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Alleviation of NaCl-induced Effects on *Chlorella vulgaris* and *Chlorococcum humicola* by Riboflavin Application

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ABSTRACT

The interactive effect of NaCl and riboflavin was investigated on the growth and other physiological parameters of *Chlorella vulgaris* Beij. and *Chlorococcum humicola* (Näg.) Rab. Low to moderate salinities (50 and 100 mM NaCl) stimulated the growth of both the species while higher levels (150-250 mM NaCl) reduced the growth of *C. humicola* only. Application of riboflavin led to a significant increase in growth and biosynthesis of pigments in salt treated algae. Salinity decreased the contents of carbohydrates and proteins while riboflavin treatments increased their contents in both tested algae. NaCl treatments increased the accumulation of proline and other free amino acids while riboflavin treatment reduced their contents in both studied algae. It was concluded that riboflavin treatment could alleviate the adverse effects of salinity on both algae.

Key Words: Algae; Riboflavin; Salinity

INTRODUCTION

Salinity represents one of the most important factors which exert stress injury on the growth and metabolism of plants. Many attempts have been undertaken to counteract the adverse effects of salt stress on plants using organic or inorganic solutes (Bejaoui, 1985; Radi *et al.*, 1989; Shaddad, 1990; Hamed & Al-Wakeel, 1995; Ali, 2000). Vitamins (e.g. ascorbic acid) were used as organic solutes for the alleviation of salinity stress (Shaddad *et al.*, 1990; Ahmed *et al.*, 1995). B-vitamins constitute a heterogeneous group of organic compounds that acts as coenzymes, and whose functions in microalgae are studied by several authors (Gopala Rao & Sastry, 1972; Kodandaramaiah & Gopala Rao, 1984). A survey of literature on B-vitamins showed that they variably enhance growth and metabolism of various plant species. Therefore, this work was conducted to investigate the capability of riboflavin to counteract the adverse effects of salinity stress on two selected algae (*Chlorella vulgaris* and *Chlorococcum humicola*), isolated from the Egyptian soils (Abel-Rahman *et al.*, 2004).

MATERIALS AND METHODS

Test organisms. Axenic cultures of *C. vulgaris* and *C. humicola* (two unicellular, non-motile, green algae) were isolated from soil sites (El-Fayoum, Egypt), with salinity range of 0.12 and 0.67 dS m^{-1}, where they flourish nearly round the year (Abdel-Rahman *et al.*, 2004).

Culture conditions. All experiments were carried out in 250 mL conical flasks, contained 100 mL Bold's basal medium (Bischoff & Bold, 1963) supplemented with sterile compressed air and kept under fluorescent light (20 µmol $m^{-2}s^{-1}$) with 16 h light period and at 25 ±2 ºC temperature.

Treatments. Exponential phase of growth of both organisms were determined in cultures, where they were grown for 14 d in media containing either NaCl at concentrations 0, 50, 100,150, 200 or 250 mM or riboflavin at concentrations 0, 0.06, 0.12 or 0.18 mM. In interactive experiments, the starting cultures were adjusted to contain 0.125 cell x 10^6 mL^{-1} medium for both organisms. Organisms were harvested (by centrifugation) after seven days from cultures, which were treated with combinations of the above mentioned concentrations of NaCl and riboflavin. Reference controls contained NaCl concentrations alone and absolute controls contained untreated culture media. All treatments were replicated thrice.

