Short Communication

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INVESTIGATION OF INDUCED SECONDARY METABOLITES IN RESPONSE TO VARIOUS TREATMENT OF ELICITOR OBTAINED FROM *HYPNEA MUSCIFORMIS* (RED ALGAE)

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Time course and dose dependent activity of elicitors (poly saccharides) obtained from *Hypnea musciformis* (red algae) was determined in terms of induced secondary metabolities in treated tissues of chickpea, followed by HPLC analysis. *H. musciformis* of class Rhodophyceae (red algae) is widely distributed at the Karachi coast of Pakistan. Carrageenan are water extractable polysaccharides isolated from *H. musciformis* (Knutsen *et al* 1995). Generally these polysaccharides are extensively used as gels and thickening agents in food and industrial preparations (Bixler 1995). It is reported in literature that polysaccharides and oligosaccharides from plants, microbial cell wall and culture filtrate can regulate defensive and developing processes (Darvill and Albersheim 1984) and known as "Elicitors". Seaweeds are rich in polysaccharides, we found worth exploring these polysaccharides as an elicitor of plant defense mechanism. Series of papers have been published for evaluating seaweed polysaccharides as an elicitor of disease resistance response in Chickpea tissues (Fatima and Seema 1999a and 2000). Antifungal properties of proteins (Agglutinins) isolated from *H. musciformis* are recently described by Melo *et al* 1997.

In this paper elicitor activity of High Molecular Weight Crude Elicitor Preparation (polysaccharides) "HMWCEP" obtained from *H. musciformis* was determined in the treated tissues of chickpea on the basis of incubation period and in response to various dilutions of elicitor. A general method of elicitor application was employed, control and treated samples were prepared as described in Fatima and Seema (1999b). Chickpea (*Cicerarietinum*) purchased from local market were elicited by HMWC polysaccharides of *H. musciformis* at concentrations of 5,25,50 and 75 μg glucose eq mL^{-1} and incubated for 24 hours. Tissues treated with 100 μg glucose eq mL^{-1} were incubated for 6,15,24 and 48 hours for induction of secondary metabolities. Concentrated ethanolic extracts prepared from each sample were analyzed by HPLC (Fatima *et al* 2001). The integrated areas of the peaks were assumed to be proportional to the amount of solute present. The results in table were recorded as the ratio of the peak area g^{-1} dry weight of treated to that of control tissues.

Typical chromatogram in Figure shows that extract were satisfactorily resolved into individual components as peak nos 1-9. Reproducibility of elution time of identified peaks was excellent. Most of the peaks were common in control and treated tissues. It was previously reported that low elicitor concentration of pathogenic fungi *Aschochyta rabei* favoured pterocarpon conjugate formation whereas high doses lead to pterocarpon aglycone accumulation (Kessman *et al* 1980). In present studies, the increases in components marked by peaks no. 3, 5, 6 8 and 9 were observed in the samples treated with elicitor concentration 25

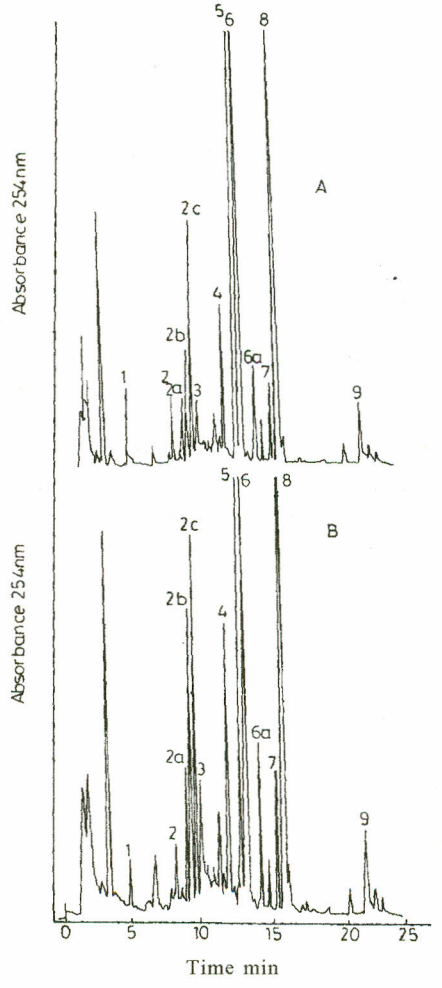

Fig 1. Typical chromatogram for separation of induced metabolities in *C. arietinum*, cotyledons. A) Control sample, B) Treated tissues.

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Table 1

Metabolities induced in *Cicer arietinum* tissues treated with HMWCEP of *H. musciformis* with different dilutions and incubation at different intervals of time

Peak No.	Rt./min	Ratio of peak area g^{-1} fresh wt. of treated/control tissue							
		Doses, *1(ug glu eq ml^{-1})				Time *2 (h)			
		5	25	50	75	6	15	24	48
1.	5.42	0.71	0.31	1.09	2.45	4.34	-	-	-
2.	8.89	1.42	0.80	1.26	0.86	2.03	1.92	0.89	4.01
3.	10.51	1.95	2.24	0.38	0.45	1.31	2.20	2.22	0.94
4.	12.46	1.12	1.17	1.29	1.31	1.27	1.09	1.54	1.61
5.	13.40	0.88	1.14	0.44	0.63	0.68	1.24	1.26	1.05
6.	13.73	0.90	1.17	0.91	1.29	1.07	1.02	0.90	1.13
7.	16.05	2.13	1.39	1.32	1.34	1.68	0.71	1.32	2.52
8.	16.52	0.91	1.14	0.91	0.86	1.07	1.00	1.12	1.15
9.	22.44	1.29	2.20	0.82	0.83	0.95	0.28	0.60	0.81

Recorded data is mean of duplicate samples, *1 Incubation period, 24 h; *2 Elicitor Conc., 100 ug glu eq ml^{-1}.

mg glu. eq mL^{-1}, whereas these compounds were low at 50 mg dilution, as shown in Table. Significant increases of peak -7 and peak-1 were shown in the lowest concentration of 5 mg and highest concentration of 75 mg respectively.

It is documented that the rapidity and magnitude with which antifungal compounds produced in response to elicitor treatment in disease resistance were important rather than magnitude alone (Anderson *et al* 1991). Result for elicitor activity as function of time (Table 1 indicated that after 6 hour of incubation high level of various components were induced especially the peak no. 1, 2, 3, 4, 6, 7 and 8. Positions remain more or less same at 15 hours, only a marked suppression was observed in peak no.7. Peak no.1 was not found in any samples incubated for longer periods. Another burst of the component was observed after 48 hours, especially peak no.2 was induced three times more in quantity. Large number of peaks marked as 2a, b, c and 6a which were induced in dose response exsperiments were either absent or induced at very low levels during time course studies, reason could be the physiological conditions of tissues. It is concluded from results that polysaccharides from *H. musciformis* induced host components quicker and at higher level in treated tissues of chickpea as compare to the control elicitor was found dose dependent.

Key words: Polysaccharides, Chickpea incubation period, *H. musciformis* integrated area.

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