

Response of *Escherichia Coli* to the Alcoholic Extract of Green Alga *Chlorococcum Humicola*

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Abstract: The response of *Escherichia coli* bacteria which was isolated from contaminated water to *Chlorococcum humicola* alcoholic algae extract was studied on growth and different parameters. The extract concentration of *Ch. humicola* was 0.07, 0.15, 0.31, 0.2, 1.25 mg/L showed a high efficiency in significantly reducing numbers of *E.coli*, the Growth Account (GA) showed a highest value at concentration 0.07 mg/L which was 303, 200,168 CFU/mL and less value at 1.25 mg/L which was 99,61,49 CFU/mL in 24, 48 and 72 h, respectively. While Removal Account (RA) appeared highest value at 1.25 mg/L 333,419,431 CFU/mL and less value at con. 0.07 mg/L which was 150,27,312 CFU/mL. Also the Mortality Percent (MP) appeared highest value at 1.25 mg/L 68,87,89 CFU/mL and less value at 0.07 mg/L which was 31,58,65 CFU/mL as well as the removing percentage of *E. coli* by green algae *C. humicola* which was 60, 80, 82% in 24, 48, 72 h, respectively. The result of study improved that the species *E.coli* is highly sensitive to *Ch. humicola* algae extracts toxicity of algal extracts were time and concentration dependent. The aim of the study refers to can be used as biodegradation agent in order to disposal of pollution by *E.coli* which represent the bacterial water pollutant with possibility to use the alcoholic extract of green algae to reduce the time and cost of water treatment.

Key words: Alcoholic algae extract, toxicity, polluted water, species, pollution, reducing

INTRODUCTION

Water sources, especially, surface water is exposed to the dangers of pollution which leads, directly or indirectly, to the ecosystem. Water pollution means any change in the physical, chemical and biological characteristics of water as well as the pathogens resulting from the effects of microorganisms such as bacteria, fungi and viruses (Cervenka *et al.*, 2006).

The rivers are the most water bodies susceptible to pollution because of the discharges resulting from the various human activities that make them unusable only after the adoption of additional treatment and liquidation units of high and cost technology.

The presence of *E. coli* and other faecal indicator organisms such as Streptococci in surface waters can indicate a human health hazard because faecal contamination increases the risk of enteric pathogenic microorganisms being endemic transport of FIOs from land to bathing water (SEPA., 2002) and to river water abstracted for irrigation of ready to eat vegetables (Beuchat, 1996). Are there for of public concern, the regulation of such contamination is covered in European Union by directives such as the bathing waters directive and more recently the water framework directive.

The *E. coli* was associated with watery or bloody diarrhea hemorrhagic colitis and hemolytic uremic syndrome (Stephan and Untermann, 1999). The organisms typically colonize the infant gastrointestinal tract within hours of life there after *E. coli* and the host derive mutual benefit. The organisms are distributed in the environment as well as in the bowel of human and animals. It is also present in water supplies as an indicator of recent fecal contamination and potential presence of enteric pathogens (Colle *et al.*, 1996).

Algae are mainly aquatic simple plants found in marine and fresh water as well as terrestrial habitats such as wet rocks or moist soils (NCERT., 2008). Microalgae can be a rich source of many chemically diverse.

Compounds that have been used as bioactive compounds as antimicrobial agents (Stengel and Connan, 2015). Making the research use it in human pathology and also in aquaculture as biodegradation which is one of the most effective methods to remediate environmental systems in both siting and engineered schemes, to reduce costs and environmentally-friendly than traditional detoxifying methods in contaminated environments (Mahmoudi, 2013).

In addition to the use of chemical sterilizers. The algae are microorganisms that are highly efficient in inhibiting the growth and effectiveness of various microorganisms (Katrırcıoğlu *et al.*, 2004).

It also contributes to the process of self-purification in the water bodies through the photosynthesis process where they releases the dissolved oxygen gas which causes the gas balance between oxygen and carbon dioxide between the atmosphere and water that have a biological, medical and economic importance and are necessary to sustain life (Likens, 2010).

As well as algae have recently received a lot of attention as a new biomass source for the production of a new energy (Zhao *et al.*, 2008). Some of the main characteristics which set algae apart from other biomass sources are that algae can have a high oil or starch content do not require agricultural land. Fresh water is not essential and nutrients that can be supplied by waste water and CO₂ by combustion gas (Marina *et al.*, 2013). The first distinction that needs to be made is between macro algae or versus microalgae (Xu *et al.*, 2004). Therefore, the aim of the study was to eliminate *Escherichia coli* using the alcoholic algae extract.

