

## DOSE DEPENDENT AND TIME COURSE ELICITOR ACTIVITY OF *CODIUM ELONGATUM* AND *ULVA LACTULUS* (GREEN ALGAE) OF KARACHI COAST

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### Abstract

High molecular weight crude polysaccharides obtained from green algal plants *Codium elongatum* and *Ulva lactulus* were evaluated as an elicitor of disease resistance response in chickpea tissues in terms of induced browning and production of induced secondary metabolites. The results were recorded as a function of time and doses of elicitor employed. Hplc method was developed for the separation of complex mixture of induced secondary metabolites.

### Introduction

As a result of infection or stress, plants exhibit some natural resistance responses (Darvill & Albersheim, 1984; Hammerschmidt, 1999). These responses may be triggered by variety of compounds isolated from cell wall, culture filtrate and cytoplasm of various parasitic and non-parasitic plants pathogens known as Elicitors (Knogge, 1996), host derived endogenous elicitors (Greenberg *et al.*, 1994) and abiotic elicitors (Setakopan *et al.*, 1992). Substances of microbial origin are diverse in nature like polysaccharides (Gunia *et al.*, 1991), proteins (Vogelsang *et al.*, 1994) and fatty acids (Castoria *et al.*, 1995). In most cases elicitor activity was associated with polysaccharide fractions of various preparations (Kessman *et al.*, 1988). The present report describes the seaweed polysaccharides as an inducer of hypersensitive response in treated tissues of chickpea.

The work presented here is based on time course and dose dependent elicitor activity of High Molecular Weight Crude Elicitor Preparations obtained from green algae viz., *Codium elongatum* and *Ulva lactulus*. Results were recorded in terms of induced browning and production of induced secondary metabolites. A simple HPLC method was developed for separation of complex mixture of these metabolites.

### Materials and Methods

**Extraction and elicitor preparation of seaweed:** *Codium elongatum* and *Ulva lactulus* (green algae) were collected from Buleji and Cap Monz in the month of March, 2002. Washed and air dried material was extracted with water, dilute acid and alkali. HMWCEP were obtained by ethanol precipitation and lyophilization.

**Elicitor activity:** A general method of elicitor application was employed (Whitehead *et al.*, 1982). Chickpea (*Cicer arietinum* L.) desi channa was purchased from local market, germinated and etiolated cotyledons were treated with 20  $\mu$ L of elicitor solution at a concentration of 5, 25, 50 and 75  $\mu$ g glucose eq/mL of two algal plants and incubated for 24 hour. In another set of experiment, cotyledons were treated with elicitor concentration of 100  $\mu$ g glc eq/mL and incubated for 6, 15, 24 and 48 hour. Control samples were

prepared by cotyledon treated with sterile water. Control and treated samples were incubated at 25°C in dark. Browning induced in treated chick pea samples was recorded with time and in response to various dilutions of elicitors.

**Extraction and production of induced secondary metabolites (ISM):** After elicitor treatment and specified period of incubation the treated and control samples were dipped in 10-15 ml of redistilled ethanol (95%) and left overnight for complete extraction, illumination was avoided as much as possible. The filtered extracts were concentrated under vacuum below 45°C, flushed with nitrogen and stored at -20°C in dark and weight of dry chickpea tissues were taken.

**Separation of ISM by HPLC:** HPLC separation was accomplished on High Chrom, 3.9 x 300 mm reverse phase C18 column, using branded instrument of Waters HPLC with a gradient system and variable UV detector. A guard column of pellicular C18 hydrocarbon chemically bonded to glass beads was placed before the analytical column. Initially 80:20 water : acetonitrile (both phases contain 1% acetic acid) was run for 5 minutes and then a gradient of 40:60 reached into 15 minutes and further goes to 100% acetonitrile in 5 minutes, stays there for another 5 minutes and returned to initial solvent system.

**Sample preparation:** Dry alcoholic extracts of treated and control samples were dissolved into 2 ml of initial solvent (40:60 water:acetonitrile, containing 1% acetic acid). 100 µL of this solution was further diluted with 1 ml of same solvent, centrifuged and filtered through 0.2 µm filters and a clear solution of 25 µL was applied on the column.

## Results and Discussion

Details of isolation, chemical analysis and elicitor activity of HMWCEP obtained from green algal plants viz., *C. elongatum*, *C. texiflora* and *U. lactulus* at a single concentration 100µg glu eq/mL were reported in our earlier report (Fatima & Seema, 1999). On the basis of this preliminary screening hot aqueous extract of *C. elongatum* with higher elicitor activity and cold NaOH (0.1N) extract of *U. lactulus* with low profile of activity were selected for dose dependent and time course studies of elicitor activity. In plant-pathogen interaction, dose dependent and time course elicitor activity was determined to optimize the elicitor concentration and conditions with possible physiological significance for subsequent production of host resistance responses (Mackenbrock *et al.*, 1993).

