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EFFECT OF EGYPTIAN GOOSEBERRY ECOTYPES (*Phyllanthus atropurpureus* L.) AND GROWTH REGULATORS ON DIRECT MICROPROPAGATION, CALLUS INDUCTION, TOTAL ALKALOIDS AND PHENOLIC CONTENT

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ABSTRACT

The effect of different Egyptian *P. atropurpureus* ecotypes (Sharkia, Ismailia and Cairo) and growth regulators on direct pathway (micro propagation), indirect pathway (callus induction), as well as alkaloids and phenolic content were determined. Murashige and skoog (MS) medium supplemented with four different growth regulators was applied for micro propagation, *i.e.* M0: (without hormone), M1: 1 mg/l Benzyl Adenine (BA), M2: 2 mg/l (BA), and M3: 4 mg/l (BA). Four different growth regulators for rooting, *i.e.* M0 (without hormone), M4: 0.50 mg/l Indol buteric acid (IBA) + 0.50 mg/l Indol acetic acid (IAA), M5: 1 mg/l (IBA) and M6: 2 mg/l (IBA). As well as four different growth regulators for callus induction, *i.e.* M0: (without hormone), M7: 2 mg/l 1-Naphthalen acetic acid (NAA) + 0.2 mg/l (BA), M8: 1 mg/l 2, 4 Dichlorophenoxy acetic acid (2, 4-D) and M9: 2 mg/l (2, 4-D). The results showed that Sharkia ecotype gave highly genetic response for micro propagation (2.91 shoots) followed by Ismailia, medium (M1) gave highly genetic response for micro propagation (2.66 shoots) followed by M0, and the interaction between Sharkia ecotype and medium (M1) gave significant for shoot number, shoot length, fresh weight, dry weight, shooting percentages, leaf number and internode number. Sharkia ecotype gave highly genetic response for rooting (4.33 roots) followed by Ismailia, medium (M4) gave highly genetic response for rooting (2.4 roots) followed by M0, and the interaction between Sharkia ecotype and medium (M4) was significant for root number, root length and rooting percentages. Sharkia ecotype gave highly genetic response for callus induction frequencies (88.8%) followed by Ismailia, medium (M8) gave highly genetic response for callus induction frequencies (81.4%) followed by M9, and the interaction between Sharkia ecotype and medium (M8) gave significant for callus fresh weight, callus dry weight, callus induction frequency and callusing Initiation time/day. Total alkaloids content (TAC) was estimated using spectrophotometer with bromocresol green (BCG) in three different parts (leaf, stem and callus). The results showed that Ismailia ecotype gave highly genetic response for TAC (1.60 mg/g) followed by Sharkia, stem gave highly genetic response for TAC (0.86 mg/g) followed by leaf. Total phenolic content (TPC) was estimated using spectrophotometer with folin- ciocalteu method in the whole plant. The results showed that Ismailia ecotype was the highest significant for TPC (2.513 mg/g) followed by Sharkia. Cairo ecotype was the lowest value in TAC and TPC.

Key words: *Phyllanthus atropurpureus* ecotypes, total alkaloids, total phenolic, direct micro propagation, callus induction.

INTRODUCTION

The name '*Phyllanthus'* means "leaf and flower" and named so because of its appearance where flower, fruit and leaf appears fused (Kumar *et al.,* 2011). *Phyllanthus amarus* and *Phyllanthus debilis* are closely related similar looking species commonly available in India. *Phyllanthus amarus* is widely distributed throughout India, while *Phyllanthus debilis* has

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its distribution restricted towards southern India (Indira and Sivarajan, 1994), Very few reports available on the tissue culture of *P. emblica*, *P. urinaria*, *P. amarus*, *P. abnormis*, *P. caroliniensis*, *P. tenellus*, *P. stipulatus* and *P. niruri*on transformed root cultures of *P. stipulatus*, *P. niruri* (Khana and Nag, 1973; Unander, 1991; Ishimaru *et al.,* 1992; Santos *et al.,* 1994; Elizabete, *et al.,* 2001; Sen *et al.,* 2009).

Genus *Phyllanthus* (Family Euphorbiaceae) is considered one of the important medicinal and ornamental plants, it has between 550 to 750 species and several of them produce useful secondary metabolites which have been extracted from whole plants (Unander, 1996). The stems, infusion of leaves and roots of *Phyllanthus* spp. are used in folk medicine for treating intestinal infections, diabetes, the hepatitis B virus and disturbances of the kidney and urinary bladder (Calixto *et al.,* 1998). *Phyllanthus amarus* is a plant of the family Euphorbiaceae and has about approximately 800 species which are found in tropical and subtropical countries of the world (Mazumder *et al.*, 2006; Tahseen and Mishra, 2013) Several compounds such as alkaloids, tannins, flavonoids, lignans, phenols and terpenes have been isolated and identified in various species of *Phyllanthus* and have shown antinociceptive action in mice and other therapeutic activities (Cechinel *et al.,* 1996). Antiviral effects against hepatitis B virus and possibly against the reverse transcriptase of retroviruses have also been reported (Venkateswaran *et al.,* 1987; Thyagarajan *et al.,* 1988; Shead *et al.,* 1992). The chemical review on genus *Phyllanthus*, reveals the presence of sterols and/ or terpenes (Lam *et al.,* 2007; Ndlebe *et al.,* 2008), lignans (Tuchinda *et al.,* 2006; Murugaiyah and Chan, 2007; Luo *et al.,* 2009), flavonoids (Than *et al*, 2006; Shakil *et al*, 2008) polyphenolic compounds and tannins (Zhang *et al.,* 2001; Shin *et al.,* 2005; Liu *et al*, 2008), in addition to minor alkaloids (Houghton *et al.,* 1996).

Phyllanthus amarus herb has found its traditional usefulness in several health problems such as diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies and wounds. Further, these are used in the treatment of kidney problems, urinary bladder disturbances, pain, gonorrhea, diabetes and chronic dysentery. Topically, it is used for several skin problems ranging from skin ulcers, sores, swelling and itchiness, wounds, bruises, scabies, ulcers and sores, edematous swellings, tubercular ulcers, ringworm, scabby and crusty lesions. Its effect in excretory system is due to its antiurolithic property and is used in the treatment of kidney/gallstones, other kidney related problems, appendix inflammation and prostate problems (Khatoon *et al.,* 2004; Ushie *et al.,* 2013).

The aqueous extract of *Phyllanthus amarus* demonstrates potent anticancer activity against 20-metylcholanthrene (20-MC) induced sarcoma development. The aqueous extract inhibits DNA topoisomerase II of mutant cell cultures and inhibited cell cycle regulatory enzyme cdc 25 tyrosine phosphatase of *Saccharomyces cerevisiae* (Rajeshkumar *et al.,* 2002). Free radicals are created when cells use oxygen to generate energy. These by-products are generally reactive oxygen species (ROS) such as super oxide anion, hydroxyl radical and hydrogen peroxide that result from the cellular redox process. At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins and DNA (Pham-Huy *et al.,* 2008).

Phyllanthus is the largest genus in the flowering plant family Phyllanthaceae. Estimates of the number species in this genus vary widely, from 750 to 1200. *Phyllanthus* has a remarkable diversity of growth forms including annual and perennial herbs, shrubs, climbers, floating aquatics, and pachycaulous succulents. Some have flattened leaf like stems called cladodes. It has a wide variety of floral morphologies and chromosome numbers (Kathriarachchi *et al.,* 2005). Family Euphorbiaceae (Spurge) represented all over the world by almost 326 genera and about 7750 species distributed mainly in both tropical and temperate regions except arctic regions. Over 60 genera and about 350 species have been reported from India (Sharma, 1993). Flora of Egypt comprises 7 genera and 52 species in this family (Ackholm, 1974). Plants of Euphorbiaceae are mostly trees or shrubs, rarely herbaceous or climber. Some are

xerophytic or cactus-like or phylloclades; some are marshy; usually the plants contain milky sap, latex or acrid juice (Benson, 1976; Sharma, 1993; Clarke and Lee, 2003).

The present study aimed to determine the optimum culture for micro propagation, callus induction, estimation of total alkaloids and phenolic contents from different ecotypes of the Egyptian gooseberry (*P. atropurpureus*).

MATERIALS AND METHODS

Collection and Sterilization of Gooseberry Ecotypes

This study was carried out in green house at Faculty of Agriculture FAC, Genetics Department, Zagazig University Egypt. on *Phyllanthus* genus*;* family Euphorbiaceae. Seedlings in the age of three years old (2013 – 2016) were cultivated in green house under natural conditions. The ecotypes healthy seedlings of uniform size of *Phyllanthus atropurpureus* (L.) were collected from three different Governorates of Egypt; Sharkia, Ismailia and Cairo. Single node cuttings (1.0-1.5 cm.) were excised from mother plant and used as the explants for further experiments. The explants were first washed with running tap water for half an hour to remove the soil particles and other extraneous tiny particles followed by 70% (*V/V*) Sodium hypochlorite with 2-3 drops of tween-20 for 20 min and finally washed 3 times with sterile double distilled water for 5, 10 and 15 min, respectively to remove all traces of Sodium hypochlorite (Ghanti *et al.*, 2004).