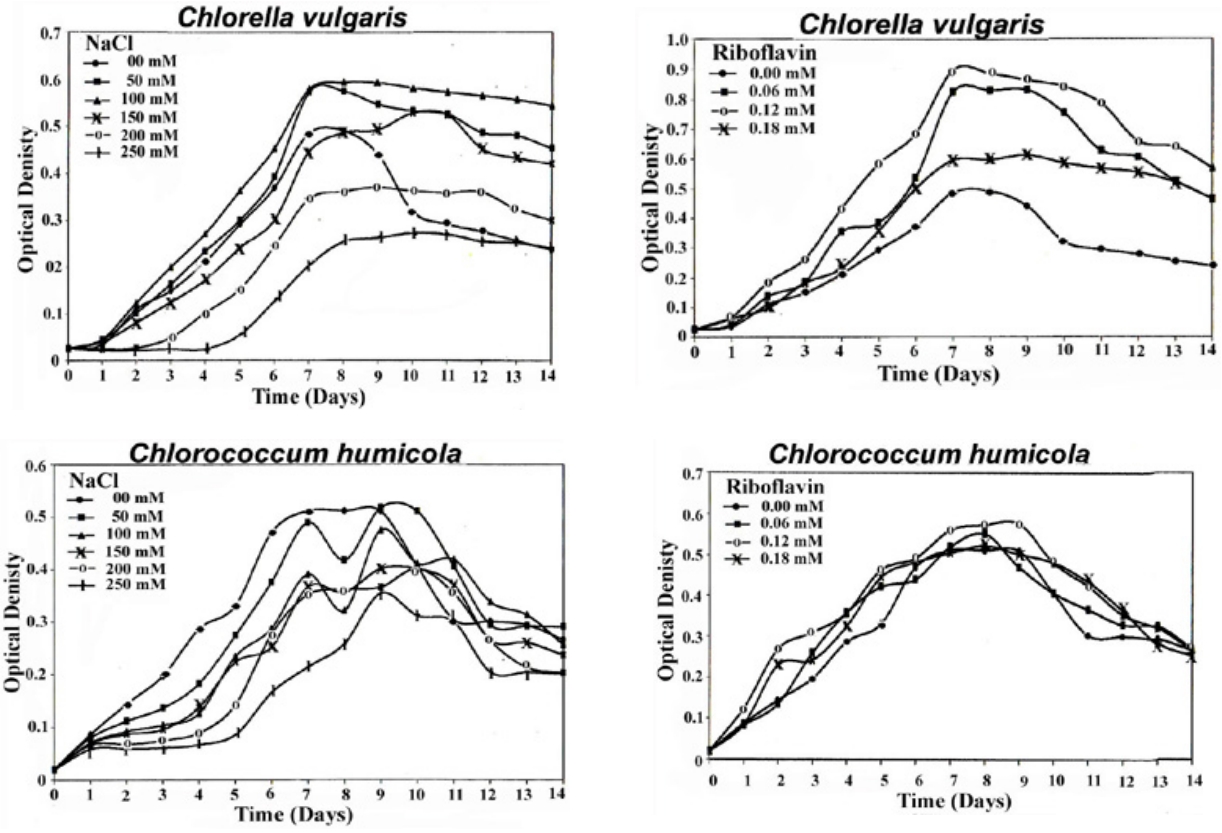
Measurements. Growth parameters included cell counts and dry weights. The cell counts were taken using haemocytometer slide or determination of optical density at 678 nm (Robert, 1979. Dry weight of cells was taken after filtering and drying overnight at 105ºC. Chlorophylls, carotenoids and total pigments were determined according to Metzner *et al.* (1965). Soluble, insoluble and total carbohydrates were determined by anthrone-sulfuric acid method (Badour, 1959). Proteins were measured according to the method of Lowry *et al.* (1951). Total free amino acids and proline were determined according to Moore and Stein (1948) and Bates *et al.* (1973), respectively.

Statistics. Data obtained were statistically analyzed using the least significant difference test (L.S.D) at 1 and 5% levels of probability.

RESULTS AND DISCUSSION

The growth of both organisms was significantly increased at low levels of salinity (Fig. 1). With a rise in

Fig. 1. Effect of different concentrations of NaCl (left) and riboflavin (right) on the growth (optical density) of *C. vulgaris vulgaris* and *C. humicola humicola*



NaCl concentrations, the growth remained steady in *C. vulgaris* but decreased in *C. humicola*. This indicated that both the algae exhibit variable response to high salinity. This conforms to the observations of Munns *et al.* (1983) who report that the effect of salt on growth of micro-algae varies dramatically between species. The salinity-induced growth reduction may be attributed to the accumulation of reactive oxygen species (Menezes-Benavente *et al.*, 2004).

