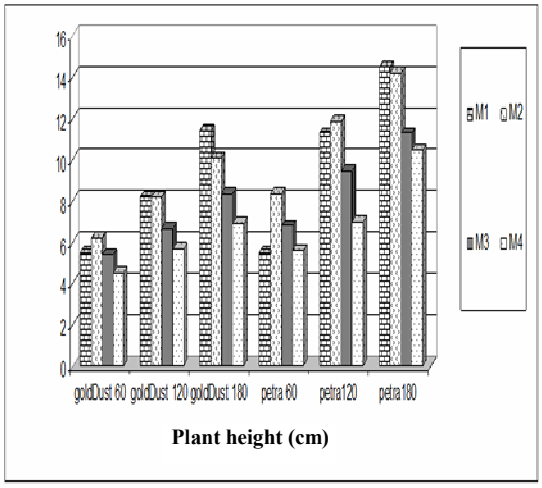


Fig. 6. Effect of media on plant height of Petra and Gold Dust croton cultivars

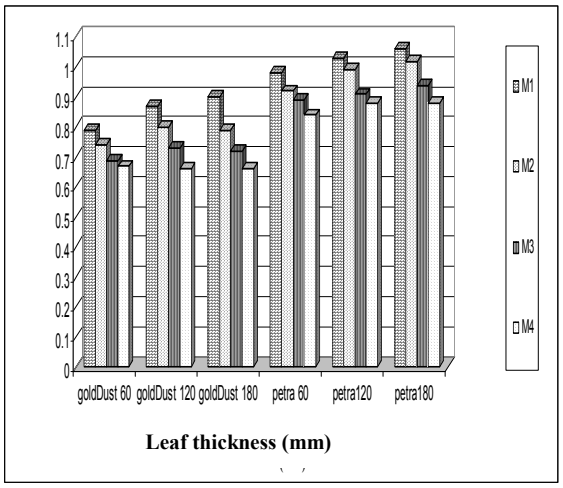


Fig. 7. Effect of media on leaf thickness of Petra and Gold Dust croton cultivars

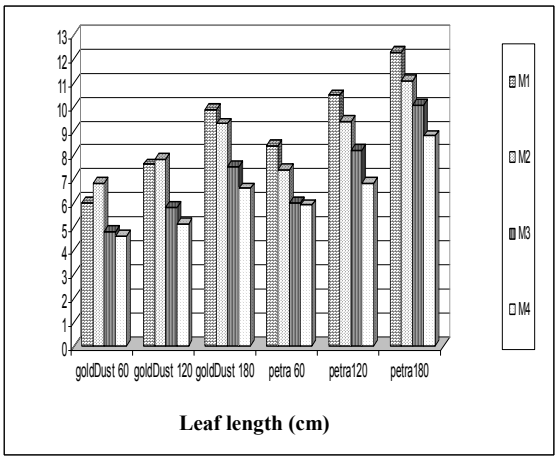


Fig. 8. Effect of media on leaf length of Petra and Gold Dust croton cultivars

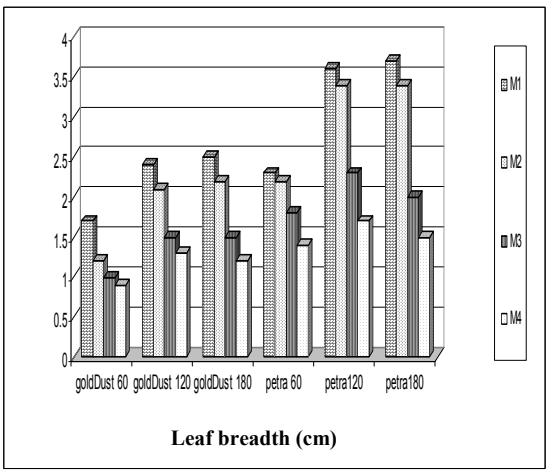


Fig. 9. Effect of media on leaf breadth of Petra and Gold Dust croton cultivars

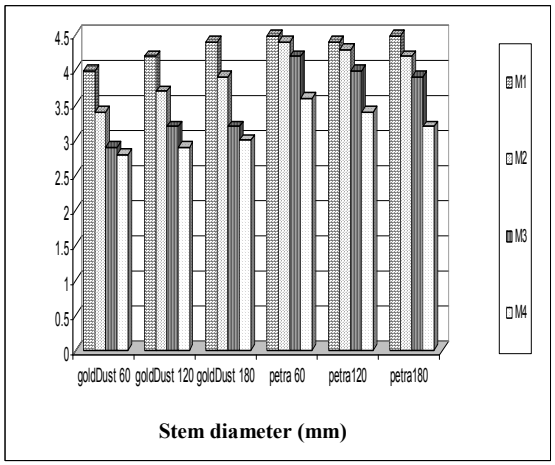


Fig. 10. Effect of media on stem diameter of Petra and Gold Dust croton cultivars