Micro Propagation of Nodal Segment

Nodal segments (1-1.5 cm) used as explants for micro propagations with four treatments of BA; M0 (without hormone), M1 (1 mg/l BA), M2 (2 mg/l BA) and M3 (4 mg/l BA). All cultures were examined after 30 day of incubation at 25±2 ºC under 16 hr. light and 8 hrs. dark provided by cool florescent light intensity of 2500 lux to record the following parameters:

- Initiation time per day.
- Shooting percentage (%).
- Number of shoots.
- Shoot length (mm).
- Number of leaves and internodes per explant.
- Fresh and dry weights (mg).

Rootlets Initiation

Propagules of *Phyllanthus atropurpureus* were tested for rooting on root induction medium with four treatments; M0 (without hormone), M4 (0.50 mg/l IBA + 0.50 mg/l IAA), M5 (1 mg/l IBA) and M6 (2 mg/l IBA)**.** All shoot lets were examined after 4 weeks of incubation at 25±2ºC under 16 hr., light and 8 hrs., dark provided by cool florescent light intensity of 2500 lux to record the following parameters:

- Initiation time per day.
- Rooting percentage (%).
- Number of roots.
- Root length (mm).

Callus Induction

Initiation of callus using internodal segments and leaves as explants

5 weeks old *in vitro* healthy plantlets were cut into leaves and internodal segments as explants. Explants (about 1 cm in length) were excised from *P. atropurpureus* plantlets and cultured on basal MS medium (without hormone) in 200 ml jars average (40- 50 ml/ jar) in addition, different concentrations of combinations of auxin with cytokinin. This experiment based on using MS medium with four treatments; M0 (without hormone), M7 (2 mg/l NAA + 0.2 mg/l BA), M8 (1 mg/l 2, 4-D) and M9 (2 mg/l 2, 4-D) and examined after 2 months of incubation at 25±2 ºC under 24 hr., dark to record the following data:

Callus Response

The initiation time of callus induction was calculated per day in the three studied ecotypes.

Callus nature

The morphological characters (Color, surface and rigidity) of calli after two months of cultivation were observed and recorded.

Callus Induction Frequency (%)

The percentage of callus formation was recorded after two months of cultivation as the

following equation. Callus formation (%) = (No of explants that formed callus ÷ Total number of explants) ×100.

Callus Fresh Weight (mg)

Fresh weight of different calli cultures were recorded at the end of the 8th week of cultivation. Three replicates of each treatment were used.

Callus Dry Weight (mg)

Fresh weight of different calli cultures were dried at 50 °C for 48 hr. using dried air oven. Three replicates of each explant were used.

Determination of Total Alkaloids

Collection of Mather plant; leaf (TAC-1), stem (TAC -2) and callus (TAC -3)

Fresh harvested leaves and stems of *P. atropurpureus* were collected. A known mass of mother plants; (leaf and stem) and the produced callus were finely chopped and subjected to determine the extraction of alkaloids. Total alkaloids content were measured with a spectrophotometer Cary 50 Bio UV-visible (Varian, Italy) associated to a software Cary win UV (Varian, Italy) (Fazel *et al.,* 2008).

Preparation of plant parts; leaf (TAC-1), stem (TAC-2) and callus (TAC-3)

The collected and identified mother plants (leaves and stem) were dried under bright sunlight for 48 hr., while, produced callus samples were dried in the mechanical dryer at 50 °C for 48 hr., until complete drying. The dried samples were ground to coarse powder with a mechanical grinder and powdered samples were kept in clean closed glass containers pending extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other foreign matter deposited on the grinder.

Extraction of the dried powdered samples

About 5 -10 grams coarse powder were weighted and subjected for extraction with 100-250 ml methanol (Absolut) using soxhlet apparatus. The process of extraction continues for 24 hr., or till the solvent in siphon tube extract become pure or clearly.

Filtration and concentration of the extracts

After the extraction process the selected plant extracts were filtered through whatman filter paper No. 1. The filtrate was collected in a beaker and then plant extracts were concentrated by evaporating the solvent using a rotary evaporator. The residue appeared as a dark brown or a dark green powder. The dried extract obtained was subjected to phytochemical screening to know the presence of alkaloids and stored in the desiccator and it was used for subsequent experiments.

Preparation of solutions

Bromo cresol green (BGC) solution was prepared by heating 69.8 mg bromo cresol green with 3 ml of 2N Na OH and 5ml distilled water until completely dissolved and the solution was diluted to 1000 ml 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 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water). Atropine standard solution was made by dissolving 1mg pure atropine (Sigma Chemical, USA) in 10 ml distilled water.

Preparation of standard curve

Accurately measure aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of atropine standard solution and transfer each to different separatory funnels. The 5ml pH 4.7 phosphate buffers and 5 ml BCG solution and shake a mixture with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10 ml volumetric flask and then diluted to adjust volume with chloroform was measured at 470 nm against blank prepared as above but without atropine.

Spectrophotometric assay

Total alkaloid content evaluation was carried out with a spectrophotometric method based on the reaction with BCG (Fazel *et al.,* 2008) with appropriate changes, A part of extracts residue was dissolved in 2 N HCL and then filtered. One ml of this solution was transferred 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 Na OH. Then, 5 ml of pH 4.7 phosphate buffer was added before 5 ml of BCG solution and shacked vigorously. Furthermore, the complex formed was extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10 ml

volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm.

Estimation of total alkaloids content of leaf (TAC-1), stem (TAC-2) and callus (TAC-3)

For the estimation of total alkaloids in the mother plants (leaf, 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 plants part samples and callus tissues samples were calculated using calibration curve ($y_{=}$ 0.0918x - 0.0026). The content of the total alkaloids in the different samples was expressed in terms of conessine (Kumbhare *et al.*, 2012).

Determination of Total Phenolic Content

Collection of mother plant

The Fresh harvested aerial parts of plant materials *Phyllanthus atropurpureus*, collected on January 2016, the cleaned dried and ground material "using grinder" was used in all experiments. All reagents were pro analysis grade, for determining the extraction of phenols from the mother plants". A known mass of mother plants of three different ecotypes were finely chopped and subjected to the phenolic extraction. The powder of plant (5 g) was in succession extracted with methanol (70%) in order of their rising polarity. Total phenolic content of methanol extracts of *P. atropurpureus* L. was evaluated with Folin- Ciocalteu method. The Folin- Ciocalteu reagent is sensitive to reducing compounds, polyphenols there by producing blue colored complex. The quantative phenolic estimation was performed at max 765 nm by change in intensity of Folin-phenolic compounds complex.

Preparation of standard curve

To prepare a calibration curve 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml of the gallic acid, stock solution was transferred to 100 ml flasks, and then diluted with water to produce gallic acid solutions, producing concentrations of 0, 25, 50 and 70% of sodium carbonate solution was added in each flask and volume was adjusted with distilled water. Readings were taken after 1 hr., at 765 nm by U.V. Spectrophotometer 1650 Shimadzu, Japan against reagent blank. The calibration curve of absorbance *vs.* concentration was plotted. 1 ml of stock solution of extracts was transferred in 25 ml flask; similar procedure was adopted as above described in preparation of calibration curve. With the help of calibration curve ($y_{=}$ 0.0018x + 0.0791), the phenolic concentration of extracts was determined (Kumbhare *et al.*, 2012).

Statistical Analysis

All studied characters for whole plants and callus inductions were analyzed by analysis of variance (ANOVA) with SPSS program version 17 in one way or two ways with three replications by SPSS program (Raja *et al.*, 2011).