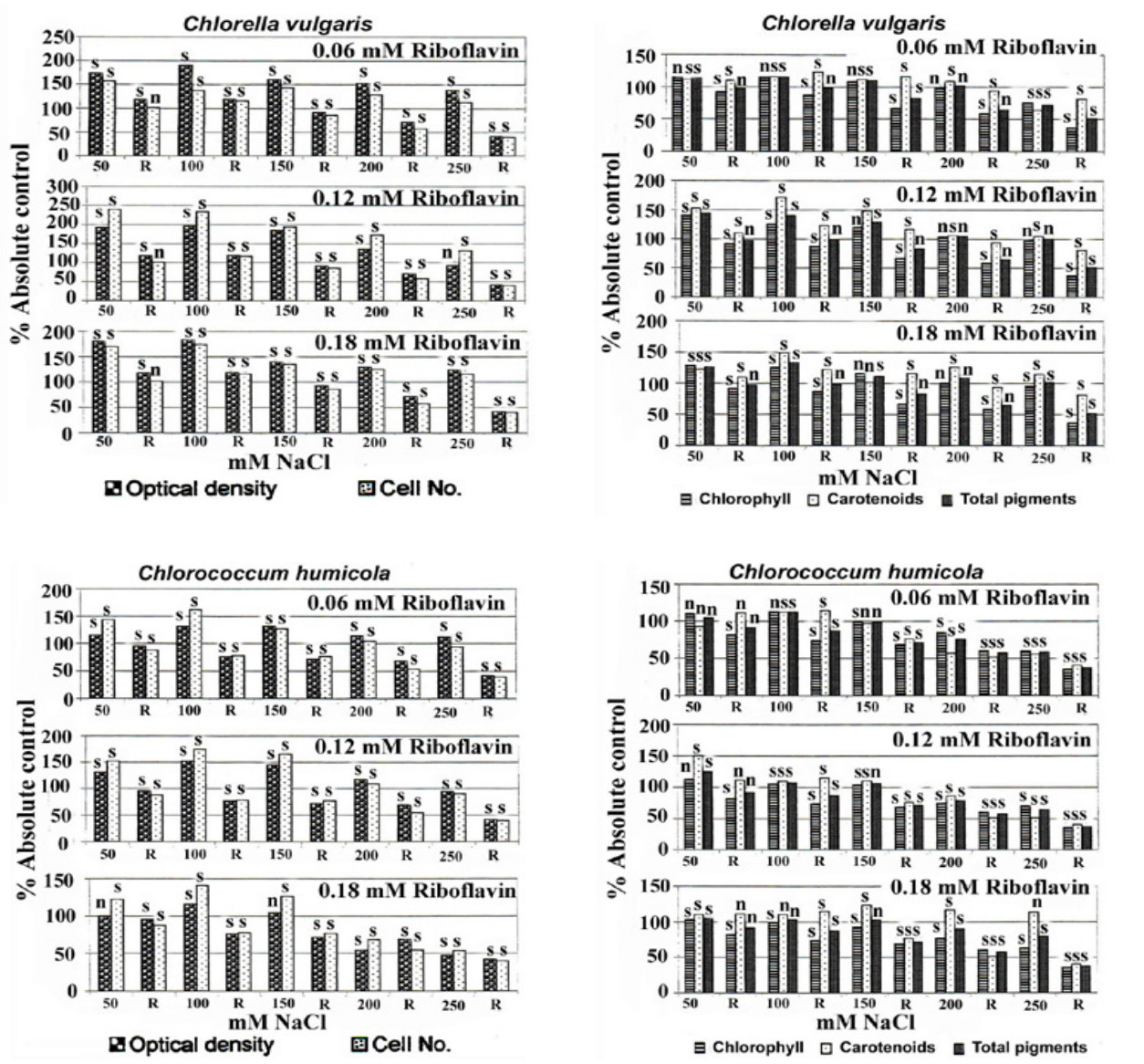
The growth of *C. vulgaris* was markedly elevated in the media containing 0.06 or 0.12 mM riboflavin. However, 0.18 mM riboflavin was higher than the reference control. However, the growth of *C. humicola*, was significantly stimulated at all concentrations of riboflavin (Fig. 1). The vitamins have been regarded as organic source for the continual growth of phytoplankton species (Swift, 1980). Under applied levels of NaCl or riboflavin, an exponential phase of growth was evident for both algae, which reached its maximum after seven days.

Riboflavin treatments, particularly at low and moderate concentrations (0.06 & 0.12 mM), alleviated the inhibitory effects of NaCl and enhanced growth and pigment contents in both the algae as compared to the reference controls (Fig. 2). Such an enhancement of pigment biosynthesis has been reported in higher plants exogenously supplied with vitamins (Shaddad *et al.*, 1989). Gopala Rao and Sastry (1972) reported that all B-group vitamins may be related to chlorophyll synthesis.

