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Short Communication

EFFECT OF COPPER ON THE GENERATION TIME AND ANTIOXIDANT POTENTIAL OF A NOVEL ISOLATE OF *CHLORELLA EMERSONII* KJ725233

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ABSTRACT

Objective: At low concentrations copper is an essential micronutrient for algal growth wherein it plays a vital role as an enzyme cofactor for photosynthetic processes but at high concentrations it functions as a toxic heavy metal. Copper exposure to microalga increases the activity of antioxidative enzymes–catalase, superoxide dismutase, peroxidase along with the induction of chlorosis at higher concentrations. The objective of the present study is to determine the effect of suboptimal concentration of copper on the generation time as well as the antioxidant potential of a novel strain-*Chlorella emersonii KJ725233*.

Methods: The growth of the microalga at the different copper concentrations was monitored by measuring the absorbance at 684 nm. Once the suboptimum copper concentration for the growth of the microalga was determined, the methanolic extracts were evaluated for Total Phenolic Content, Total Flavonoid Contents as well as the antioxidant potential by employing standard methods such as phospho- molybdenum method for Total Antioxidant Capacity and Ferric Reducing Antioxidant Potential.

Results: Results indicated that 0.1 µmol of copper stimulated the growth of *Chlorella emersonii* KJ725233, which was evident from the reduced generation time of the microalga as compared to that of the control and other copper concentrations. The study also proved a 45.69% increase in the antioxidant activity of the microalga on exposure to 0.1 µmol of copper.

Conclusion: The present study indicated copper at 0.1 µmol concentration not only acts as a micronutrient but also levies stress resulting in increased antioxidant activity of this novel isolate *Chlorella emersonii* KJ725233.

Keywords: *Chlorella emersonii* KJ725233, Copper, Generation time, Antioxidant

Copper is an essential micronutrient for plants and microalgae which function as a cofactor for proteins and enzymatic reactions. It is also an important component of the photosynthetic and respiratory electron transport chain (in the form of plastocyanin). Copper is also a significant part of Reactive Oxygen scavenging (ROS) system and is responsible for the oxidative stress caused due to an increased production of ROS [1]. Copper undergoes the Haber-Weiss and Fenton reactions thus inducing the production of hydrogen peroxide, hydroxyl radicals and other ROS which damages the membrane lipids and proteins [2, 3]. To combat oxidative stress, the organisms respond by antioxidant enzymes, hydrophilic as well as lipophilic antioxidant compounds [4].

Changes in cell membrane permeability, chromatin structure, protein synthesis, enzymatic activity, photosynthesis and respiratory processes are all known to be associated with an increased copper concentration in the growth environment [5]. An excessive copper concentration damages the cell membrane function causing depletion in the internal potassium ion concentrations thus affecting the osmotic membrane permeability. With the increase in ionic copper concentration, more copper binds to the chloroplast and other cell proteins decreasing the synthesis of chlorophyll leading to an irrevocable damage to the chloroplast lamellae–photosynthetic apparatus thus preventing photosynthesis eventually culminating in cell death [6, 7].

With this in view, the present study designs a suboptimal dosage of copper in the form of an inorganic salt $CuSO_4.5H_2O$ on a novel isolate of *Chlorella* species–*Chlorella emersonii* KJ725233 and studies its effect on the antioxidant capacity of this microalga. As per our earlier studies, sonicated methanolic extract showed higher antioxidant activity as compared to the other solvents used hence; the present study was designed using methanolic extraction of the bioactive from the microalga [8].

In the present study, *Chlorella emersonii* KJ725233 was mass cultured in Chu's medium no.10 in the presence of five different concentrations of an inorganic salt of copper ($CuSO_4.5H_2O$) beginning with 0.075, 0.1, 0.3, 0.5 and 1.0 µmol of free copper concentrations. The culture was incubated under artificial light with a 12 h photoperiod and aeration. The growth was monitored by measuring the absorbance at 684 nm after every 48 h of incubation [8]. The growth rate of the microalga was calculated using optical densities [9].

After 30 d of growth, the culture was centrifuged at 5000 rpm for 20 min to obtain the biomass, which was then dried at 60 °C for 48 h. The dried powder obtained was suspended in methanol to yield a concentration of 0.1 g/ml. These suspensions were then sonicated on ice for 40 min using an LABMAN sonicator and then centrifuged (EPPENDORFF) at 5000 rpm for 15 min. The extraction was repeated thrice. The supernatants were dried at 30 °C and then suspended in 3 ml of dimethyl sulfoxide. The extracts were kept in sealed eppendorfs at 4 °C until used [8]. These extracts were then used to determine the modulation of Total Antioxidant Capacity (TAC) [12], Ferric Reducing Antioxidant Potential (FRAP) [12], Total Phenolic Content (TPC) [10] and the Total Flavonoids Content (TFC) [11] of *Chlorella emersonii* KJ725233 in the presence of copper.