RESULTS AND DISCUSSION

Effect of BA on Plant Micro propagation of *P. atropurpureus* Plant *In vitro* Using Shoot Tips and Nodal Segments as Explants

Nodal segment explants incubated on MS medium with four different concentrations of BA "M0 (without hormone), M1 (1 mg/l BA), M2 (2 mg/l BA) and M3 (4 mg/l BA)" for shoot proliferation. Three replicates of each concentration were done. Data were recorded after 30 day of incubation of cultures in 16/8 hr., light at 25°C, while dry weight was obtained by drying of explants in the mechanical dryer at 50ºC for 48 hr., until complete drying. The best ecotype was Sharkia ecotype for all *in vitro* criteria (Except for criteria of initiation time) on MS medium with the four treatments of BA followed by Ismailia ecotype (Fig. 1). Cairo ecotype was the lowest value for all studied characters with four treatments of BA. The data presented in Tables 1, 3 and 5 indicates highly significant differences among all characters of all ecotypes of Egyptian *P. atropurpureus*. Morphological studies carried out; number of shoots per explant, length of shoots, number of leaves and number of internodes per explant, fresh and dry weight, initiation time and shooting percentage with 4 treatments; on MS medium with four different concentrations of BA and the means of studied criteria of all ecotypes which are collected from different regions of Egypt are presented in Tables 2, 4 and 6. Heritability in broad sense was (94.67 - 99.72 %). whereas, shoot

E1 E2 E3

Fig. 1. The effect of media (M1) on micro propagation for *in vitro* criteria, *i.e.* No. shoots / explant, shoot length (mm), No. leaves, No. internodes and others of three different ecotypes of Egyptian *P. atropurpureus.* L (E1. Sharkia, E2. Ismailia and E3. Cairo)

Table 1. Mean of squares (MS) and heritability in broad sense for *in vitro* criteria, *i.e.* No. shoots/explant, length of shoots and fresh weight of three different ecotypes of Egyptian *P. atropurpureus* on four different media using nodal segments as explants after 30 day

SOV	df	MS		
		No. shoots/explant	Shoot length (mm)	Fresh weight (mg)
Rep	2	3.003086**	280.3364	21340.89*
Tre	11	6.245511**	1863.74**	107080.6**
Ecotype (V)	2	20.97531**	8668.614**	525845.5**
Media (M)	3	6.966049**	715.037**	24494.03*
(V × M)	6	0.975309*	169.7994	8785.61
Error	22	0.296016	102.4509	6518.761
h^2		98.66	98.8541276	98.79

NS= Not-significant,*=Significant at P < 0.05, **=Significant at P<0.01. Values are means of three replicates.

Table 2. Means for *in vitro* criteria, *i.e.* number of shoots/ explant, shoot length (mm) and fresh weight (mg) of three different ecotypes of Egyptian *P. atropurpureus* on four different media using nodal segments as explants after 30 day

Treatment (mg/l)	No. shoots/explant			Mean	Shoot length (mm)			Mean	Fresh weight (mg)			Mean
	E1	E2	E3		E1	E2	E3		E1	E2	E3	
M0	3.33	1.11	0.4	1.6	61.1	12.2	3.33	25.54	471.1	81.11	22.2	191.4
M1	4.66	2.55	0.7	2.66	71.1	19.4	9.44	33.31	488.8	88.44	26.2	201.1
M2	2.33	0.66	0	0.9	50.4	8.33	0	19.59	390.3	66.66	0	152.3
M3	1.33	0.66	0	0.6	30.7	6.22	0	12.33	240.4	28.33	0	89.59
Mean	2.91	1.24	0.3	1.4	53.3	11.5	3.192	22.69	397.69	66.135	12.1	158.6
LSD 0.05	0.92134123			-	17.1404021			-	136.724279			-
0.01	1.25229552			-	23.2973933			-	185.836905			-

Values are means of three replicates.

Table 3. Mean of squares (MS) and heritability in broad sense for *in vitro* criteria, *i.e.* dry weight, shooting percentages (%) and initiation time of three different ecotypes of Egyptian *P. atropurpureus* on four different media using nodal explants after 30 day

SOV	df	MS		
		Dry weight (mg)	Shooting percentage (%)	Initiation time/day
Rep	**2**	722.0586*	1882.716**	6.361111
Tre.	**11**	2738.347**	3793.49**	22.59343**
Ecotype (V)	**2**	11252.2**	16882.72**	60.58333**
Media (M)	**3**	1598.826**	1069.959*	8.151235
(V × M)	**6**	470.1564*	792.1811	17.15123**
Error	**22**	173.9038	333.8945	4.371212
h^2		98.53	98.14	94.67

Values are means of three replicates.

Table 4. Means for *in vitro* criteria, *i.e.* dry weight, shooting percentage (%) and initiation time/day of three different ecotypes of Egyptian *Phyllanthus atropurpureus* L. on four different media using nodal explants after 30 day

Treatment (mg/l)	Dry weight (mg)			Mean	Shooting percentage (%)			Mean	Initiation time/ day			Mean
	E1	E2	E3		E1	E2	E3		E1	E2	E3	
M0	81.66	24.44	5	37.03	100	66.66	44.44	70.366	6	5.11	5	5.37
M1	84.33	26.22	5.77	38.77	100	66.66	55.55	74.07	6	5.11	5	5.37
M2	52.55	10.66	0	21.07	100	66.66	0	55.553	6	5.11	0	3.703
M3	26.88	5.88	0	10.92	100	55.55	0	51.85	8.333	9.33	0	5.887
mean	61.3	16.8	2.69	26.94	100	63.8	24.99	62.96	6.58	6.165	2.5	5.082
LSD 0.05	22.3314852			-	30.9433803			-	3.54049564			-
0.01	30.3531615			-	42.058529			-	4.81227445			-

Values are means of three replicates

Table 5. Mean of squares (MS) and heritability in broad sense for *in vitro* criteria, *i.e.* No. leaves /explant and No. internodes/explant, of three different ecotypes of Egyptian *P. atropurpureus* on four different media using nodal explants after 30 day

SOV	df	MS	
		No. leaves	No. internodes/explant
Rep	2	1.231481*	50.84259**
Tre	11	31.89983**	89.30051**
Ecotype (V)	2	117.1204**	287.8148**
Media (M)	3	29.54218**	70.00309**
(V × M)	6	4.671811**	32.77778*
Error	**22**	0.335859	9.832492
h^2	**-**	99.72	97.02

NS = Not-significant, * = Significant at P < 0.05, ** = Significant at P < 0. 01. Values are means of three replicates

Table 6. Means for *in vitro* criteria, *i.e.* number of leaves/explant and number of internodes/ explant of three different ecotypes of Egyptian *P. atropurpureus* on four different media using nodal explants after 30 day

Treatment (mg/l)	No. leaves/ explant			Mean	No. internodes / explant			Mean
	E1	E2	E3		E1	E2	E3	
M0	8.555	3	1	4.185	10.666	3.666	0.444	4.925
M1	10	4.222	1.44	5.220	18.333	4.222	0.555	7.703
M2	4.888	1.111	0	1.999	6	1.444	0	2.481
M3	3.222	0.888	0	1.37	3.5555	0.777	0	1.444
Means	6.6662	2.30525	0.61	3.193	9.63862	2.52725	0.24975	4.138
LSD 0.05		0.98138937		-		5.31000481		-
0.01		1.33391352		-		7.21740769		-

Values are means of three replicates.

tip explants at four media can't produce any shoots neither in Sharkia ecotype nor in other ecotypes. These results confirmed with (Johnson and Alias, 2007), they washed the explants thoroughly under running tap water for 5 min, and then washed with the commercial detergent tween-20 for 3 min. This was followed by thorough washing with sterile distilled water, carried out surface sterilization in the presence of mercuric chloride solution (0.5% *W/V*) for 2 min and then washed thrice with sterile distilled water.

Shoot number

Results in Table 2 show that the numbers of induced shoots increased by decreasing BA concentration till 1.0 mg/l while, decreased by higher concentrations. The best ecotype was Sharkia (2.91 shoots) and the best media was M1; 1.0 mg/l BA (2.66 shoots). Nodal segment explants gave higher number of shoots 4.66 at the interaction between M1 (1.0 mg/l BA) and Sharkia ecotype.

Shoot length

Results in Table 2 show that the best ecotype was Sharkia (53.3 mm) and the best media was M1; 1.0 mg/l BA (33.31 mm). The maximum shoot length (71.11 mm) was induced by nodal segment explants at the interaction between M1 (1.0 mg/l BA) and Sharkia ecotype followed by (61.11 mm) at the media M0 (without hormone) with Sharkia ecotype. At the same time, the shortest shoot (3.33 mm) was produced at M0 (without hormone) in the Cairo ecotype.

Leaf and internode number

Data in Table 6 show that the best ecotype was Sharkia (6.6662 and 9.63862) and the best media was M1; 1.0 mg/l BA (5.220 and 7.703). The nodal explants gave higher number of leaves and internodes (10 and 18.333) at M1 (1.0 mg/l BA) with Sharkia ecotype followed by, (8.555 and 10.666) leaves and internodes at M0 (without hormone) in the Sharkia ecotype, While, the lowest number of leaves (0.888) was recorded with effect of M3 (4.0 mg/l BA) by Ismailia ecotype the lowest number of internodes (0.444) was recorded with effect of M0 with Cairo ecotype.

