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Effect of aqueous extract of *Croton bonplandianum* Baill. on *Lycopersicon esculentum* Mill. studied in relation to some macromolecules

Ajoy Ghosh, Padma Chatterjee*

Plant Biochemistry, Molecular Biology & Advance Plant Physiology Research Laboratory, Department of Botany, University of Kalyani, Kalyani 741235, Nadia, West Bengal, (INDIA)

E-mail: schatterjeecal2003@yahoo.co.in

ABSTRACT

Some biochemical parameters in relation to growth and development were analysed in *Lysopersicon esculentum* Mill. plants in response to pre-soaking of seeds followed by foliar spray done with aqueous extract of *Croton bonplandianum* Baill. In the study it was observed that the aqueous extract of *Croton bonplandianum* enhanced the chlorophyll and soluble sugar content and reduced the DNA content per gram of tissue, whereas it had no effect on RNA content.

KEYWORDS

Croton bonplandianum;
Aqueous extract;
Biochemical parameters;
Macromolecules,
Lycopersicon esculentum.

INTRODUCTION

Croton bonplandianum is a widely distributed weed of the family Euphorbiaceae. The plant has many harmful effects on other plants. It contains two phytotoxic compounds (related to abscisic or phaseic acid) which affects the growth of *Chrysopogon aciculatus, Cynodon dactylon, Eupatorium odoratum, Evolvulus nummularius* and *Mikania cordata*[1]. The plant also affect the growth and metabolism of *Parthenium* by decresing the chlorophyll, sugars, protein and lipid contents and increasing the organic and amino acid content[2]. In one of our[3] studies we reported promotive effect of the aqueous extract of *Croton bonplandianum* on some morphological parameters of *Lycopersicon* plants that was observed after spraying at the initial age of the plants. So we decided to find out the effect of the extract on some macro molecules that are concerned in growth and development.

EXPERIMENTAL

Healthy *Croton bonplandianum* plants were collected from the campus of the University of Kalyani during the month of June –July 2011. The collected plants were washed thoroughly with the distilled water. Plants were sun dried and powdered with electric blender. 100 gm. of the powered plant material was soaked in 1000 ml. of distilled water for overnight and then filtered. The filtrate was labelled as 100% extract and was used for the study. Tomato seeds were pre-soaked in different concentration (25%, 50%, and 100%) of the said extract along with distilled water (control) for 3-4 hours, separately. Then the seeds were transplanted to the loam soil and irrigated adequately for germination. On germination plants ware irrigated as usual. Organic manure was applied to the plants. As the plants grew, different concentrations of extract were sprayed (after 20 days from the day of seed pre-soaking) on the leaves of respective plants, weekly for 4

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consecutive weeks.

Then the following tests were done from the leaves of the plants. Study was done at normal growing season of *Lycopersicon* . No pesticide was applied.

Chlorophyll content

Reagents

80% acetone

Procedure

From the experimental plants laminas of the healthy mature leaves were excised, cut into small pieces and mixed up. From the mixture 1gm of material was taken out and used for chlorophyll content measurement following the method of Arnon (1949)[4]. The material was taken into a clean mortar and grind to a fine pulp with addition of 20 ml 80% chilled acetone. It was centrifuge at 5,000 rpm for 5 min and the supernatant was transferred to a 100 ml volumetric flask. The residual was further grind with 80% acetone, centrifuge and the supernatant was collected. The process was repeated until the residual became colourless. Final volume of the collected supernatant was made up to 100 ml with 80% acetone. Absorbance was taken at the weave length 645 nm and 663 nm against 80% acetone as blank. Amount of chlorophyll per gram of tissue was calculated using the following formula

mg of total Chlorophyll = 22.2 (A_{645}) +

$$\textbf{8.02 } (A_{663}) \times \frac{V}{1000 \times W}$$

where A = absorbance at specific weavelengths; V = final volume of chlorophyll extract; W = fresh weight of tissue extracted.