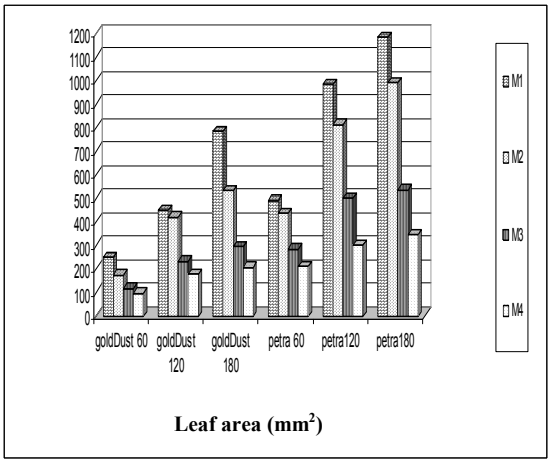


Fig. 11. Effect of media on leaf area of Petra and Gold Dust croton cultivars

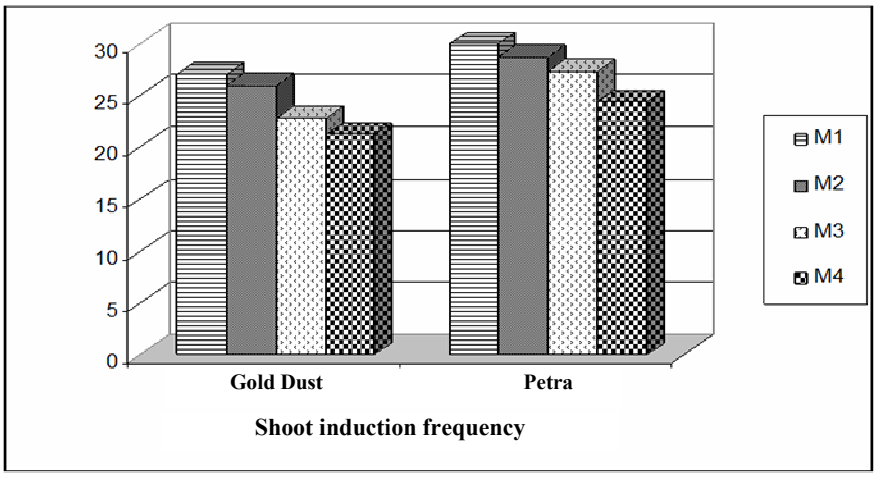


Fig. 12. Effect of media on shoot induction frequency of Petra and Gold Dust croton cultivars

Table 8. Means of squares (MS) and heritability in broad sense for micro propagation criteria *i.e.* number of roots/explant and root length (cm) of two cultivars under study of croton plant after 15 days on rooting media

SOV	df	MS	
		No. roots/explant	Root length(cm)
Reps	2	0.66	0.5*
Treats	5	15.33**	2.48**
Genotype	1	231.33**	2.14**
Media	2	36.55**	4.44**
G×M	2	113.94**	0.69**
Error	10	1.46	0.13
h^2		94.6%	

SOV = Source of variation, df=degree of freedom, Msmean sum of squere, * = Significant at P <0.05, ** = Significant at P < 0.01, Values are means of three replicates

Table 9. Average Means for micro propagation criteria *i.e.,* number of roots/explant and root length (cm) of two cultivars under study of croton plant after 15 days on rooting media

Treatment	No. roots/explant		Mean	Root length (mm)		Mean
	Gold Dust	Petra		Gold Dust	Petra	
M1	6	9	7.5	8.5	10.5	9.5
M2	7	10	8.5	8	9	8.5
M3	18	21	19.5	3.3	4.5	3.9
Mean	10.33	13.33		6.6	8	
LSD 0.01	1.426		-	0.697		-
LSD 0.05	1.002		-	0.490		-

LSD = Least significant deference, Values are means of three replicates

Table 10. Means of squares (MS) and heritability in broad sense for micro propagation criteria *i.e.* number of roots/explant and root length (cm) of two cultivars under study of croton plant after 30 days on rooting media