Fresh and dry weight/ mg

Results in Tables 2 and 4 show that the fresh and dry weight obtained from nodal segment explants increased by decreasing BA concentration then decreased at higher concentrations. The best ecotype was Sharkia (397.69 and 61.3) and the best media was M1; 1.0 mg/l BA (201.1 and 38.77). The highest fresh and dry weight of shoots increased to (488.888 and 84.333) at M1 (1.0 mg/l BA) with Sharkia ecotype followed by (471.111 and 81.666 mg) at M0 (without hormone) in the same ecotype by nodal explants whereas, the lowest fresh and dry weight were obtained (22.222 and 5 mg) with M0 (without hormone) by Cairo ecotype.

Initiation time

Results in Table 4 show that the best ecotype was Cairo (2.5 days) and the best media was M0

and M1; 1.0 mg/l BA (5.37 days).The fastest shoots were obtained by the interaction between and M1 (1.0 mg/l BA) and the Cairo ecotype and also M0 (without hormone) after 5.0 days followed by Ismailia ecotype (5.11 days) but Sharkia was the latest ecotype (6.0 days).

Shooting percentage (%)

Results in Table 4 show that the best ecotype was Sharkia (100%) and the best media was M1; 1.0 mg/l BA (74.07%). The maximum response 100% was obtained from all studied media in Sharkia ecotype, followed by 66.66% which given by act of M0, M1 and M2 with nodal segment explants in Ismailia ecotype, While Cairo ecotype was the lowest ecotype that gave response 44.44% at M0 (without hormone).These results confirmed with the finding of (Johnson and Alias, 2007), they found that the maximum percentage of multiple shoots formation (80%) was achieved after 4 weeks on (MS) with 1.0 mg/l BAP.

Effect of Growth Regulators and Different Ecotypes on Rooting Stages Using propagules of *P. atropurpureus*

Four concentrations of IBA "M0 (without hormone), M4 (0.50 mg/l IBA + 0.50 mg/l IAA), M5 (1 mg/l IBA) and M6 (2 mg/l IBA)" were prepared for rooting of *P. atropurpureus.* The data presented in Tables 7 and 9 indicates highly significant variations among all characters of all ecotypes of Egyptian *P. atropurpureus* recorded after 30 days. Heritability in broad sense was (72.22 - 97.84 %). All ecotypes gave responses with M0 (without hormone), M4 (0.50 mg/l IBA + 0.50 mg/l IAA) but they didn't give any response at other media; M5 (1 mg/l IBA), M6 (2 mg/l IBA) in the same conditions.

Rooting percentage

Results in Table 10 show that the best ecotype was Sharkia (94.44%) and the best media was M4; 0.50 mg/l IBA + 0.50 mg/l IAA (66.66%). The means of studied criteria of all ecotypes which are collected from different regions of Egypt are presented in rooting percentage at two different treatments. Highest percentage of rooted shoot lets (Ratio of rooted propagules from total inoculated propagules was 100% with interaction between M4 and Sharkia ecotype followed by, 88.88% that was recorded on MS basal salt medium M0 in the same ecotype. The lowest ecotype was Cairo (Fig. 2). The results confirmed with Rajagobal (2010) who found that Rooting (87%) of the shoots was the best and achieved in 1/2 MS + IBA (0.5 mg/l) + IAA (0.5 mg/l).

Root initiation time

Data presented in Table 10 show that the fastest rooting response occurred after 7.33 days with Ismailia ecotype at M4 and M0 followed by Cairo ecotype (8.55 days) while, the latest response took place after 17.33 days with Sharkia ecotype at the same medium.

Root number

Results in Table 8 show that the best ecotype was Sharkia (4.33 roots) and the best media was M4; 0.50 mg/l IBA + 0.50 mg/l IAA (2.40 roots). The highest number of roots (6 roots) was recoded at M4 followed by, 2.66 roots on M0 by Sharkia ecotype followed by, 0.88 roots by Ismailia ecotype on M0. While the lowest number of roots (0.44 roots) was obtained with Cairo ecotype on medium: M4 and M0.

Root length

Results in Table 8 indicate significant variation in root length under different treatments. The best ecotype was Sharkia (16.99 mm) and the best media was M4; 0.50 mg/l IBA + 0.50 mg/l IAA (8.99 mm). The longest roots (18.11 mm) were gained at M4 followed by, (15.88 mm) at M0 with Sharkia ecotype followed by, (55.55mm) at M4 and M0 with Ismailia ecotype while, the shortest roots (33.33 mm) were obtained by M4 and M0 with the Cairo ecotype.

Effect of Growth Regulators and Different Ecotypes on Callus Induction Using Leaves and Internodal Segments

Callus induction

Leaves and internodal segment explants cultured under aseptic conditions on MS basal salt medium supplemented with 3% sucrose M0 (without hormone) and different concentrations of, M7 (2 mg/l NAA + 0.2 mg/l BA), M8 (1 mg/l 2,4-D) and M9 (2 mg/l 2,4-D). Data obtained from 3 replicates to each concentration. Data were recorded after 2 months of incubation of cultures under 24 hr., complete dark at 25°C.

E1 **E2** **E3**

Fig. 2. The effect of media; M4 (0.50 mg/l IBA + 0.50 mg/l IAA) on rooting for *In vitro* criteria, *i.e.* number of roots, root length (mm), root percentage (%) and initiation time/day for three different ecotypes of Egyptian *P. atropurpureus* L. (E1. Sharkia, E2. Ismailia and E3. Cairo). (View from the above side)

Table 7. Mean of squares (MS) and heritability in broad sense for *in vitro* criteria, *i.e.* No. roots and root length (mm) of three different ecotypes of Egyptian *Phyllanthus atropurpureus* L. on two different media using propagules after 30 day

SOV	df	MS	
		No. roots /explant	Root length (mm)
Rep	2	0.932099	89.00617
Tre	5	14.34691**	130.558
Ecotype (V)	2	27.52469**	322.6914*
Media (M)	1	5.191358*	2.469136
(V × M)	2	5.746914**	2.469136
Error	10	0.732099	71.86173
h^2		97.84	81.89

NS = Not-significant, * = Significant at P < 0.05, ** = Significant at P < 0. 01. Values are means of three replicates.

Table 8. Means for *in vitro* criteria, *i.e.* No. roots and root length (mm) of three different ecotypes of Egyptian *P. atropurpureus* on two different media using propagules after 30 day

Treatment (mg/l)		No. roots			Mean	Root length (mm)			Mean
		Sharkia	Ismailia	Cairo		Sharkia	Ismailia	Cairo	
M0		2.66	0.88	0.44	1.32	15.88	5.55	3.33	8.25
M4		6	0.77	0.44	2.40	18.11	5.55	3.33	8.99
Mean		4.33	0.825	0.44	1.86	16.99	5.55	3.33	8.62
LSD	0.05		1.55092995		-		15.3658353		-
	0.01		2.20763002		-		21.8720899		-

Values are means of three replicates.

Table 9. Mean of squares (MS) and heritability in broad sense for *in vitro* criteria, *i.e.* rooting percentage (%) and initiation time/day of three different ecotypes of Egyptian *P. atropurpureus* on two different media using propagules after 30 day

SOV	df	MS	
		Rooting percentage (%)	Initiation time/day
Rep	2	432.0864	175.284
Tre	5	1691.383	71.41728
Ecotype (V)	2	4135.864*	178.5432
Media (M)	1	61.7284	0
(V × M)	2	61.7284	0
Error	**10**	728.4272	68.64691
h^2		85.21	72.22

NS= Not-significant, * = Significant at P < 0.05, ** = Significant at P < 0.01. Values are means of three replicates.

Table 10. Means for *in vitro* criteria, *i.e.* rooting percentage (%) and initiation time/day of three different ecotypes of Egyptian *P. atropurpureus* on two different media using propagules after 30 day

Treatment (mg/l)	Rooting percentage (%)			Mean	Initiation time/day			Mean
	Sharkia	Ismailia	Cairo		Sharkia	Ismailia	Cairo	
M0	88.88	55.55	44.44	62.95	17.33	7.33	8.55	11.07
M4	100	55.55	44.44	66.66	17.33	7.33	8.55	11.07
Mean	94.44	55.55	44.44	64.81	17.33	7.33	8.55	11.07
LSD 0.05	48.9215728				-			
0.01	69.6361126				-			

Values are means of three replicates.

