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Secondary Metabolites of *Chlorella* Vulgaris Under Saline Stress

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ABSTRACT

In the present study, the effect of NaCl on Secondary Metabolites of Chlorella vulgaris Beijerink was investigated. The C. vulgaris was treated with different concentrations of NaCl viz., 0.1, 0.2, 0.3 and 0.4M besides control over 10, 20 and 30 days. The results exhibited maximum increase in the Phenol, Flavonoid and Alkaloid up to 0.3 M for all the cultures over all the durations. The study revealed that, increased activity of Phenol, Flavonoid and Alkaloid content may provide a strategy to enhance salt tolerance and protection against oxidative damage.

Keywords: Chlorella, Phenol, Flavonoid, Alkaloid, NaCl

I. INTRODUCTION

Enhanced salt concentrations change the growth conditions in a manner unfavorable for most organisms. But, algae differ in their adaptability to salinity and based on tolerance extent they are grouped into halophytic and halo tolerant. In either case the algae produce some metabolites to protect from salt injury and also to balance as per the surrounding osmotica (Richmond, 1986). Hence microorganisms have often been used as model organisms to study basic physiological process in living cells.

In plants, salt stress leads to the over production of highly reactive and toxic reactive oxygen species (ROS) which damage proteins, lipids, carbohydrates and DNA in turn resulting in oxidative stress. Salt stress leads to an imbalance of the cellular ions resulting in ion toxicity and osmotic stress; leading to retardation of growth either directly by salt or indirectly by oxidative stress induced by ROS (Emad et al 2010). The antioxidant defense machinery protects algae against oxidative stress damages. Algae similar to plants, possess very efficient enzymatic (superoxide, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydro ascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaicol peroxidase, GOPX and glutathione-S-transferase, GST) and non-enzymatic (ascorbic acid, ASH; glutathione, GSH; phenolic compounds, carotenoids, alkaloids, non-protein amino acids and αtocopherols) antioxidant defense systems that protects plant cells from oxidative damage by scavenging ROS.

In addition to being rich sources of proteins, carbohydrates and fatty acids, microalgae are considered as promising alternative source for antioxidants such as carotenoids, flavonoids and phenols (Li et al. 2007; Natrah et al.,2007; Hajimahmoodi et al., 2010; Rodriguez-Garcia and Guil-guerrero 2008; Chacón-Lee and González-Mariño 2010; Lee et al., 2010).

Algal organisms are rich sources of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interests in the pharmaceutical industry (Ely et.al, 2004; Febles et.al, 1995 and Tuney et.al., 2006). Algae produce a number of secondary metabolites as a chemical defense against predation, herbivory and competition for space (DeLara-Isassi et.al., 2000; De Nys et. al., 1998).

Hence, in the present study, an effort has been made to analyze the effect of NaCl on Secondary Metabolites of Chlorella vulgaris Beijerink.

II. MATERIALS AND METHODS

The organism used in the present study i.e., Chlorella vulgaris Beijerinck was isolated from the garden soil of Gulbarga University, Gulbarga. De's modified Beneck's medium was best suited for the growth of C. vulgaris in the laboratory. Axenic cultures were maintained at temperature of 26 ± 2oC. Further to investigate the effect of NaCl, the experiments were carried out in 250 ml conical flasks, contained 100 ml of De's modified Beneck's basal medium. The exponentially growing algal suspension was centrifuged and inoculated in the flasks containing different concentrations of NaCl such as 0.1, 0.2, 0.3 and 0.4 M besides control and kept for observation to 30 days. The samples were drawn periodically during growth (10th, 20th and 30th day) from control and different concentrations of NaCl and were subjected for the analysis of Phenol, Flavonoid and Alkaloid. The phenols were estimated by Folin-Ciocalteaus method (Bray and Thorpe, 1954). The flavonoids from algae quantitatively estimated by Swain and Hills method (1959). The total alkaloid contents of algae were estimated by Ikans method (1981).