SOV	df	MS	
		No. roots/explant	Root length(cm)
Reps	2	0.66	0.5*
Treats	5	15.33**	2.48**
Genotype	1	231.33**	2.14**
Media	2	36.55**	4.44**
G×M	2	113.94**	0.69**
Error	10	1.46	0.13
h^2		94.6%	

SOV=source of variation, df=degree of freedom, MS=mean sum of squere, * = Significant at P <0.05,
** = Significant at P < 0.01, Values are means of three replicates

2 mg/l was best convenient for the induction of roots because the higher number and length of roots were done on that composition, while the higher concentration of IBA (2 mg/l) failed to produce response significantly. The media with IAA showed root induction in comparatively shorter time. IAA also induced callus in a very low concentrations. This is in agreement with the earlier findings of **(Nasib *et al.*, 2008)**.

Root Number

Results in Table 11 and illustrated in Fig. 13 show that the best cultivars was Petra (15.33 roots) and the best media was M3 : 1mg/l NAA +1 mg/l IBA (22.50 root). The highest number of roots (24 roots) was recoded at M3 followed by, 12 roots on M1 by Petra cultivars. While the lowest number of roots was obtained with Gold Dust cultivars on medium M2 (8 roots).

Root Length

Results in Table 11 indicate significant variation in root length under different hormones, the best cultivars was Petra (9.56 mm) and the best media was M1:1mg/l IBA (10.5 mm). The longest roots (11.5 mm) were gained at M2 followed by (10.5 mm) while, the shortest roots (4.5 mm) obtained by the Gold Dust cultivar.

Acclimatization

Plantlets with at least 2 to 3 roots were transferred to the green house for acclimatization. The potting mix used were comprised of 80% sand and 20% farmyard manure, routinely used in the nursery of our institute, was found suitable for the hardening of the plants. The survival rate percentage of the *In vitro* grown plants was 80% as showing in Fig. 14.

Conclusion

Formation has been achieved for enhanced shoots and roots by using the (MS) medium with 1 mg/l of BAP. Also the *in vitro* roots were successfully induced when used (1 or 2) mg/l of IBA which gave a longest and few roots, while with 1 mg/l of NAA which gave roots but shorter and more. Successfully has been acclimation in pots consisting of 80% sand and 20% farm yard manure. The alkaloids extracts can be used as antimicrobial agents against different pathogens bacteria. The present results give a hope and support to traditional uses of *C. variegatum*a medicinal plants as a promising source for antibacterial activity.

Table 11. Average means of squares (MS) and heritability in broad sense for micro propagation criteria *i.e.* number of roots/explant and root length (cm) of two cultivars under study of croton plant after 30 days on rooting media

Treatment	No. roots/explant		Mean	Root length(cm)		Mean
	Gold Dust	Petra		Gold Dust	Petra	
M1	9	12	10.5	10.5	10.5	10.5
M2	8	10	9	7.5	11.5	9.5
M3	21	24	22.5	4.5	6.7	5.6
Mean	12.66	15.33		7.5	9.56	
LSD 0.01	1.774		-	0.535		-
LSD 0.05	1.247		-	0.376		-

LSD = Least significant deference, Values are means of three replica

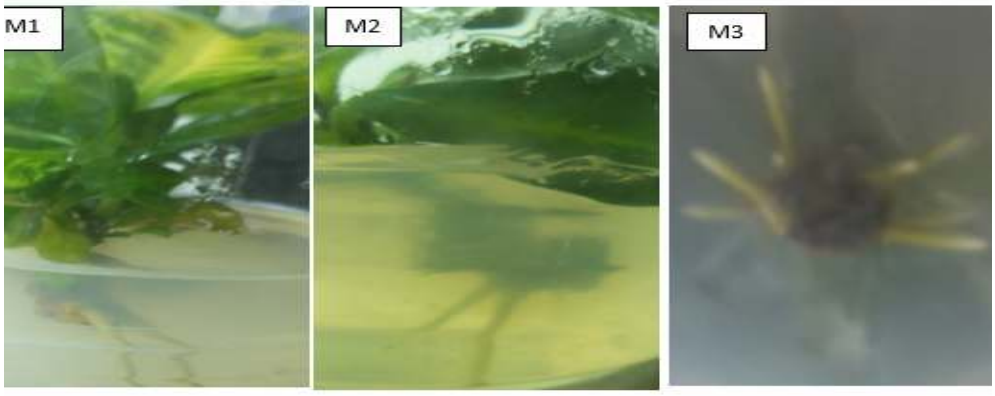


Fig. 13. The effect of three media; M1, M2, and M3 on root multiplication for the two cultivars

Pots (b) **Glass Cup(a)**

Fig. 14. The effect of acclimatization for the two cultivars

REFERENCES

Analytical Software (2008). Statistix Version 9, Analytical Software, Tallahassee, Florida, USA.