MATERIAL AND METHODS

Isolation and diagnosis of bacterial: The bacteria *Escherichia coli* were isolated from the drainage water of the Rustmiya Sewage Treatment Plant in Baghdad using Prescott method (Kumar *et al.*, 2010). Grown on the nutrient agar medium then 1 mL were taken from pure isolation into glass vials containing broth nutrient medium and incubated at 37°C.

Preparation of dried algae: *Chlorococcum humicola* was isolated from small stream near the University of Baghdad-Jadriya from different places where several samples were collected, depends on method by Stein (1973). Then the alga was laboratory diagnosed using a microscope based on the diagnosis method (APHA *et al.*, 2005).

The alga was cultured under constant laboratory conditions and temperature of 25±2°C, 3000 lux and 16:8 light: dark as photo system in Chu 13 media (Yamaguchi *et al.*, 1987) (incubated in the incubation room for 18 days, harvested by centrifuge with of 3000 rpm speed for 15 min, the sedimentation dried in oven at 50°C for 48 h, all samples keeps in 25°C antal used (Jawad, 1982; Eppley *et al.*, 1977).

Extraction of active substances from algae: About 15 g of the dried algae was dissolved in 250 mL of chloroform and then placed in shaking incubator at 25°C with 170 rpm for 15 min and then dried at 40°C (Taskin *et al.*, 2007).

Preparation of concentrations of algae extract: About 1 mL of bacterial suspension was taken for each isolation of bacterial isolates and placed in a sterile glass flask

containing different concentrations of the extract: 0.07, 0.15, 0.31, 0.62 and 1.25 mg/L prepared from the primary concentration by the dilutions and then the volume was completed to 100 mL of sterile sewage water with (SEPA., 2002) repeated of each bacterial isolation. In addition to the control treatment which was free of the algae extract and incubated at 37°C for 72 h (Kumar *et al.*, 2010).

The number of bacterial cells was calculated in 1 mL of bacterial suspension using the method of hemocytometer (Chamber counting) where the calculation was daily for 3 days to draw growth curve and account the Growth Account (GA), Removal Account (RA), Mortality Percent (MP) and Removing Percentage (RP) according to (Dahiru and Obidoa, 2007).

RESULTS AND DISCUSSION

The result showed exposure of *E. coli* bacteria to different concentrations of *Ch. humicola* alcoholic extract clearly effect on the growth curve decreed depends on these cells within 72 h. From exposure a decrease was observed within increased emphasis and register a growth curve rapprochement and non-significant differences of the cells number at concentrations 0.13, 0.62, 1.25 mg/L in 48 h Fig. 1 and 2. While Growth Account (GA) showed a clear decline as significant for all concentrations compared with control witch was not exposed to algae extract witch registered 168,200,303 and 133,177,303 CFU/mL in concentration of 0.07 mg/L and 100,100,248 CFU/mL by concentration of 0.15 mg/L and 100,100,248 CFU/mL by concentration of 0.31 mg/L while registered the two concentration 1.25,0.62 mg/L value reached 61,72,180 and 49,61,99 after 24, 48 and 72 h. Respectively while the control which was register 480 CFU/mL after same period Table 1.

Also register the Removal Account (RA) a value with significant and increased his concentration and the value converged with non-significant after 24, 48 and 72 h. Between 1.25,0.62 mg/L and the signification between the high concentration compared with the less two concentration which used at the study witch all the values significant compared with control Table 2.

As well as Mortality Percent (MP) register values reached 31,58,65% at concentration 0.07 mg/L, 37,63,71% at 0.15 mg/L and 47,74,79% at 0.31 mg/L 6285,87% at concentration 0.62 mg/L after 24, 48 and 72 h, respectively Table 3. While the removing percentage registered 60, 80 and 82% after 24, 48 and 72 h. Which refers to increased the removal rate.