III. RESULTS

C. vulgaris when treated with different concentrations of NaCl viz., 0.1, 0.2, 0.3 and 0.4M over 10, 20 and 30 days exhibited increase in phenol content with maximum of 0.341, 0.353 and 0.365 mg/g at 0.3 M and with minimum of 0.303, 0.313 and 0.327mg/g at 0.4M for all the cultures(Table and Fig 1). Whereas flavonoid content enhanced with maximum of 0.632, 0.737 and 0.837mg/g at 0.3M and with minimum of 0.333, 0.411 and 0.443 mg/g at 0.4M for all the cultures (Table and Fig 2). Similarly, maximum level of alkaloid content was reported at 0.3M with 0.843, 0.953 and 1.047 mg/g and minimum of 0.512, 0.553 and 0.637 mg/g at 0.4M for all the cultures (Table and Fig 3).

IV. DISCUSSION

Our results on phenol, flavonoid and alkaloid contents of C. vulgaris exposed to different concentrations of NaCl indicated gradual increase up to 0.3 M thereafter showed decrease in all the cultures over all the durations.

Similar observations were made by Ali et al. (2003) in Barly seedlings. Several earlier studies reported that, phenolics in marine algae serve as ROS scavengers, metal chelators and enzyme modulators preventing lipid peroxidation (Rodrigo and Bosco, 2006). Rezazadeh et al. (2012) reported increased flavonoid content in Cynara scolymus L. under saline stress. It has been indicated that total flavonoid contents in salt-stressed seedlings of rice varieties were increased. It has been speculated that flavonoids may have

protective role under stress condition. Flavonoids are frequently induced by abiotic stress and protect plants (Dixon and Paiva, 1995; Grace and Logan, 2000). Similarly Moussa, (2004) indicated that NaCl stress causes significant increase in the alkaloid content of soybean plants when compared with control samples. Jaleel et al. (2007a) reported that antioxidant mechanisms may provide a strategy to enhance salt tolerance in plants