Antonio, S., L. Maria, S. Faria and N. Giuseppina (2007). Traditional uses, chemistry and pharmacology of croton species (*Euphorbiaceae*). J. Brazilian Chem. Soc., 18 (1): 11-33.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck (1966). Antibiotic susceptibility testing by a standardized single disc method. Ame. J. Clin. Pathol., 45(4): 493-496.

Christopher, L. and A. Offeibea (2014). Anticancer properties of some ornamental plants on kust campus, kumsi, Ghana. Int. J. Phytopharmacol., 5 (5): 366-370.

Deng, M., J. Chen, R.J. Henny and Q. Li (2010). Genetic relationships of codiaeum variegatum cultivars analyzed by amplified fragment length polymorphism markers. Hort., 45(6): 868–874.

Deshmukh, S.D. and M.N. Borle (1975). Studies on the insecticidal properties of indigenous plant products. Indian J. Entomol., 37(1):11-18.

Faridah, Q.Z., A.H.A. Abdelmageed, K. Nor Shuhada and A.A. Julia (2012). Callus induction and plant regeneration of *Michelia champaca* (Magnoliaceae): A multipurpose tree. J. Med. Plants Res., 6 (17): 3338-3344.

George, E.F. (1993). Plant propagation by tissue culture: The technology. London: Exegetics Limitd, 273-343.

Hassan, E.M., R.A. Hassan, J.Y. Sakib, S.M. Mahamed and S.A. El-Toumy (2013). Chemical constituents and cytotoxic activity of *Codiaeum variegatum* CV petra. J. Appl. Sci. Res., 9(8): 4884-4888.

Jackie, K.O., W. Atte, O. Jimmy, K. Jussi and T.K. Carina (2016). Antimicrobial activity of croton macrostachyus stem bark extracts against several human pathogenic bacteria. J. Pathogens, Article ID 1453428, 5.

Kupchan, S.M., I. Uchida, A.R. Branfman, R. G. Dailey and B.Y. Fei (1976). Antileukemic principles isolated from Euphorbiaceae plants. Sci. 191 (4227): 571-572.

Landa, F., L.S. de, R. Paiva, P.D.O. Paiva and J.S. Bueno Filho (1999). *In vitro* inductíon of callus on leaf explantes of pequizeiro (*Caryocar brasüiense* Camb.). Thesis, Lavras: UFLA, Agron./Plant Physiol., 73.

Lima, E.C., R. Paiva, R.C. Nogueira, F.P. Soares, E.B. Emrich and Á.A.N. Silva (2008). Callus induction in leaf segments of croton urucurana Baill. Ciência e. Agrotecnologia, 32(1): 17-22.

Maciel, M.A.M., A.C. Pinto, S.N. Brabo and M.N. Da Silva (1998). Terpenoids from croton cajucara. Phytochem., 49(3): 823-828.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-9.

Nasib, A., K. Ali and S. Khan (2008). *In vitro* propagation of Croton (*Codiaeum variegatum*). Pak. J. Bot., 40 (1): 99-104.

Norhanom, A.W. and M. Yadav (1995). Tumour promoter activity of Malaysian Euphorbiaseae. British J. Cancer, 71 (4): 776-779.

Puebla, P., J.L. López, M. Guerrero, M.L. Martí, L. San Román and A. San Feliciano (2003). Neo-clerodane diterpenoids from Croton schiedeanus. Phytochem., 62(4): 551-555.

Resende, C.F.D., R.E. Bianchetti, A.M.S.D. Oliveira, V.F. Braga and P.H.P. Peixoto (2015). *In vitro* propagation and acclimatization of Lippia rotundifolia, an endemic species of Brazilian Campos Rupestres. Revista Ciência Agronômica, 46 (3): 582-589.

Sana, S., S. Mathew and R.S. Krishnapriya (2012). Organogenesis and Somatic embryogenesis in various cultivars of *Codiaeum variegatum* L. Global Advan. Res. J. Biotechnol., 1(3): 40-47.

Santiago, E.J.A (2003). Morphological and biochemical callus characterization of long pepper (piper hispidinervium Candolle, De Candolle) Thesis (Doctorate in crop Sience)-Universidade Federal de lavras, MG, 162p.