The overall response to plant regulators in internodal segments was superior, while leaf explants didn't result any response in medium of callus induction at the same conditions. Friable greenish callus was successfully induced from wound sites in the young internodal segments explants. The experiment included four concentrations with combination of NAA, BAP, and 2, 4-D with 3 ecotypes. All treatments were established with a fresh weight at least 22 mg of callus cultures except M0 (without hormones); no callus induction response was noted in the leaves and internodal segments explants and leaves didn't give any response with all treatments. The data presented in Tables 11 and 13 indicates highly significant variations among all characters of all ecotypes of Egyptian *P. atropurpureus*. Heritability in broad sense was (91 - 98%). All ecotypes gave responses with M7 (2 mg/l NAA + 0.2 mg/l BA), M8 (1 mg/ l 2, 4-D) and M9 (2 mg/l 2, 4-D) but they didn't give any response at other media; M0 (without hormone), in the same conditions. These results confirmed the importance of some ecotypes for alkaloids production and possibility the selection between different genotypes for improvement of total alkaloid production (Figs. 3 and 4).

Callus induction frequency (%)

In this experiment, ratios of responded explants from total inoculated explants were presented in Table 14 which appeared that the best ecotype was Sharkia (88.883%) followed by Ismailia. The best media was M8; 1.0 mg/l 2, 4-D (81.476%). The maximum responses (100%) was obtained from the interaction between Sharkia ecotype and M8 (1 mg/l 2, 4-D) using internodal segment as explants followed by Sharkia ecotype with M9 (2 mg/l 2, 4-D) (88.88%). The poorest responses (22.22%) was obtained by the interaction between Cairo ecotype and M9 (2 mg/l 2, 4-D), but no response was recorded by M0 (MS basal salt medium without hormone) with leaves and internodal segments, respectively.

Callus nature

Results showed that the nature of callus that obtained from inter-nodal explants with M7 (2 mg/l NAA + 0.2 mg/l BA) were yellowish, smooth and friable in the Sharkia ecotype and white yellowish and smooth and friable in Ismailia ecotype but with M8 (1 mg/l 2, 4-D) were brownish, smooth and friable in Sharkia ecotype, yellow brownish, smooth and compact in Ismailia ecotype, and were white, smooth and friable in Cairo ecotype. While with M9 (2 mg/l 2, 4-D) were banana, smooth and compact in Sharkia ecotype, white greenish, smooth and compact in Ismailia ecotype and yellowish, smooth and compact in Cairo ecotype (Figs. 3 and 4).

Callus fresh and dry weight/mg.

Results in Table 12 show that the best ecotype was Sharkia (219.62 and 54.903 mg) and the best media was M8; 1.0 mg/l 2, 4-D (149.320 and 37.326 mg). Fresh and dry weights obtained from intermodal segments were increased by decreasing 2, 4-D concentration. Greatest fresh and dry weights (312 and 78.05mg) were noted on M9 (2 mg/l 2, 4-D) in the Sharkia ecotype followed by (260 and 65 mg) at M8 (1 mg/l 2, 4-D) in the Sharkia ecotype but no response with M7 (2 mg/l NAA + 0.2 mg/l BA) in the Cairo ecotype, After sub culture of the produced callus there is no increasing in the mass of the fresh weight and dry weight. There is no increasing in the mass of callus fresh weight and dry weight. Color of callus will be converted to black or brown after 6 weeks, so several numbers of internodal segments were cultured on media for callus induction to determine total alkaloids (TAC. 3). The results confirmed with (Malayaman *et al.*, 2014) they found that the leaf segments produced maximum callus induction on 45 days (82.5%) when MS medium was fortified with BAP (3.5 mg/l), NAA (2.5 mg/l), 2, 4-D (0.5 mg/l) and the best response was observed on inter nodal callusing (80%) when the MS medium contained 3.0 mg/l BAP, 2.0 mg/l NAA and 0.5 mg/l 2, 4-D. It was observed that the explants produced scanty callus when the concentration of the hormones are low.

Initiation time

Results in Table 14 show that, the best ecotype was Sharkia (21.333 day). The best media was M7; 2 mg/l NAA + 0.2 mg/l BA (17.444 day). The fastest response (21.33 days) was obtained when internodal segment explants of Sharkia ecotype incubated at 24 hr., dark, 24 ± 1°C with M7 followed by (31 day) that reported at the same concentration with internodal segment of Ismailia ecotype while the latest response (44 days) with M8 and M9 by Cairo ecotype.

Estimation of Total Alkaloids Content (TAC)

Effect of different ecotypes from leaf, stem and produced callus on TAC (mg/DW)

The results of total alkaloid content (TAC) in leaf, stem and callus of all ecotypes are presented in Table 16. TAC was expressed in milligram atropine equivalent (mg). The results of BCG assay showed that the best ecotype was Ismailia ecotype (1.604917 mg), the best plant part was the stem; TAC. 2 (0.862778 mg). Higher mean of TAC was presented with the interaction between the leaf extract and Ismailia ecotype and valued 1.391 mg, followed by callus extract of the same ecotype (1.373333 mg) while TAC in stem extract of the same ecotype was (1.142333 mg). Sharkia ecotype was higher in stem (0.921 mg) than leaf and callus. Cairo ecotype was higher in stem (0.525 mg) than leaf and callus. Heritability in broad sense of total alkaloids content to all ecotypes was (92 - 99%) (Table 15).

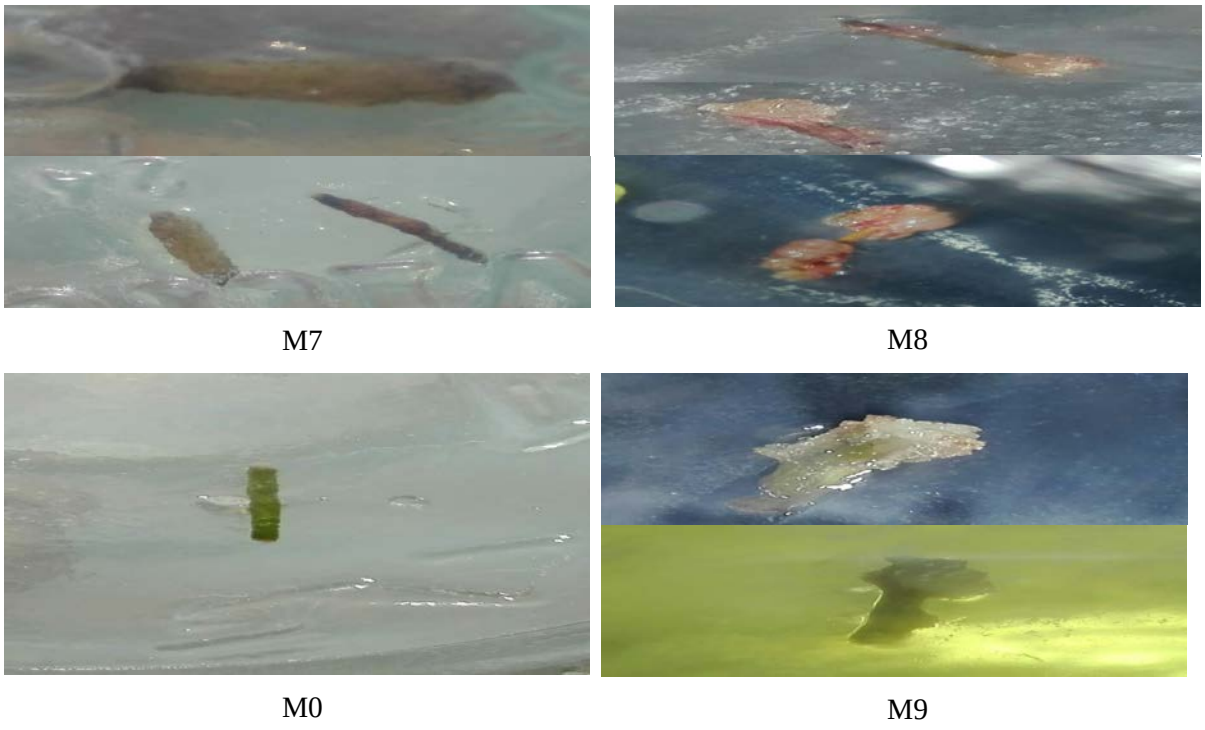

Fig. 3. The effect of four media; M0, M7, M8 and M9 on callus induction for *in vitro* criteria, *i.e.* callus percentage (%), callus fresh weight/mg and callus dry weight/mg with Ismailia ecotype (E2)