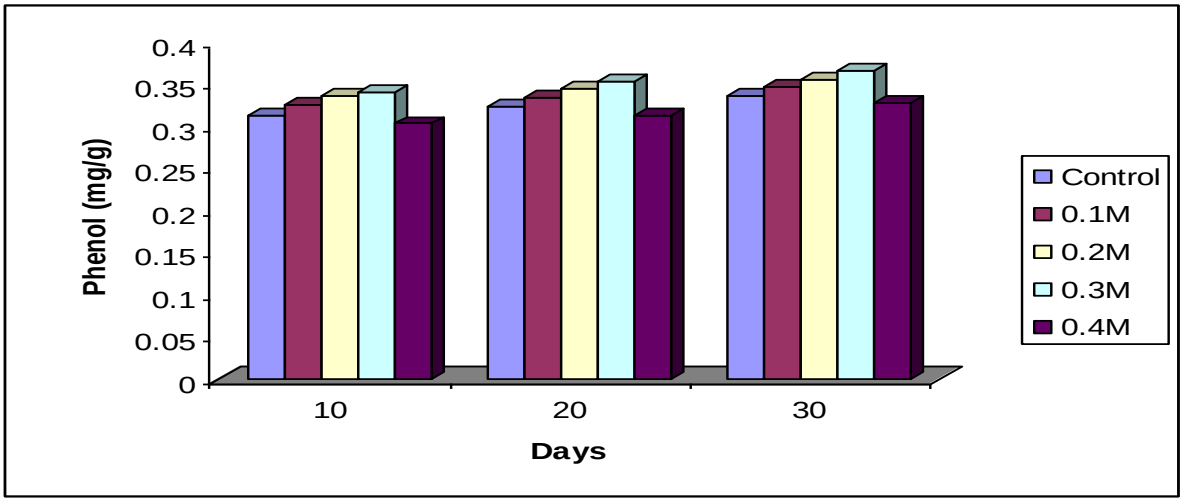
V. CONCLUSION

Our results indicated increase in the Phenol, Flavonoid and Alkaloid contents of C. vulgaris when exposed to different concentrations of NaCl except at 0.4 M. Several earlier studies reported that, phenolics in marine algae serve as ROS scavengers, metal chelators and enzyme modulators preventing lipid peroxidation. Flavonoids are frequently induced by abiotic stress and these compounds accumulated in plant tissue could help to protect from damaging effects. The study revealed that, increased activity of Phenol, Flavonoid and Alkaloid may provide a strategy to enhance salt tolerance and protection against oxidative damage.

Table–1: Effect of different concentrations of NaCl on Phenol content of Chlorella vulgaris

NaCl con. (M)	Phenols (mg/g)		
	10 days	20 days	30 days
Control	0.312 ± 0.11	0.323 ± 0.02	0.336 ± 0.05
0.1	0.325 ± 0.03	0.334 ± 0.03	0.347 ± 0.03
0.2	0.336 ± 0.03	0.344 ± 0.03	0.355 ± 0.04
0.3	0.341 ± 0.03	0.353 ± 0.02	0.365 ± 0.02
0.4	0.303 ± 0.04	0.313 ± 0.17	0.327 ± 0.04

Fig.1 : Effect of different concentrations of NaCl on Phenol content of Chlorella vulgaris

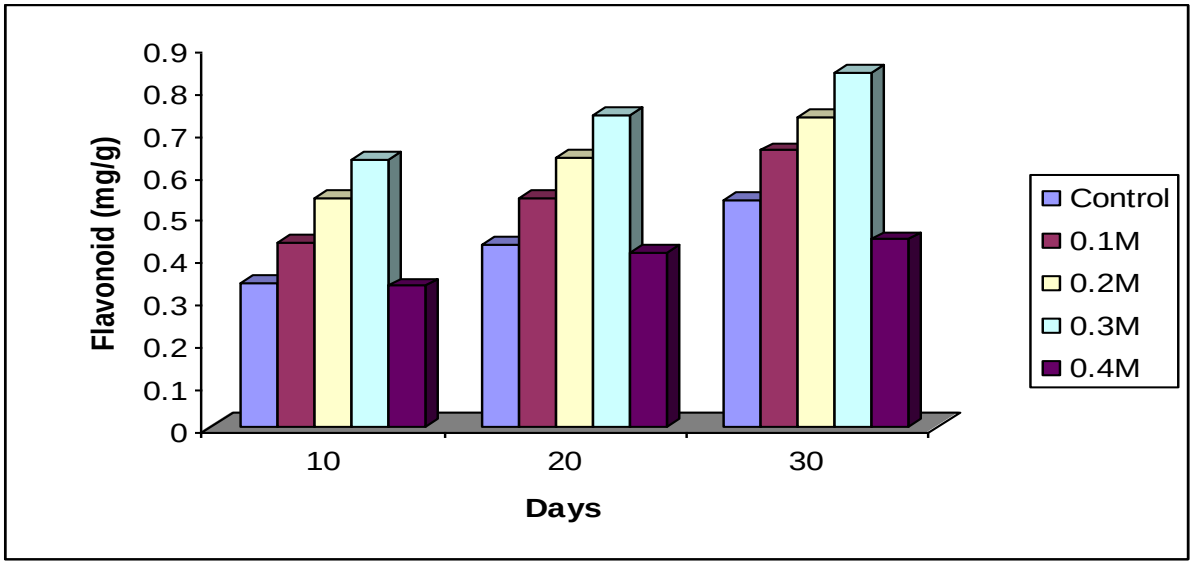


Chlorella vulgaris

Table -2: Effect of different concentrations of NaCl on Flavonoid content of *Chlorella vulgaris*