The study showed that after 30 d of incubation, cells growing in the presence of 0.075 µmol and 0.1 µmol of copper were found to have intact chloroplasts with a reduced generation time. Thus, it can be concluded that concentrations of 0.3 µmol and above proved toxic to their growth which was evident from an increased generation time and reduced biomass as mentioned in table 1. In addition to this, chlorotic symptoms like oxidative bleaching of the chlorophyll in the cells were observed.

In order to survive heavy metal stress, algal cells are known to synthesize more of carotenoids than chlorophyll [7]. Since 0.1 µmol of copper enhanced the growth of the microalga as seen from the generation time, this concentration was thus subsequently used to assay its effect on the cells antioxidant system.

The results revealed a 57.38 % increase in total phenolics in the presence of copper as compared to that of the control. Also, the flavonoids were enhanced by 27.61 % as a response to copper stress as denoted in table 2.

Table 1: Generation time and dried biomass of *Chlorella emersonii* KJ725233 in the presence of varying copper concentrations

	Control	0.075 µmol	0.1 µmol	0.3 µmol
Generation time (h)	12.93	10.80	9.40	17.01
Biomass (g)	0.3289±0.023	0.3687±0.048	0.3987±0.052	0.2535±0.065

Results are expressed as mean±SD (n = 3).

Table 2: Total phenols and flavonoids content of methanolic extract of *C. emersonii* KJ725233

Sample	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	TAC (mg AAE/g DW)	FRAP (mg AAE/g DW)
Normal	7.186±0.148	5.276±0.269	14.29±0.645	13.986±0.336
Copper (0.1 µM)	11.31±0.706	6.7331±0.123	21.33±0.257	20.773±0.981
Percent Increase	57.38	27.61	49.26	48.52

Results are expressed as mean±SD (n = 3).

The TAC and FRAP of *Chlorella emersonii* KJ725233 under normal growth conditions i.e. 14.29 mg/g and 13.986 mg/g respectively were increased by 49.26 % and 48.52 % in the presence of 0.1 µmol of copper as summarized in table 2. From the results, it is evident that 0.1 µmol copper induces oxidative stress on the microalga leading to enhanced production of antioxidants. The antioxidant activity of *C. emersonii* KJ725233 can be attributed to the phenolics, flavonoids, carotenoids in the microalga.

Table 3: Correlation of the total antioxidant capacity with the total phenolic content and the correlation of the ferric reducing antioxidant potential with the total phenolic content

Sample	TAC	TPC	Correlation R^2	FRAP	TPC	Correlation R^2
Normal	14.29±0.645	7.186±0.148	0.721	13.986±0.336	7.186±0.148	0.918
Copper 0.1 µM	21.33±0.257	11.31±0.706	0.795	20.773±0.981	11.31±0.706	0.999

Results are expressed as mean±SD (n = 3). TAC and FRAP expressed as mg ascorbic acid/gDW whereas TPC and TFC expressed as mg gallic acid/gDW and mg quercetin/gDW respectively.

From the correlation coefficient as seen in table 3, it is evident that the phenolic compounds are major contributors to the ferric reducing antioxidant potential (R^2 = 0.918; 0.999). On the other hand, there may be other low molecular weight compounds contributing to the total antioxidant capacity of *Chlorella emersonii* KJ725233 as evident from the correlation coefficient (R^2 = 0.721; 0.795) of the phenolic content to that of the total antioxidant capacity.

The ability of copper to act as a micronutrient or as a cytotoxic agent has been cited widely in literature Li *et al.* 2013 [13]. In the present study, copper concentrations above 0.1 µM were found to cause chlorosis in the case of *Chlorella emersonii* KJ725233 whilst concentrations of 0.075 and 0.1 µM stimulated growth as compared to the control. However, a decrease in generation time for the latter as compared to 0.075 µM indicated the increase in stress levels due to exposure to the higher concentration and hence a probable boost in its antioxidant activity. The reduction in the growth at higher copper concentrations has been attributed to the inhibition of normal cell division [7].

The antioxidant activity of methanolic extracts of *Chlorella emersonii* KJ725233 was found to be 13.98 mg/g for the control and 21.33 mg/g ascorbic acid equivalence in the presence of 0.1µM of copper. These values obtained are almost 22 folds higher than those reported by Manivannan *et al.* 2012 [14] and Hemlatha *et al.* 2013 [12] for *Chlorella marina*. Also, the phenolic content of *Chlorella emersonii* KJ725233 in the absence as well as the presence of copper was found to be almost four times higher as compared to that reported for methanolic extract of *Chlorella salina* by Saranya *et al.* 2014 [15].

The findings for the first time report a novel strain of *chlorella* with an inherent higher antioxidant capacity which has been further boosted by using a copper salt. The present isolated–*Chlorella emersonii* KJ725233 thus serves as a potential candidate for the scale-up and production of various antioxidants that could find application in food and pharma as a substitute for chemical antioxidants.

CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interest.

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