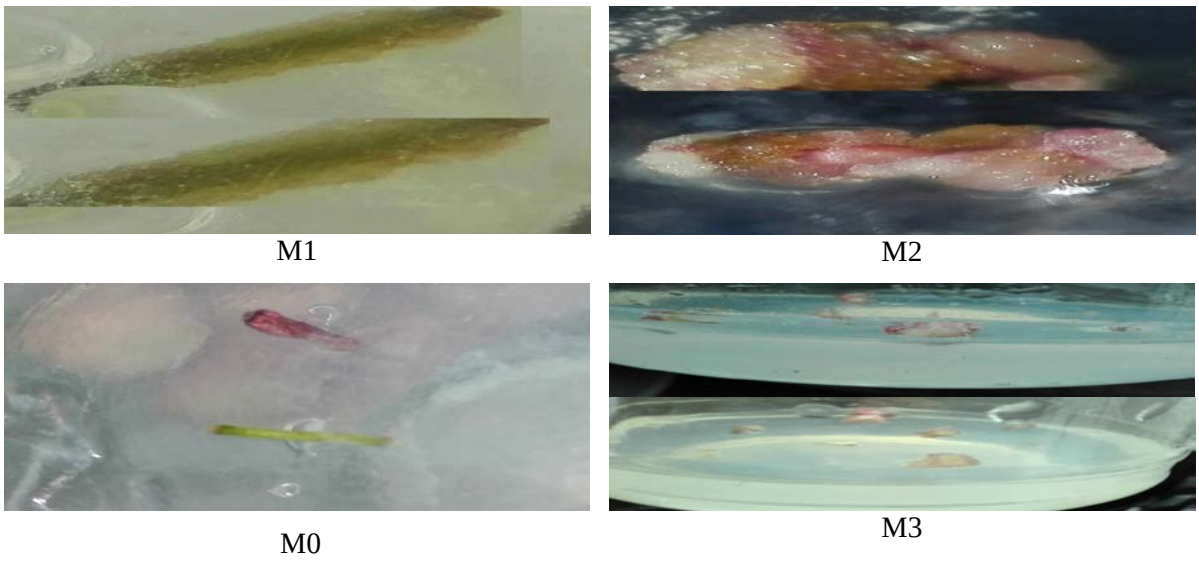

Fig. 4. The effect of four media; M0, M7, M8, and M9 on callus induction for *in vitro* criteria, *i.e.* callus percentage (%), callus fresh weight/mg and callus dry weight/mg with Sharkia ecotype (E1)

Table 11. Mean of squares (MS) for *in vitro* criteria, *i.e.* callus fresh weight /mg and callus dry weight/mg of three different ecotypes of Egyptian *P. atropurpureus* L. on three different media using internodal segments as explants after 2 months

SOV	df	MS	
		Fresh weight (mg)	Dry weight (mg)
Rep	2	36486.01966*	2280.376*
Tre	8	33220.15889**	2076.26**
Ecotype (V)	2	81947.06219**	5121.691**
Media (M)	2	34996.23914*	2187.265*
(V × M)	4	7968.667124	498.0417
Error	16	7536.405464	471.0253
h^2		92.26	92.26

NS= Not-significant, * = Significant at P < 0.05, ** = Significant at P < 0.01. Values are means of three replicates

Table 12. Means for *in vitro* criteria, *i.e.* callus fresh weight/mg and callus dry weight/mg of three different ecotypes of Egyptian *P. atropurpureus* on three different media using internodal segments as explants after 2 months

Tre. (mg/l)	Callus fresh weight/mg			Mean	Callus dry weight/mg			Mean
	E1	E2	E3		E1	E2	E3	
M7	86.666	34.444	0	40.37	21.66	8.61	0	10.09
M8	260	118.3333	69.6296	149.320	65	29.58	17.40	37.326
M9	312.222	88.888	41.11	147.406	78.05	22.22	10.27	36.846
Means	219.62	80.5551	41.60453	113.929	54.903	20.136	10.393	28.477
LSD 0.05		149.144227		-		37.2860568		-
0.01		205.425068		-		51.3562669		-

Values are means of three replicates.

Table 13. Mean of squares (MS) for *in vitro* criteria, *i.e.* callus induction frequencies and callus initiation time/day of three different ecotypes of Egyptian *P. atropurpureus* on three different media using internodal segments as explants after 2 months

SOV	df	MS	
		Callus induction frequencies (%)	Callus Initiation time/day
Rep	2	658.4362*	51.68313
Tre	8	3065.844**	466.6739**
Ecotype (V)	2	8065.844**	326.2387*
Media (M)	2	2757.202**	542.2757**
(V × M)	4	720.1646**	499.0905**
Error	**16**	149.177	81.21091
h^2		98.3304548	91.0416645

NS= Not-significant, * = Significant at P < 0.05, ** = Significant at P < 0.01. Values are means of three replicates

Table 14. Means for *in vitro* criteria, *i.e.* callus percentage (%) and callusing initiation time/day of three different ecotypes of Egyptian *P. atropurpureus* on three different media using internodal segments as explants after 2 months

Treatments (mg/l)	Callus induction frequency (%)			Mean	Callusing initiation time/day			Mean
	E1	E2	E3		E1	E2	E3	
M7	77.77	66.66	0	48.143	21.333	31	0	17.444
M8	100	77.77	66.66	81.476	21.333	31	44	32.111
M9	88.88	55.55	22.22	55.55	21.333	36.88	44	34.071
Means	88.883	66.66	37.033	64.192	21.333	32.96	44	32.764
LSD 0.05	21.1417581			-	4.23163097			-
0.01	29.1197801			-	5.82847285			-

Values are means of three replicates.

Table 15. Analysis of variance and heritability in broad sense of total alkaloids for aerial part of whole plant (Leaf; TAC. 1), (Stem; TAC. 2), (Callus TAC. 3) and total phenolic for aerial part of whole plant; whole plant (TPC) of three different ecotypes of Egyptian *P. atropurpureus*

SOV	df	MS			
		Leaf; TAC. 1	Stem; TAC. 2	Callus; TAC. 3	Whole plant; TPC
Replications	2	0.000514	0.00131	0.02131	0.001925
Ecotypes	2	0.731792**	0.293452**	0.791073**	0.309447**
Error	4	0.000349	0.001184	0.021362	0.001095
h^2 in broad sense	-	99.8572	98.79916	92.31404	98.94578

*and ** Significant at 5 and 1% levels, respectively.

Table 16. Means of total alkaloids (mg/DW) for whole plant (Leaf; TAC. 1), (stem; TAC. 2), (callus; TAC. 3) and total phenolic (mg/DW) for whole plant; whole plant (TPC) of three different ecotypes of Egyptian *P. atropurpureus*

Ecotypes	Leaf; TAC. 1	Stem; TAC. 2	Callus (TAC. 3)	Whole plant (TPC)	Mean
Sharkia	0.774667	0.921	0.675	2.423333	1.1985
Ismailia	1.391	1.142333	1.373333	2.513	1.604917
Cairo	0.414333	0.525	0.372	1.917333	0.807167
Mean	0.860	0.862778	0.806778	2.284556	-
LSD 0.05	0.04232325	0.07799559	0.33127795	0.0750072	-
0.01	0.0701932	0.1293558	0.549425	0.1243995	-

Values are means of three replicates.

The amount of total alkaloids in leaves, stem and callus tissues samples of three ecotypes was estimated using calibration curve. Table 17 show that the means of TAC was higher in stem extract (9.426773 mg/ml) followed by leaf extract (9.396514 mg/ml) while, callus extract was the lowest value (8.816751 mg/ml). Ismailia ecotype was the best value (14.21375 mg/ml) followed by Sharkia ecotype (8.636407 mg/ml) while, Cairo ecotype was the lowest value (4.789881 mg/ml), (Fig. 5). The results confirmed with (Sarg *et al*., 2012) they said that the chemical review on genus *Phyllanthus*, reveals the presence of sterols and/ or terpenes, lignans, flavonoids, polyphenolic compounds and tannins, in addition to minor alkaloids.

Estimation of Total Phenolic Content (TPC)

Effect of different ecotypes from whole plant on TPC (mg/DW)

The results of total phenolic content (TPC) in whole plant of all ecotypes are presented in Table 16. Using the Folin- Ciocalteu reagent and Gallic acid. The results showed that the best ecotype was Ismailia ecotype. The higher mean of TPC was presented in the extract of Ismailia ecotype was (2.513 mg/g) followed by extract of Sharkia ecotype was (2.423333 mg/g), While TPC in Cairo ecotype (1.917333 mg/g). Effect of character variation was significant in total phenolic content (TPC) for whole plant (areal parts) that illustrated by analysis of variance and heritability in broad sense of total phenolic content to all ecotypes was 98.94578% (Table 15).

The amount of total phenolic in the whole plant of three samples was calculated using calibration curve. The means of TPC in Table 17; TPC was higher in Ismailia ecotype extract (1.35 mg/ml) followed by Sharkia ecotype extract (1.30 mg/ml). Cairo ecotype showed the lowest in Total phenolic content (1.02 mg/ml) (Fig. 6). The results confirmed with Luiz *et al.* (2003) they used a simple spectrophotometric method based on the development of an adequate methodology for the quality control of *Phyllanthus niruri* aqueous extracts.