The result of qualitative estimation of elicitor activity in terms of induced browning are presented in Fig. 1 and 2. Difference in browning were significant in treated and control samples, also level of browning was different in response to various dilutions of the same elicitors employed. Maximum browning was produced by the elicitors of *C. elongatum* at 25µg and 100µg concentration as compared to browning induced by other elicitor dilutions. Time course study of elicitor activity was carried out over a period of 48 hour. No significant browning was produced by the treated and control samples at 6 hour of incubation however browning appeared after 15 hour in response to *C. elongatum* as compared to control samples (Fig. 2). A distinct and definite browning was produced at 24 hour in response to both plants, intensity of browning was further increased and no significant difference remained in treated and control samples after 24 hour. Level of browning was high in the samples treated with elicitor preparations of *C. elongatum* than the *U. lactulus* (Fig. 1 & 2).

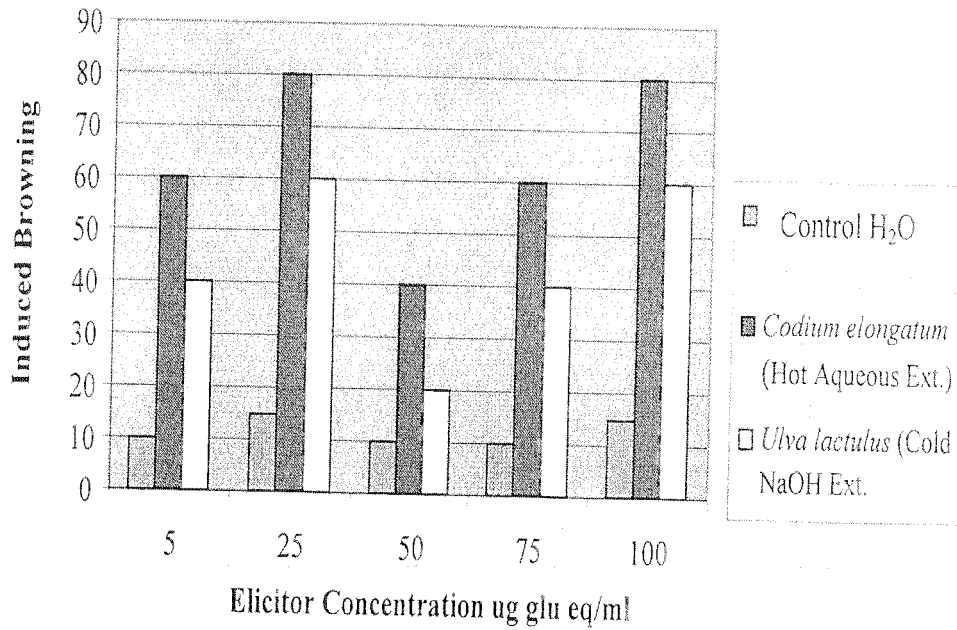


Fig. 1. Induced browning exhibited by Chickpea tissues on treatment with various dilutions of HMW Crude elicitor preparation of *C. elongatum* and *U. lactulus*.