Salinized cells of both algae treated with riboflavin showed an increase in the contents (fractions & total) of carbohydrates and proteins more than those subjected only to salinity (Fig. 3). A stimulatory effect of riboflavin on soluble carbohydrates was more pronounced than those of the insoluble ones, and the increase in the protein fractions was even more than the control cultures. However, the response was greater in *C. vulgaris* than in *C. humicola*. Other reports indicate that similar changes in carbohydrates levels in response to vitamin treatments were related to an increase in endogenous hormones (particularly cytokinin) or to enzymes activities related to carbohydrate metabolism (Back & San Pietro, 1968; Gopala Rao & Sundersanam, 1984). Makled (1995) reported that application of thiamin (B_1) similarly enhanced protein accumulation in *C. vulgaris vulgaris* and *Ankistrodesmus falcatus*.

Fig. 4 shows that NaCl treatments increased the accumulation of proline and free amino acids in both the algae, and this accumulation was greater with increased

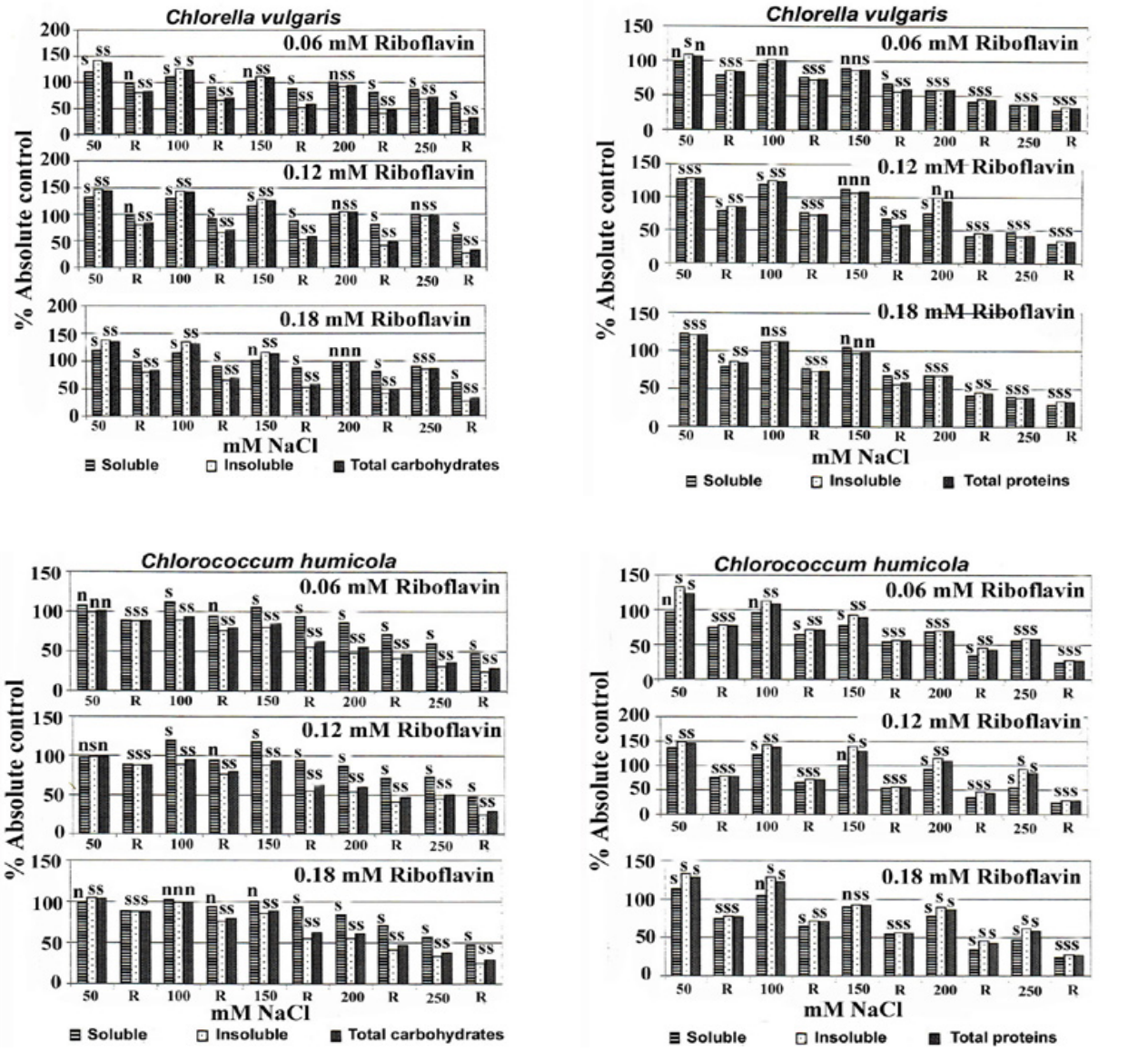
Fig. 2. The interactive effects of NaCl and riboflavin on growth (left) and pigments (right) in *C. vulgaris vulgaris* and *C. humicola humicola*. R = Reference control. Letters on the columns indicate the statistical analysis of the original data where: n = Non significant and s = significant at least at P> 0.05