Table 17. Comparison of total alkaloid content amount (mg/ml) among intact (Leaf; TAC. 1), (stem; TAC. 2), (callus; TAC. 3) and total phenolic for whole plant/TPC of three different ecotypes of Egyptian Gooseberry

Ecotype	Leave; TAC. 1	Stem; TAC. 2	Callus; TAC. 3	Whole plant; TPC	Mean
Sharkia	8.466957	10.061	7.381264	1,30	8.636407
Ismailia	15.18083	12.47204	14.98838	1,35	14.21375
Cairo	4.541757	5.747277	4.08061	1,02	4.789881
Mean	9.396514	9.426773	8.816751	1.22	7.21501

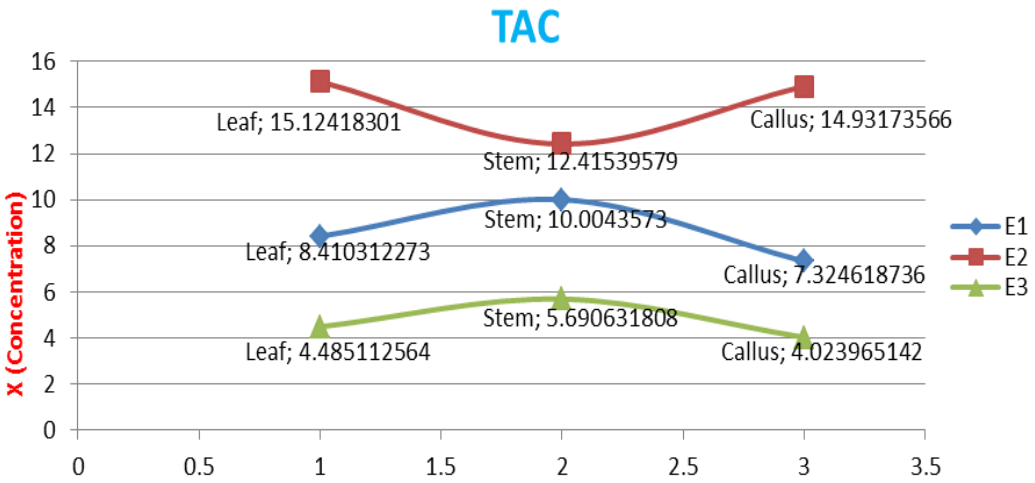

Fig. 5. Conc. TAC (mg/ml) among intact (leaves and stem) and callus of three different ecotypes means of Egyptian Gooseberry *(P. atropurpureus* L.)

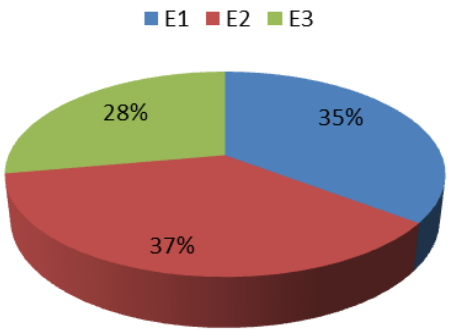

Fig. 6. Conc. TPC among intact whole plant of three different ecotypes means of Egyptian gooseberry *(P. atropurpureus* L.)

REFERENCES

Ackholm, V. (1974). Student's Flora of Egypt. Cairo Univ., Egypt 2nd Ed.

Benson, L. (1976). Plant Classification. 2nd Ed.

Oxford and IBH publishing company, New Delhi, Calcutta and Bombay, 159.

Calixto, J.B., A.R.S. Santos, F.V. Cechinel and R.A. Yunes (1998). A review of the plants of the genus *Phyllanthus*: Their chemistry, Pharmacol and Therapeutic Potential, John Wiley and Sons, Inc., Med. Res. Rev., 4: 225-258.

Cechinel, F.V., A.R.S. Santos, R.O.P. Campos, O.G. Miguel, R.A. Yunes, F. Ferrari, J. Messana and J.B. Calixto (1996). Chemical and pharmacological studies of *Phyllanthus caroliniensis* in mice. J. Pharm. Pharmacol., 48: 1231- 1236.

Clarke, I. and H. Lee (2003). Name that Flower: The Identification of Flowering Plants 2nd Ed., Melbourne Univ. press, Aus., 139.

Elizabete, C., F.O. Michel and M.V. Ana (2001). *In vitro* culture of *Phyllanthus stipulatus* (Euphorbiaceae), Revta brasil. Bot., São Paulo., 24 (1): 25-34.

Fazel, S., M. Hamidreza, G. Rouhollah and V. Mohammadreza (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J. Pharm. Sci., 32: 17-20.

Ghanti, K.S., B. Govindaraju, R.B. Venugopal, S.R. Rao, C.P. Kaviraj, F.T.Z. Jabeen, A. Barad and S. Rao (2004). High frequency shoot regeneration from *Phyllunthus amarus* Schum. and thonn. Indian J. Biotechnol., 3: 103 – 107.

Houghton, J., Z. Woldemariam, S. O'Shea and P. Thyagarajan (1996). Two securinega-type alkaloids from *Phyllanthus amarus*. Phytochem., 43: 715-717.

Indira, B. and V.V. Sivarajan (1994). Ayurvedic Drugs and Their Plant Sources. Ist Ed., Oxoford and IBH publishing company PVT. Ltd., New Delhi.

Ishimaru, K., K. Yoshimatsu, T. Yamakawa, H. Kamada and K. Shimomura (1992). Phenolic constituents in tissue cultures of *Phyllanthus niruri*. Phytochem., 34: 2015-2016.

Johnson, M. and A. Alias (2007). Somoclonal variation studies on *Phyllanthus amarus* Schum and Thonn. Iranian J. Biotechnol. Res., 5 (4) : 240 - 245.

Kathriarachchi, H., P. Hoffmann, R. Samuel, K. J. Wurdack and M.W. Chase (2005). Molecular Phylogenetic of Phyllanthaceae inferred from five genes (plastide atpB, matK, 3' ndhF, rbcL and nuclear PHYC). Molecular Phylogenet. and Evol., 36: 112-134.

Khatoon, S., V. Rai and A. Rawat (2004). Comparative pharmacognostic studies of three *Phyllanthus* species. J. Ethnopharmacol., 104 : 79-86.

Khana, P. and T.N. Nag (1973). Isolation, identification and screening of phyllemblin from Emblica officinalis Gaertn. tissue culture. Indian J. Pharmacol., 35: 23-25.

Kumar, S., H. Choudhary and C. Seniya (2011). *In vitro* antibacterial study of aqueous and methanolic extracts of some selected medicinal plants. J. Chem. and Pharmac. Res., 3:854.

Kumbhare, M.R., V. Guleha and T. Sivakumar (2012). Estimation of total phenolic content, cytotoxicity and *in-vitro* antioxidant activity of stem bark of *Moringa oleifera*. Asian Pacific J. Tropical Dis., 144 - 150.

Lam, H., Y. Wang, C. Kuang and S. Lee (2007). Chemical investigation of *Phyllanthus reticulates* by HPLC-SPE-NMR and conventional methods. Phytochem. Anal., 18: 251 - 255.

Liu, X., C. Cui, M. Zhao, J. Wang, W. Luo, B. Yang and Y. Jiang (2008). Identification of phenolics in the fruits of emblica (*Phyllanthus emblica* L.) and their antioxidant activities. Food Chem., 109 : 909 - 915.

Luiz, A.L.S., L.B. Valquíria, G.O. George and R.P. Pedro (2003). Total Flavonoid Determination for the Quality Control of Aqueous Extractives from *Phyllanthus niruri* L. Lat. Am. J. Pharm., 22(3): 203-207.

Luo, W., M. Zhao, B. Yang, G. Shen and G. Rao (2009). Identification of bioactive compounds in *Phyllenthus emblica* L. fruit and their free radical scavenging activities. Food Chem., 114: 499-504.

Malayaman, V., B. M. Ghouse and B. K. Amzad, (2014). An efficient callus induction from *Phyllanthus debilis* Klein Ex Willd- A Wild Medicinal Plant of Eastern Ghats, India. J. Pure App. Biosci., 2 (2): 181-186

Mazumder, A., A. Mahato and R. Mazumder (2006). Antimicrobial potentiality of *Phyllanthus amarus* against drug resistant pathogens. Nat. Prod. Res., 20 (4): 323–326.

Murugaiyah, V. and L. Chan (2007). Determination of four lignans in *Phyllanthus niruri* L. by a simple high-performance liquid chromatography method with fluorescence detection. J. Chrom., 1154: 198-204.

Ndlebe, V.J., N.R. Crouch and D.A. Mulholl (2008). Triterpenoids from the African tree *Phyllanthus polyanthus*. Phytochem. Letters, 1: 11-17.