NaCl con. (M)	Flavonoids (mg/g)		
	10 days	20 days	30 days
Control	**0.342 ± 0.14**	**0.430 ± 0.12**	**0.537 ± 0.01**
0.1	**0.437 ± 0.17**	**0.543 ± 0.13**	**0.653 ± 0.08**
0.2	**0.543 ± 0.08**	**0.637 ± 0.13**	**0.731 ± 0.16**
0.3	**0.632 ± 0.31**	**0.737 ± 0.22**	**0.837 ± 0.09**
0.4	**0.333 ± 0.08**	**0.411 ± 0.08**	**0.443 ± 0.09**

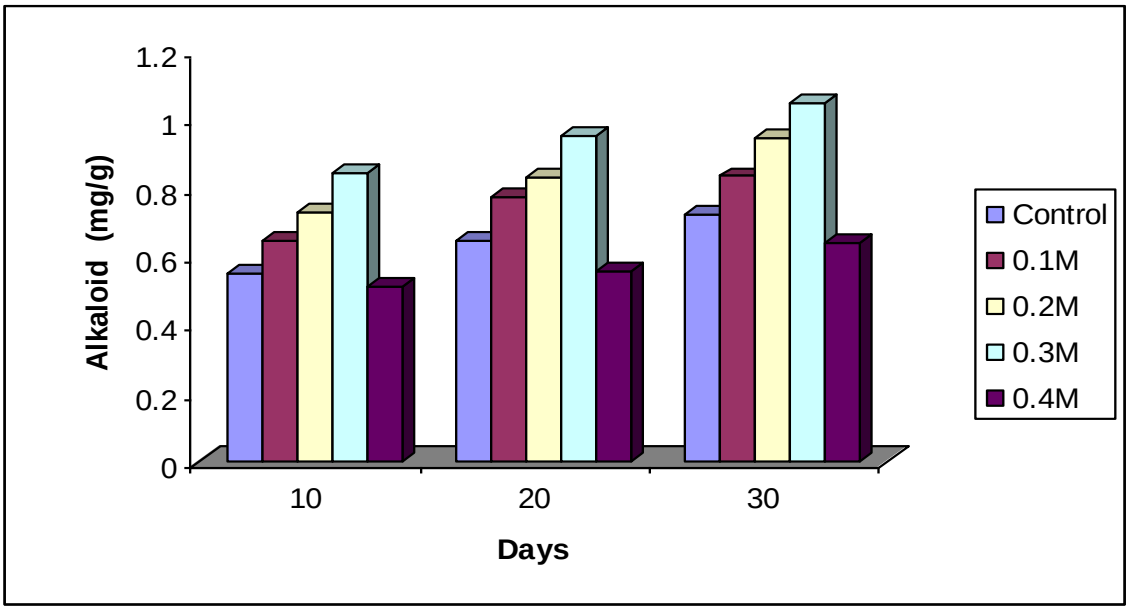
Fig. -2 : Effect of different concentrations of NaCl on Flavonoid content of *Chlorella vulgaris*



Chlorella vulgaris

Table–3: Effect of different concentrations of NaCl on Alkaloid content of Chlorella vulgaris

NaCl con. (M)	Alkaloids (mg/g)		
	10 days	20 days	30 days
Control	**0.551± 0.07**	**0.643 ± 0.08**	**0.721 ± 0.05**
0.1	**0.642 ± 0.07**	**0.771 ± 0.07**	**0.833 ± 0.03**
0.2	**0.730 ± 0.06**	**0.830 ± 0.06**	**0.943 ± 0.04**
0.3	**0.843 ± 0.03**	**0.953 ± 0.10**	**1.047 ± 0.09**
0.4	**0.512 ± 0.07**	**0.553 ± 0.03**	**0.637 ± 0.06**



Chlorella vulgaris

Fig.–3:Effect of different concentrations of NaCl on Alkaloid content of Chlorella vulgaris

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