

Effect of Algal Extracts on the Growth of Two Bacterial Types Isolated from Pollutants Discharge

Thaer Mohammed Ibrahim¹, Raad Abdul Hadi Nayyef², Buthaina Abdul- Aziz Hassan Al-Magdamy¹

¹College of Education for Pure Sciences- Ibn- Al-haitham, University of Baghdad, Iraq,

²Ministry of Education, Open Educational College, Iraq

Abstract

Effect of *Chlorococcum humicola* alcoholic algae extract was studied on the growth of, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, which were isolated from contaminated water. The extract of *Ch. humicola* showed a high efficiency in reducing the numbers of the two types of bacteria. The removal rate of *K. pneumonia* were 0.0, 48.4 and 57.0, The removal rate of *P. aeruginosa* were 63.1, 79.8 and 82.9% after 24, 48, 72 h respectively. The results improved that the *K. pneumonia* is more sensitive than *P. aeruginosa* for algae extract concentrations used in study, and the best effective time is 24h for the two bacterial species. The aim of the study was to eliminate microorganisms using the Alcoholic algae extract. Especially *P. aeruginosa* and *K. pneumonia* of bacterial isolates that cause many diseases for human and animals.

Keywords: Alcoholic algae extract, toxicity, polluted water

Introduction

Klebsiella pneumonia infections may occur at almost all body sites, but the highest incidence was found in the urinary and respiratory tracts. The main population at risk are neonates and patients predisposed by prior surgery, diabetes, malignancy, etc. [1]. Also it is found in mammalian mucosal surfaces and opportunistic pathogens and the principal pathogenic reservoirs of infection are the gastrointestinal tract of patients and the hands of hospital personnel organisms can spread rapidly. Often leading to nosocomial [2]. As well as *K. pneumonia* occurs in the lungs. Where they cause destructive changes inflammation, hemorrhage and sometimes producing a thick, bloody, mucoid sputum described as currant jelly sputum [3].

Pseudomonas aeruginosa causes disease in healthy persons. Most infection occurs in compromised hosts or only when they reach tissue outside the intestinal tract, particularly the urinary tract, biliary tract, lung, eye, kidney, ear intestine and damaged or burned skin and causing inflammations at these sites [4].

P. aeruginosa infection occurs in three stages the bacterial attachment and colonization followed by local invasion and dissemination and systemic disease, And two mechanisms contribute to the complex pathogenesis of these respiratory infections with *P. aeruginosa*: first the bacterial adhesion to the respiratory epithelial cells initiates infections. And secondly inhibition of phagocytosis by macrophages and polymorph leucocytes for its progression. And during both mechanisms. Bacterial cells become mucoid by secreting an exopolysaccharide containing alginate [5].

From another view the water environment has a great importance in the world through covering an area of about 75% of the world. Therefore, attention to water resources is necessary for the purpose of ensuring the requirements of human use in all fields.

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 [6].

Corresponding Author :

Buthaina Abdul- Aziz Hassan Al-Magdamy

E-mail: buthena.a.hasan17@gmail.com

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.

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 [7].

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 [8].

As well as algae have recently received a lot of attention as a new biomass source for the production of a new energy [9]. 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 [10]. The first distinction that needs to be made is between macro algae or versus microalgae. [11].

Therefore, the aim of the study was to eliminate some microorganisms, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* using the Alcoholic algae extract.

Material and Method

1. Isolation and diagnosis of bacterial

The bacteria, *P.aeruginosa*, and *K.pneumoniae* bacteria were isolated from the drainage water of the Rustmiya Sewage Treatment Plant in Baghdad using Prescott method [12]. They were grown on the Nutrient agar medium, then 1ml were taken from pure isolation into glass vials containing broth nutrient medium and incubated at 37°C.

2. 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, according to [13].

Then the algae was laboratory diagnosed using a microscope based on the diagnosis method [14].