Pham-Huy, L.A., H. He and C. Pham-Huy (2008). Free Radicals, Antioxidants in

disease and Health. Int. J. Biomed. Sci., 4(2): 89- 96.

Raja, A., J.A. Blessy, R. Sathiya, N. Jayabalan, (2011). *In vitro* Regeneration of *Phyllanthus maderaspatensis* L.-A Traditional Medicinal Plant. Indian J. Nat. Sci., 4: 0976-0997.

Rajagobal, E.V. (2010). *In vitro* organogenesis in *Phyllanthus amarus* Schum. and Thunn. the Asian and Aust. J. Plant Sci. and Biotech., 4(1): 74-76.

Rajeshkumar, N.V., K. Joy, G. Kuttan, R.S. Ramsewak, M.G. Nair and R. Kuttan (2002). Antitumour and anticarcinogenic activity of *Phyllanthus amarus* extract. J. Ethnopharmacol., 81 (1): 17-22.

Sarg, T., A. Abdel- Ghani, R. Zayed and M. El-Sayed (2012). Bioactive compounds from *Phyllanthus atropurpureus*. J. Nat. Prod., 5 (10): 0974 – 5211.

Santos, A.R.S., F.V. Cechinel, A.M. Viana, F.N. Moreno, M.M. Campos, R.A. Yunes and J. B. Calixto (1994). Analgesic effects of callus culture extracts from selected species of *Phyllanthus* in mice., J. Pharm. Pharmacol., 46: 755-759.

Sen, A., M.M. Sharma, D. Grover and Batra (2009). *In vitro* regeneration of *Phyllanthus amarus* Schum and Thonn. An Important Med. Plant. Our Nat., 110-115.

Shakil, A., J. Kumar, K. Pandey and B. Saxena (2008). Nematicidal prenylated flavonones from *Phyllanthus nirur*. Phytochem., 69 : 759-764.

Sharma, O.P. (1993). Plant Taxonomy. Tata McGraw-Hill publishing company Ltd., New Delhi, New York, San Francisco, Paris, London, Tokyo, 377-382.

Shead, A., K. Vickery, A. Pajkos, R. Medhurst, J. Freiman, R. Dixon and Y. Cossart (1992). Effects of *Phyllanthus* plant extracts on duck hepatitis B virus *in vitro* and *in vivo*. Antiviral Res., 18: 127-138.

Shin, S., H. Kang and I. Lee (2005). A flavonoid from medicinal plants blocks hepatitis B virus-e antigen secretion in HBV-infected hepatocytes. Antiviral Res., 67: 163- 168.

Tahseen, M. and G. Mishra (2013). Ethnobotany and diuretic activity of some selected indian medicinal plants. The Pharma Innov., 2 : 112 - 119.

Than, N., S. Fosto, B. Poeggeler, R. Hardeland and H. Laatsch (2006). Niruriflavone, a new antioxidant flavone sulfonic acid from *Phyllanthus niruri*; Z. Naturforsch, 61 : 57-60.

Thyagarajan, S.P., S. Subramanian, T. Thirunalasudary, P. S. Venkastewaran and B. S. Blumberg (1988). Preliminary study: the effect of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. Lancet, 2: 764-766.

Tuchinda, P., A. Kumkao, M. Pohmakotr, S. Sophasan, T. Santisuk and V. Reutrakul (2006). Cytotoxic arylnaphthalide lignan glycosides from the aerial parts of *Phyllanthus toxodiifolius*. Planta Med., 72: 60-62.

Unander, D.W. (1991). Callus induction in *Phyllanthus* species and inhibition of viral DNA polymerase and reverse transcriptase by callus extracts., Plant Cell Reports., 10 : 461 - 466.

Unander, D. W. (1996). *Phyllanthus* pecies: *in vitro* culture and production of secondary metabolites., In Biotechnology in agriculture and forestry (YPS. Bajaj, ed.)., Springer-Verlag, Berlin., 37: 304 - 318.

Ushie, O., P. Neji and E. Etim, (2013). Phytochemical screening and antimicrobial activities of *Phyllanthus amarus* stem bark extracts. Int. J. Mod. Biol. and Med., 3: 101-112.

Venkateswaran, P.S., D.W. Unander, B.S. Blumberg, T. Halbherr, D. Sharager, L. Dahl, M. Kraus, C. Rissinger and H.H. Simmons (1987). Potential antiviral agents for the treatment of hepatitis B virus and retroviruses. Scientia Rep., 300-301.

Zhang, J., T. Abe, T. Tanaka, R. Yang and I. Kouno (2001). Phyllanemblinins A-F, new ellagitannins from *Phyllanthus emblica*. J. Nat. Prod., 64 : 1527-1532.

تأثيـر الطـرز البيئيـة للأملـج المصـري ومنظمـات النمو علـى الإكثـار الدقيق المباشر وإنتاج الكالس والمحتـوى الكلـى للقلـويـدات والفينولات

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الهدف من البحث هو تحديد تأثير الطرز البيئية المختلفة للأملج المصرى (الشرقية والإسماعيلية والقاهرة) ومنظمات النمو على المسـار المباشـر (الإكثـار الدقيـق) والمسار غير المباشـر (إنتاج الكالـس) وكذلك على المحتـوى الكلى للقلويدات والفينولات، بالنسبة لمرحلة الإكثار الدقيق تم استخدام بيئة (MS) بأربعة تركيزات مختلفة من منظمات النمـو : M0 (without hormon)-M1 (1 mg/l BA) - M2 (2 mg/l BA) - M3 (4 mg/l BA)، أما بالنسبة لمرحلة التجزير فقد أستخدم أربعة تركيزات مختلفة من منظمات النمو: M0 - M4 (0.50 mg/l IBA +0.50 mg/l IAA)- M5 (2 mg/l IBA)- M6 (4 mg/l IBA) أما بالنسبة لإنتاج الكالس أستخدمت أربعة تركيزات مختلفة من منظمات النمو:M0 - M7 (2 mg/l NAA) + 0.2 mg/l (BA) - M8 (1 mg/l 2, 4 –D)– M9 (2mg/1 2, 4-D) ، وقد أظهرت النتائج أن الطراز البيئى للشرقية كان أفضل وراثيا بالنسبة لمرحلة الأكثار الدقيق حيث أعطى (2.91 shoots) ويليه طراز الإسماعيلية، وأفضل بيئة للإكثار الدقيق كانت M1 (1 mg/l BAP) حيث أعطت (2.66 shoots) ويليها M0 (without hormon) وطراز الشرقية مع بيئة M1 أعطى قيمة معنوية مع كل من الصفات المدروسة وهى عدد الفروع وطول الفروع والوزن الطازج والوزن الجاف ونسبة الفروع وعدد الأوراق والسلاميات، أيضا الطراز البيئى للشرقيه اعطى اعلى استجابه وراثيه بالنسبة لمرحلة التجزير (4.33 roots) ويليه طراز الإسماعيلية أما أفضل بيئة للتجزير كانت M4 (0.50 mg/l IBA +0.50 mg/l IAA) حيث أعطت (2.4 roots) ويليها (without hormon) M0 وطراز الشرقية مع بيئة M4 أعطى قيمة معنوية مع كل من الصفات المدروسة وهى عدد الجذور وطول الجذر ونسبة التجذير، كما أعطى الطراز البيئى للشرقيه أعلى استجابة وراثية بالنسبة لمرحلة إنتاج الكالس حيث أعطى (88.8%) ويليه طراز الإسماعيلية أما أفضل بيئة لإنتاج الكالس كانت M8 (1 mg/l 2, 4 –D) حيث أعطت (81.4%) ويليها M9 وطراز الشرقية مع بيئة M8 أعطى قيمة معنوية مع كل من الصفات المدروسة وهى الوزن الطازج والوزن الجاف ونسبة إنتاج الكالس وموعد بدء الكالس، المحتوى الكلى للقلويدات تم حسابه بطريقة أخضر برومو كريزول باستخدام الأسبكتروفوتوميتر لكل من (الأوراق- الساق- الكالس)، وقد أظهرت النتائج ان الطراز البيئى الإسماعيلية هو أعلى الطرز البيئية المدروسة فى إجمالى المحتوى الكلى من القلويدات (1.60 mg/g) ويليها طراز الشرقية، وكانت الساق هي أفضل جزء من النبات لانتاج القلويدات (0.86 mg/g) ويليها الورقة، المحتوى الكلى للفينولات تم حسابه بطريقة Folin- Ciocalteu method ، وقد أظهرت النتائج أن الطراز البيئى الإسماعيلية هو أعلى الطرز البيئية المدروسة في إجمالى المحتوى الكلى من الفينولات (2.513 mg/g) ويليها طراز الشرقية، في حين كان الطراز البيئي القاهرة اقل في معدل المحتوى الكلى للقلويدات والفينولات.

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