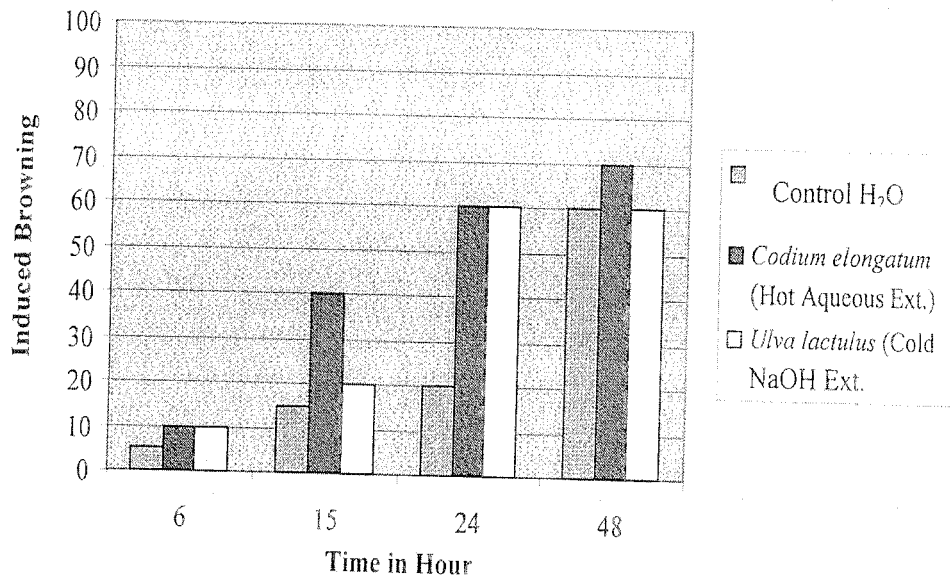


Fig. 2. Induced browning exhibited by Chickpea tissues on treatment with various dilutions of HMW Crude elicitor preparation of *C. elongatum* and *U. lactulus* at a single concentration and incubated at different interval of time.

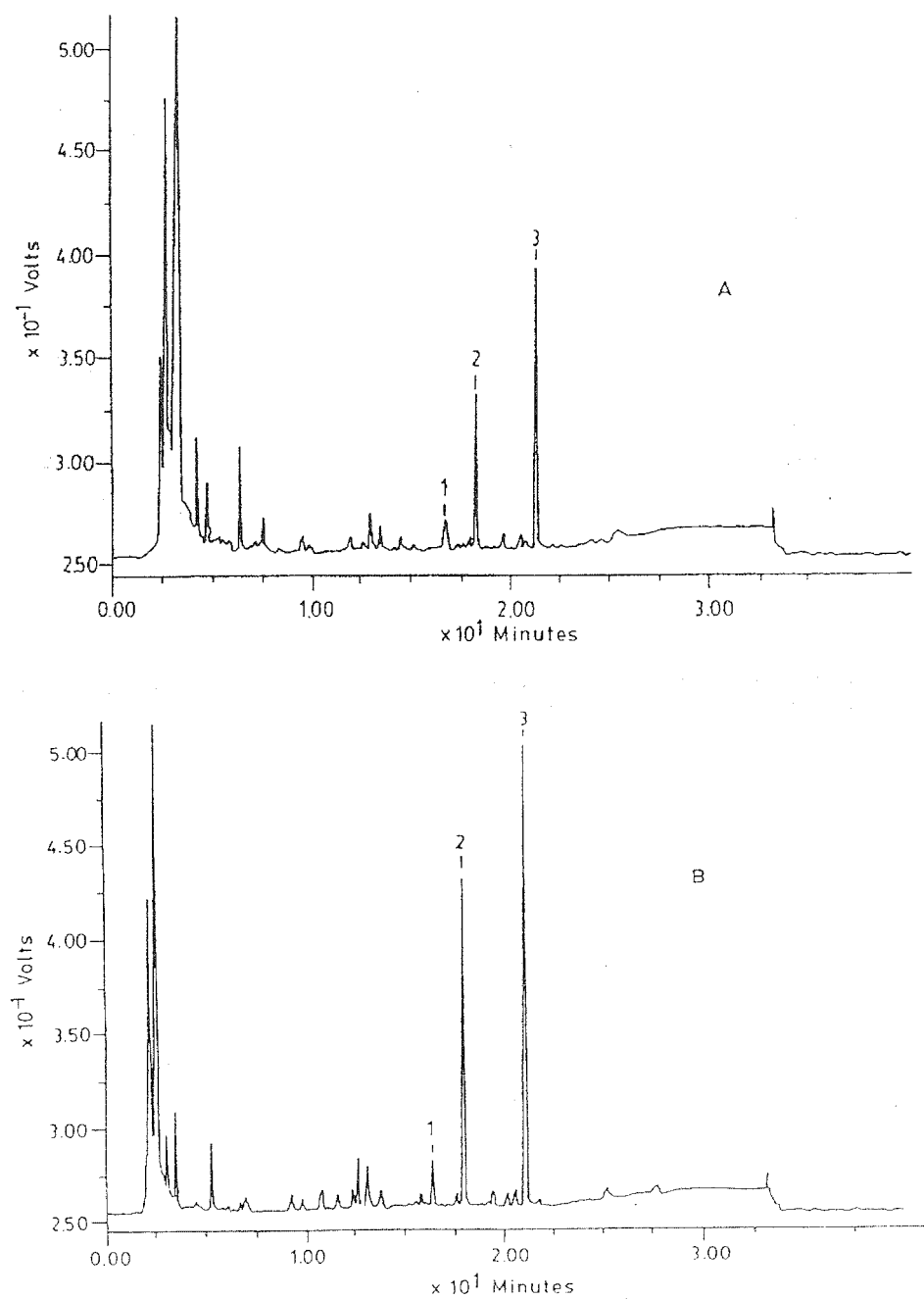


Fig. 3. HPLC separation of induced secondary metabolites in chickpea (*Cicer arietinum*) cotyledons in dose response experiment. (A) Control sample (B) Tissues treated with *Codium elongatum*.