NaCl concentrations. Our results are in agreement with those obtained by Lin and Kao (1996) for rice. It is likely that the accumulation of free proline and total free amino acids could be one of the major mechanisms of salinity tolerance in these algae. The role of riboflavin in modifying the salt stress induced decrease in proline and other free amino acids contents was also revealed for both algae (Fig. 4). Cells of both these salt stressed algae when exposed to riboflavin exhibited the accumulation of carbohydrates and proteins (Fig. 3) while free proline and total free amino acid content were reduced (Fig. 4). This implies that the incorporation of free amino acids into protein was markedly enhanced by riboflavin treatment in both algae.

In conclusion, the application of riboflavin could alleviate the adverse effects of salinity on *C. vulgaris* and *C. humicola*. The stimulation of growth of *C. vulgaris* and *C. humicola* by riboflavin may be attributed to induction of some metabolic enzyme activities as has been noted for *Dunaliella tertioleca* (Jahnke & White, 2003). However, further studies are imperative on the effects of salinity and other vitamins on certain other organisms so as to establish

Fig. 3. The interactive effects of NaCl and riboflavin on the contents of carbohydrates (left) and proteins (right) in *C. vulgaris vulgaris* and *C. humicola humicola.* R = Reference control. Letters on the columns indicate the statistical analysis of the original data where: n = Non significant and s = significant at least at P> 0.05



the role of these factors on the levels of endogenous vitamins and enzymes.

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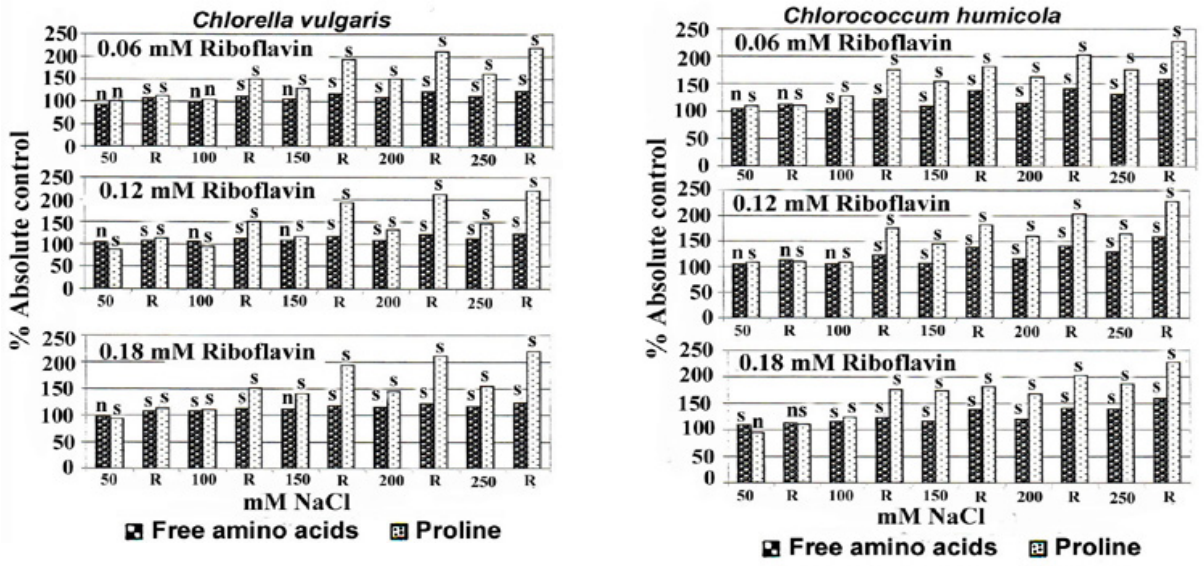
Fig. 4. The interactive effects of salinity (NaCl) and riboflavin on the free amino acids and proline in *C. vulgaris vulgaris* and *C. humicola humicola*. R = Reference control. Letters on the columns indicate the statistical analysis of the original data where: n = Non significant and s = significant at least at P> 0.05



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