Total soluble sugar content

Reagents

1 80% ethanol

2 0.2% anthrone in conc. H_2SO_4

Procedure

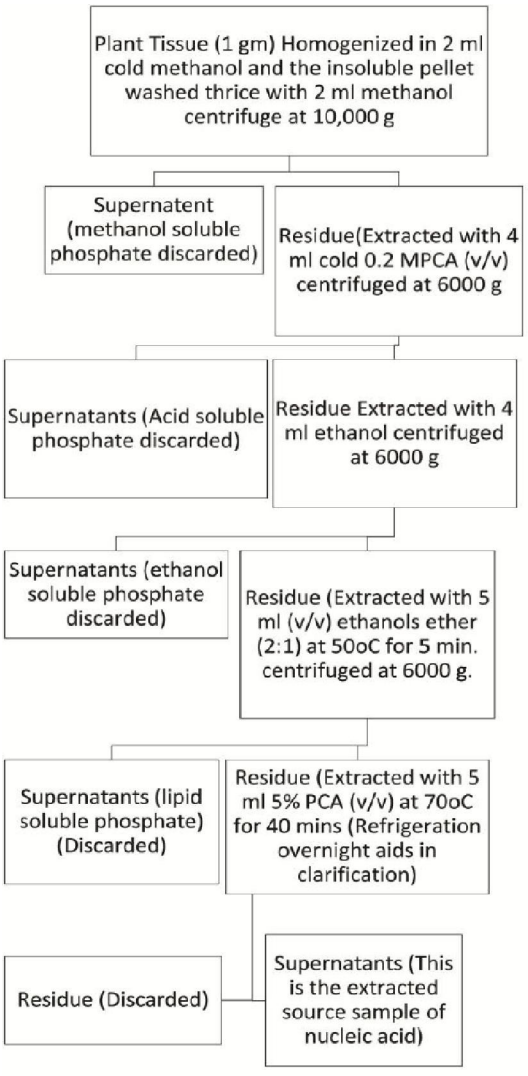
Determination of soluble sugar content was done following the method of Mc Cready *et al*. (1950)[5]. For this 0.5 gms of leaf samples were extracted with 80% boiling ethanol in a mortar with pestle. Extract was centrifuged at 6000 g for 10 mins. The supernatant was taken in a test tube. This was repeated thrice. The resultant supernatant was the carbohydrate source. 1 ml of each source sample was taken in separate test tubes after necessary dilution of the stock solution. In each of them 4 ml freshly prepared, precooled (0.2% in conc. H_2SO_4, w/v) anthrone reagent was added. After 30 mins, the intensity of green colour in terms of OD was measured in a spectrophotometer at 630 nm. Actual quantity was evaluated from the standard curve of glucose

Extraction and determination of nucleic acid content

(a) Extraction of nucleic acid

Procedure

Extraction of nucleic acid was made from 1 gm of leaf material following the methods described by Cherry (1963)[6] with some modification. An outline of extraction procedure is as follows :



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(b) Estimation of nucleic acid content

(i) Estimation of DNA content

Reagents

1 Standard DNA
2 Diphenylamine reagent : 100 ml glacial acetic acid (BDH, AR) added to 2.7 ml conc. H_2SO_4 and 1 gm AR grade diphenylamine.

Procedure

DNA content was estimated following the method of Burton (1968)[7] 1 ml of nucleic acid extract was mixed with 5 ml freshly prepared DPA reagent. The mixture was heated in a boiling water bath for 30 min. with a glass marble at the top of the test tube. After cooling in running tap water, intensity of blue colour was measured spectrophotometrically at 600 nm. DNA content was quantified from the OD values of a standard curve prepared with calf thymas DNA.

(ii) Estimation of RNA content

Reagent

1 Standard RNA solution – 0.2 mg/ml.
2 Orcinol reagent : 1 gm AR grade orcinol dissolved in 100 ml conc. HCl containing 0.1% $FeCl_3.6H_2O$.

Procedure

For the estimation of RNA, 3 ml of each of leaf extract (in 5% PCA) in separate test tubes was treated with an equal volume of freshly prepared orcinol reagent and boiled in a water bath for 20 mins. with glass marbles at the top of the test tubes. The mixture was then cooled, the colour intensity of the blue green colour was measured at 660 nm in the spectrophotometer. The blank used contained a mixture of 3 ml distilled water and 3 ml orcinol reagent, which was treated in an identical manner. RNA content was calculated from a standard curve prepared with yeast RNA. This method was done following Markham (1955)[8], modified by Choudhuri and Chatterjee (1970)[9].