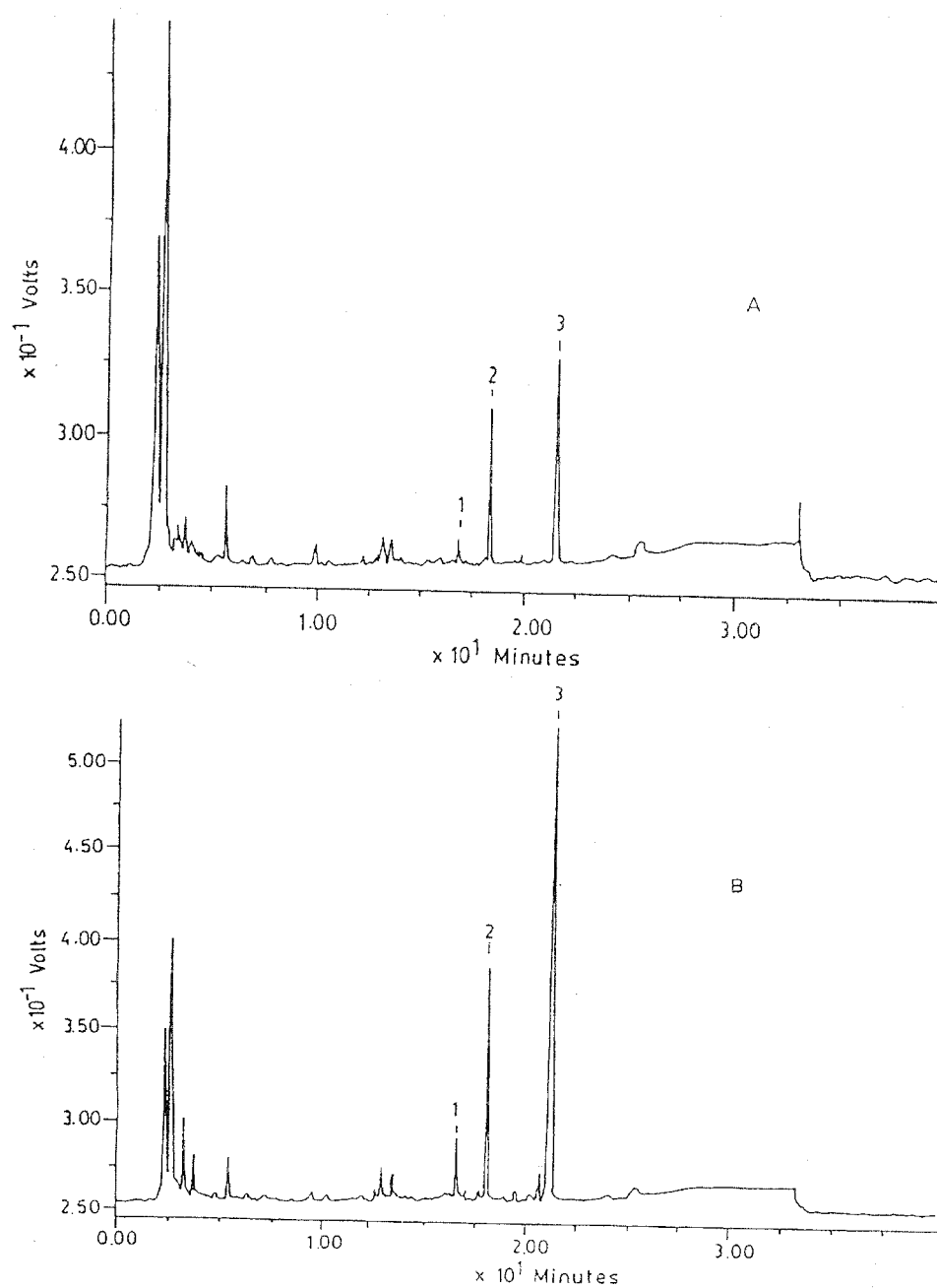


Fig. 4. HPLC separation of induced secondary metabolites in chickpea (*Cicer arietinum*) cotyledons in time course experiment. (A) Control sample (B) Tissues treated with *Codium elongatum*.