Also the study agrees with Taskin *et al.* (2010) which indicated the ability of *Cladophora* extract to reduce the number of *E. coli* bacteria, the reason is due to the secretion of many substances such as ferpenoids, fatty acids, acids, amino which have inhibitory effect on

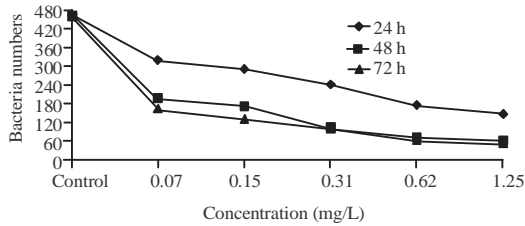


Fig. 1: The decreasing of *E. coli* numbers using algae extract during 72 h

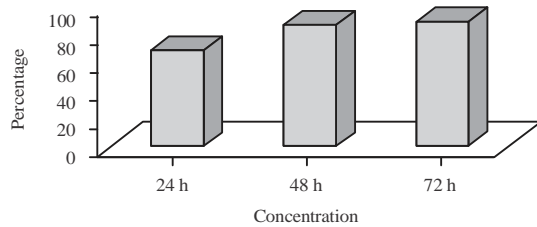


Fig. 2: Removing percentage (%) of *E. coli* under different concentration of green alga *C. humicola*

Table 1: The growth account for *E. coli* under different concentration of green alga *Ch. humicola*

Con. mg/L	GA		
	24 h	48 h	72 h
Control	480±11.36 ^{A*}	480±10.41 ^{A*}	479.3±9.9 ^{A*}
0.07	303±3.33 ^{a*}	200±11.55 ^{c*}	168.0±17.50
0.15	303±40.44 ^{a*}	177±16.80 ^{c*}	133.0±11.00
0.31	248±21.28 ^{b*}	100±1.0	100±5.77
0.62	180±14.43 ^{b*}	72.0±2.3 ^{d*}	61.0±10.51
1.25	99±7.2	61.0±2.1 ^{d*}	49.0±6.51

GA = Growth Account, Small letter * = No significant in the same column; Capital letter = No significant in same row at 0.05 level

Table 2: The removal account for *E. coli* under different concentration of green alga *Ch. humicola*

Con. mg/L	RA		
	24 h	48 h	72 h
Control	0.0±0.0	0.0±0.0	0.0±0.0
0.07	150±13.20 ^{a*}	276±19.43 ^{c*}	312.0±13.0 ^{b*}
0.15	180±4.62 ^{a*}	303±3.51 ^{c*}	347.0±29.6 ^{b*}
0.31	230±7.51	380±10.44	380.0±40.7 ^{b*}
0.62	300±7.53 ^{b*}	408±5.29 ^{c*}	419.0±19.0
1.25	333±9.53 ^{b*}	419±14.53 ^{d*}	431.0±2.00

GA = Growth Account, Small letter * = No significant in the same column; Capital letter = No significant in same row at 0.05 level

Table 3: The Mortality Percent (MP) for *E. coli* under different concentration of green alga *C. humicola*

Con. mg/L	MP		
	24 h	48 h	72 h
Control	0.0±0.00	0.0±0.00	0.0±0.0
0.07	31.3±0.43	58.3±0.33	65.0±2.52
0.15	37.5±5.01	63.1±1.13	71.96±1.28
0.31	47.9±0.53	79.1±0.04	79.17±0.17
0.62	62.5±2.02	85.0±1.73 ^{a*}	87.3±0.84 ^{b*}
1.25	68.8±4.79	87.3±0.29 ^{a*}	89.76±1.76 ^{b*}

GA = Growth Account, Small letter * = No significant in the same column; Capital letter = No significant in same row at 0.05 level

bacteria (Xu *et al.*, 2004). And they produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against the other selling organisms (Rao *et al.*, 2007). These active metabolites also known as biogenic compounds such as halogenated compounds, alcohols, aldehydes, terpenoids are produced by several species of marine macro and microalgae and have antibacterial, anti-algal and anti-fungal properties which are effective in the prevention of bio fouling and have other uses in therapeutics (Dahiru and Obidoa, 2007).

The algae extract has an inhibitory effect against the positive and negative bacteria (Gram stain) by inhibiting the enzyme of phosphatase protein which plays an important role in the process of inserting substances into the body of the organism (Katrıcioğlu *et al.*, 2004).

The active substances found in the algae extract have an effect on the positive bacteria of (Gram stain) rather than on the negative bacteria.

As indicated by Ordog *et al.* (2004). The reason is that the negative bacteria are less sensitive than the active compounds found in positive bacteria of (Gram stain) due to their cell wall structure which consists of many layers (Abd-Elnaby, 2010).

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