RESULT AND DISCUSSION

Chlorophyll content

Chlorophyll content was measured four times (on 51st day, 67th day, 80th day and 98th day from the day of seed presoaking) from the actively growing phase of the plants. It was found that chlorophyll content gradually increase in all the sets (TABLE 1), except in few cases (50% and 10% extract treated sets) where, chlorophyll content decreased at the last stage.

TABLE 1 : Total Chlorophyll content of different treatment sets.

sets		Total Chlorophyll Content mg/g of tissue at the age of			
		51 days	67days	80 days	98 days
Control	Mean	0.72	0.78	0.94	0.93
	Std. Deviation	0.03	0.03	0.02	0.03
25% extract treated	Mean	0.87	1.00	1.02	1.11
	Std. Deviation	0.05	0.02	0.03	0.04
50% extract treated	Mean	0.71	0.86	1.16	0.81
	Std. Deviation	0.03	0.02	0.04	0.03
100% extract treated	Mean	0.98	1.05	1.12	0.93
	Std. Deviation	0.04	0.03	0.03	0.02
Total	Mean	0.82	0.92	1.06	0.95
	Std. Deviation	0.12	0.12	0.09	0.12

TABLE 1 (a) : Anova of chlorophyll content

			Sum of Squares	df	Mean Square	F	Sig.
at the age of 51 days * sets	Between Groups	(Combined)	0.16	3	0.05	42.80	0.000
	Within Groups		0.01	8	0.00		
	Total		0.17	11			
at the age of 67days * sets	Between Groups	(Combined)	0.15	3	0.05	78.92	0.000
	Within Groups		0.01	8	0.00		
	Total		0.15	11			
at the age of 80 days * sets	Between Groups	(Combined)	0.08	3	0.03	30.34	0.000
	Within Groups		0.01	8	0.00		
	Total		0.09	11			
at the age of 98 days * sets	Between Groups	(Combined)	0.14	3	0.05	52.58	0.000
	Within Groups		0.01	8	0.00		
	Total		0.15	11			

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Moreover, the treated plants generally containedgreater (sometimes equal) amount chlorophyll than the control plants.

Statistical analysis of the variance (ANOVA) also supports the observation(TABLE 1.a) i.e. the similarity among the sets were at the level of P=0.00. Hence it can be said that the observed variation was statistically significant and the treated plants contains more chlorophyll than the control plants.

Total soluble sugar content

Chlorophyll content was also measured four times (on 51st day, 67th day, 80th day and 98th day from the day of seed presoaking) from the actively growing phase of the plants.

TABLE 2 : Soluble sugar content of different treatment sets

sets		Content of total soluble Sugar mg/gm. of tissue at age of			
		51 days	67 days	80 days	98 days
Control	Mean	5.75	8.32	26.82	8.58
	Std. Deviation	0.67	0.50	1.48	0.86
25% extract treated	Mean	21.23	25.33	39.83	7.37
	Std. Deviation	0.86	2.52	1.26	0.30
50% extract treated	Mean	16.58	13.86	38.74	3.96
	Std. Deviation	0.65	1.03	1.10	0.90
100% extract treated	Mean	21.69	13.97	36.18	5.48
	Std. Deviation	1.51	1.50	1.46	0.60
Total	Mean	16.31	15.37	35.39	6.35
	Std. Deviation	6.76	6.60	5.47	1.94

It was found that up to the 80 days age of the plants the treated plants always contains more soluble sugar than the control plants (TABLE 2). But at the older age (i.e. at 98 days), the treated plants contain less sugar than the control.

TABLE 2(a) : Anova of soluble sugar conten

		Sum of Squares	df	Mean Square	F	Sig.
51 days * sets	Between Groups	494.31	3.00	164.77	168.93	0.00
	Within Groups	7.80	8.00	0.98		
	Total	502.11	11.00			
67 days * sets	Between Groups	459.44	3.00	153.15	61.89	0.00
	Within Groups	19.80	8.00	2.47		
	Total	479.24	11.00			
80 days * sets	Between Groups	314.90	3.00	104.97	59.08	0.00
	Within Groups	14.21	8.00	1.78		
	Total	329.11	11.00			
98 days * sets	Between Groups	37.42	3.00	12.47	24.85	0.00
	Within Groups	4.01	8.00	0.50		
	Total	41.43	11.00			

Analysis of variance (ANOVA)of sugar content (TABLE 2.a) also reveals that the different treatment sets possess very insignificant similarity (at the level of <1%) among them. That means different sets always differ significantly in respect of sugar content.