**Table 1. Metabolites in extracts of *Cicer arietinum* cotyledons treated with various dilutions of HMWCEP of green algae.**

Plant	Peak No.	Rt/min.	Ratio of peak area/gm fresh wt. of treated/control tissues			
			Elicitor Conc. $\mu\text{g glu eq/mL}$			
			5	25	50	75
S-1	1	16.38	2.32	1.63	2.35	2.38
S-2			0.93	0.18	0.21	0.36
S-1	2	17.83	3.23	2.06	2.97	3.00
S-2			1.37	2.01	2.13	1.50
S-1	3	21.08	2.07	1.12	2.10	2.30
S-2			0.85	1.75	2.10	1.45

S-1 = *Codium elongatum*, S-2 = *Ulva lactulus*

**Table 2. Metabolites in extracts of *Cicer arietinum* cotyledons treated with HMWCEP of Green algae at different interval of time.**

Plant	Peak No.	Rt/min.	Ratio of peak area/gm fresh wt. of treated/control tissues			
			Incubation Period (hours)			
			6	15	24	48
S-1	1	16.07	0.74	0.76	4.04	0.22
S-2			0.48	1.27	2.77	3.77
S-1	2	17.70	2.00	2.26	2.95	1.21
S-2			0.61	1.01	1.21	2.35
S-1	3	20.84	1.79	3.91	3.95	1.14
S-2			0.51	1.11	1.21	2.23

S-1 = *Codium elongatum*, S-2 = *Ulva lactulus*

Rapidity and magnitude with which antifungal compounds are produced are important in disease resistance (Anderson *et al.*, 1991). HPLC method is successfully used for separation and quantification of induced secondary metabolites (ISM) known as 'Phytoalexins' in response to various elicitor preparations (Mackenbrock *et al.*, 1992, Fatima *et al.*, 2001, Fatima & Seema, 2002). Dose response and time course elicitation of petrocarbons 'phytoalexin' in cell suspension culture of *Cicer arietinum* by a fungus *Aschochyta rabie* and other phytoalexin capsidiol induced by the fungal derived elicitor has been reported (Kessman *et al.*, 1988).

Typical chromatogram in Fig. 3 and 4 showed the resolution of alcoholic extracts of elicited tissues of chickpea by HPLC analysis. Intensity of peaks was high in treated samples as compared to the control samples. The integrated area of the peaks were assumed to be proportional to the amount of solute present. The results in Table 1 and 2 were recorded as the ratio of the peak area/g dry weight of treated to that of control tissues. Prominent peaks 1,2,3 eluted in the organic phase of the gradient, possibly could be the flavonoids. Dose response activity of elicitors is given in Table 1. All three peaks

were induced at higher level at a concentration of 5 $\mu$ g in response to elicitor of *C.elongatum*, and induction was low at 25 $\mu$ g concentration again increases were observed at 50 $\mu$ g which remained almost the same at 75 $\mu$ g elicitor concentration. Same pattern was not followed by the tissues treated with elicitor preparation of *U. lactulus*. Peak 2 and 3 showed a regular increase from 5-50 $\mu$ g elicitor concentration and decreased at 75 $\mu$ g. Peaks 1 of this plant for all elicitor preparations were below the control level.

To examine the production rate of various major and minor components of induced secondary metabolites, a time course study was carried out over a period of 48 hour. These ISM in chickpea tissues were also estimated spectrophotometrically (Fatima & Seema, 2000). Results in Table-2 showed that after elicitor treatment and at 6 hour of incubation, except peak-1 other two peaks, 2 and 3 were induced at higher levels in sample treated with *C. elongatum* whereas in samples treated with elicitor preparation of *U. lactulus* all three peaks were below the control level. The metabolites concentration increased upto 24 hour in response to the elicitor preparations of both the plants. Results for 48 hours showed a regular decrease of metabolites in samples treated with *C. elongatum* whereas increments were observed in all the peaks treated with *U. lactulus*. Results for peak-1 in *C. arietinum* at 24 and 48 hours are a bit strange, as peak-1 was induced four times to the control at 24 hours and decreased sharply at 48 hours of incubation. Induced browning and induction of secondary metabolites also depend on the physiological condition of plant tissues.

These results showed that polysaccharides from green algal plants are potentially active elicitors especially elicitor preparations from *C. elongatum* induced higher level of browning and secondary metabolites in the treated tissues of chickpea. These preparations are active at low concentration i.e. 5 $\mu$ g glu eq/mL and exhibited responses as early as 6 hour of incubation.

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