Shamsa, F., H. Monsef, R. Ghamooshi and M. verdian-rizi (2008). Spectrophotometric determination of total alkaloids in some Iranian Medicinal Plant. Thai J. Pharm. Sci., 32: 17-20.

Simona, D.M., G. Fulvio, Z. Franco, V. Sara, F. Gelsomina, V. Francesco and Maria I. (2008). Identification of minor secondary metabolites from the latex of croton lechleri (Muell-Arg) and evaluation of their antioxidant activity. Molecules, 13 (6): 1219-1229.

Somchit, N., I. Reezal, I.E. Nur, A.R. Mutalib (2003). *In vitro* antimicrobial activity of ethanol and water extract of *Cassia alata*. J. Ethnopharmacol., 84 (1): 1-4.

Vermani, K. and S. Garg (2002). Herbal medicines for sexually transmitted diseases and AIDS," J. Ethnopharmacol., 80 (1): 49–66.

WHO (2002). World Health Organization "WHO traditional medicine strategy 2002–2005," Tech. Rep. WHO/EDM/TRM/ 2002.1,World Health Organization, Geneva, Switzerland, 2002.

تأثير التراكيب الوراثية المختلفة لنبات الكروتون ومنظمات النمو على إنتاج الكالوس والإكثار الدقيق والأنشطة المضادة للبكتريا

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الهـدف مـن هـذه الدراسـة هـو تحديـد تـأثير التراكيـب الوراثيـة المختلفـة لنبـات الكروتـون (العدسـي والبلـدي) وهرمونات النمو على إنتاج الكالوس والإكثار الدقيق والأنشطة المضادة للبكتريا، استخدمت بيئة الـ MS مع أربع تركيبات مختلفة من هرمونات النمو في بيئة إنتاج الكالوس، M1 (2 mg/l 2, 4-D), M2 (3 mg/l 2, 4-D), M3 (2mg/l NAA), M4 (1mg/l 2, 4-D + 1 mg/l NAA)، وللإكثار الدقيق تم إستخدام أربع توازنات هرمونية مختلفة M1 (1 mg/l (Benzyl adenine) BA + 25 mg/l peptone), M2 (1mg/l BA + 50mg/l peptone), M3 (3mg/l BA + 25mg/l peptone), M4 (3mg/l BA + 50mg/l peptone). وبخصوص إنتاج الجذور تم أستخدم مجموعات من منظمات النمو على النحو التالى Mr1 (1mg/l IBA), Mr2 (2mg/l IBA), and Mr3 (1mg/l IBA +1mg/l NAA)، وأظهرت النتائج أن أعلى استجابة وراثية لإنتاج الكالوس شوهدت للتركيب الوراثى للبلدى (٩١,٢٥%) يليه العدسى، وبيئة M3 أظهرت أعلى استجابة لإنتاج الكالوس (٨٢,٤%) تليها بيئة M1، التفاعل بين التراكيب الوراثية والبيئات كل على حده كانت معنوية جدا فى صفة وزن الكالوس الطازج، وبخصوص الإكثار الدقيق فإن التركيب الوراثى للبلدى أعطى أعلى استجابة وراثية يليه العدسى، أيضا بيئة M1 أعطت أعلى استجابة للإكثار الدقيق يليها بيئة M2، التجذير المعملى تم بنجاح باستخدام (١أو٢) مجم/لتر من الـ IBA والتي أعطت جذور أطول وأقل في العدد، في حين أن ١مجم/لتر من الـ NAA أعطى جذور أقصر ولكن بعدد أكبر،ثم نقل النباتات التى كونت جذور للصوبة للتأقلم في أصص بها تربة رملية مخلوطة بمخلفات حيوانية عضوية، تم الكشف عن الأنشطة المضادة للبكتيريا للقلويدات المستخرجة من الكالوس لكلا النوعين من التراكيب الوراثية ضد البكتيريا الموجبة والسالبة لجرام، وأعطيت القلويدات المستخرجة من الكالوس للبيئات M1 وM2 أعلى نشاط مضاد للبكتريا، وأخيرا يقدم التقييم الحالي دليلا علميا على التركيب المثالي للبيئات لإنتاج مستخلصات القلويدات من صنفى نبات الكروتون العدسى والبلدى كنبات طبى لإستخدامها كعوامل مضادة للبكتريا ضد مسببات الأمراض المختلفة.

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