By means of LSD we have also found that all the treatment sets always contain more soluble sugar than that of the control except at the days of 98 (TABLE 2.b). At this stage 50% and 100% extract treated sets contain significantly less amount of sugar.

TABLE 2 (b) : Comparison of different treatment setsby LSD

Dependent Variable: Sugar content at	(I) sets	(J) sets	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
51 days	Control	25% extract treated	-15.48*	0.81	0.00	-17.34	-13.62
		50% extract treated	-10.83*	0.81	0.00	-12.69	-8.97
		100% extract treated	-15.94*	0.81	0.00	-17.80	-14.09
67 days	Control	25% extract treated	-17.01*	1.28	0.00	-19.97	-14.05
		50% extract treated	-5.54*	1.28	0.00	-8.50	-2.58
		100% extract treated	-5.65*	1.28	0.00	-8.61	-2.69
80 days	Control	25% extract treated	-13.01*	1.09	0.00	-15.52	-10.50
		50% extract treated	-11.91*	1.09	0.00	-14.42	-9.40
		100% extract treated	-9.36*	1.09	0.00	-11.87	-6.85
98 days	Control	25% extract treated	1.20	0.58	0.07	-0.13	2.54
		50% extract treated	4.62*	0.58	0.00	3.28	5.95
		100% extract treated	3.10*	0.58	0.00	1.76	4.43

*** The mean difference is significant at the .05 level.**

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Estimation of DNA content

DNA content was estimated from the leaf of the plants at the age of 70 days.

TABLE 3 : µg of DNA/gm. tissue of the plant

sets	Mean	Std. Deviation
control	7.42	0.40
25% extract treated	6.19	0.60
50% extract treated	5.65	0.55
100 % extract treated	5.13	0.55
Total	6.10	0.99

TABLE 3 (a) : Anova of µg of DNA/gm tissue of the plant

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.631016	3	2.877	10.233	0.004
Within Groups	2.249165	8	0.281		
Total	10.88018	11			

It was found that in the control plant the amount of DNA was 7.42µg/ gm. tissue. It was gradually reduced to 5.13 µg/gm. tissue in the 100% extract treated plants (TABLE 3). Analysis of variance (TABLE 3.a) reveals that the similarity among the groups was at 0.4 % level. Hence it can be said that the DNA content varies significantly among the treatments.

When we have compared the mean of different treatment with the mean of control by the method of LSD (TABLE 3.b); we have found that all the treated sets possess significantly (25% extract treated set at the level of 0.05, rest two at the level of 0.01) less amount of DNA than that of the control sets.

Estimation of RNA content

RNA content was also estimated from the leaf of the plants at the age of 70 days.

TABLE 3 (b) : Comparison of µg of DNA/gm tissue of the plant by LSD.

LSD (I) sets	(J) sets	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lowe Bound	Upper Bound
control	25% extract treated	1.22*	0.43	0.02	0.23	2.22
	50% extract treated	1.76*	0.43	0.00	0.77	2.76
	100 % extract treated	2.28*	0.43	0.00	1.29	3.28

*** The mean difference is significant at the .05 level.**

Estimation of RNA revealed that all the plants contain about 18 µg of RNA/gm.tissue of the plant (TABLE 4). Analysisof variance also supports that the variation of RNA content among the different sets were insignificant.

TABLE 4 : µg of RNA/gm. tissue of the plant

sets	Mean	Std. Deviation
control	18.21	0.22
25% extract treated	18.10	0.20
50% extract treated	17.87	0.27
100 % extract treated	18.01	0.21
Total	18.05	0.23

The above findings may be cited as a new report of promotive effect of aqueous extract of *Croton bonplandianum* on two important physiological and metabolic regulators (chlorophyll, Sugar) of plant growth and development. However the DNA content / gm. of tissue was lowered and no effect was observed on the RNA content.

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