The algae was cultured and purified in 10ml test tube with Chu13 medium by using dilution method and streaking plating under constant laboratory conditions (temperature of 25±2 °C, 16:8h light:dark, 3000 LUX) [15]. The axenic algal culture was incubated in the incubation room for 18 days, the harvesting where by Centrifuge with of 3000 rpm/minute speed for 15 minute. Then the sediment dried in 45°C for 48h and saved in dark until used.

3. Extraction of active substances from algae

1.5 g of the dried algae was dissolved in 250 ml of Chloroform and then placed in shaking incubator at 25 °C with 70 rpm/minute for 15 min and then dried at 40 °C [16].

4. Preparation of concentrations of algae extract

(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 (2) 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 hours [17].

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 72 hours [18].

Results and Discussion

The results showed that the algae extract showed a clear effect on the microorganisms used, where the numbers of were decreased that treated with different concentrations of *C. humicola* extract (1.25, 0.62, 0.31, 0.15, and 0.07) mg / l within 72 hours.

The algae extract showed no effect on the number *K. pneumoniae* in 24 hours. On the 48 hours, the number of bacteria decreased from 470 cells/ml to 422, 400, 386, 330, and 290, cells/ml. On the 72 hours, the number of bacteria was 350, 300, 290, 276, and 200 cells/ml. As shown in Fig.(1).

The effect of algae extract on *P. aeruginosa* bacteria, using different concentrations (0.07, 0.15, 0.31, 0.62, 1.25) mg / l, was the decrease in the number of cells on the first day of the total 410 to (390, 280, 220, 176, 155) cell / ml. The decrease in the number of bacteria on the 48 hours was (350, 264, 230, 100, 85) Cell / ml. On the 72 hours, the numbers were as follows: (300, 210, 120, 83, and 72) cell / ml. As shown in, Fig.(2)

The study results showed the effect of algae extract towards bacteria *P. aeruginosa*, while the *K. pneumoniae* showed resistance to the effect of the extract was low.

As compared to other types of bacteria. This is because they contain complex layers in their walls, making them more difficult to penetrate into the cell walls [19].

The studies also indicated that the algae extract had a clear inhibitory effect on *P. aeruginosa* and, and the reason is due to their contain of the peptides rings and alkaloids, in addition to polysaccharides [20].

The study agrees with [2], who pointed to the ability of the algae extract to reduce the bacterial numbers, where these fatty acids interact with membrane proteins causing deformation in the structure and effectiveness of the membrane.

Also the study agrees with [21], which indicated the ability of *Cladophora phare* extract to reduce the number of *E. coli* bacteria, the reason is due to the secretion of many substances such as Ferpenoids, fatty acids. amino acids, , which have inhibitory effect on bacteria [22]. 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 [23]. 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 [24].

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 [25].

The active substances found in the algae extract have an effect on the positive bacteria of (Gram stain) rather than on the negative bacteria [26].

As indicated by [27]. 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.

The results of the study showed that the concentration of 1.25 mg / l was the most concentrated effect on bacterial cell count, where *Klebsiella pneumoniae* was the most resistant to the effect of the extract, and the effect of the algae extract was more effective on *E. coli*. [28]

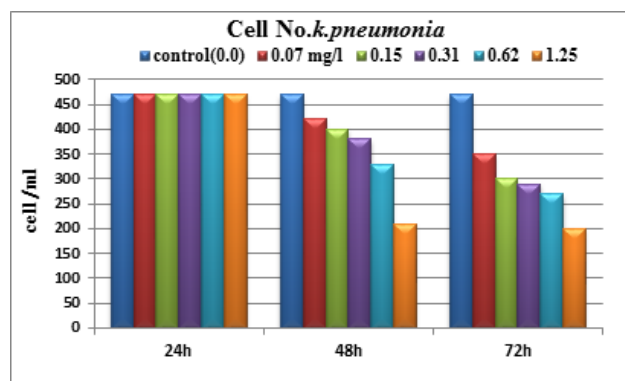


Fig. (1) Effect of Algal extractions on *K. pneumonia* Growth

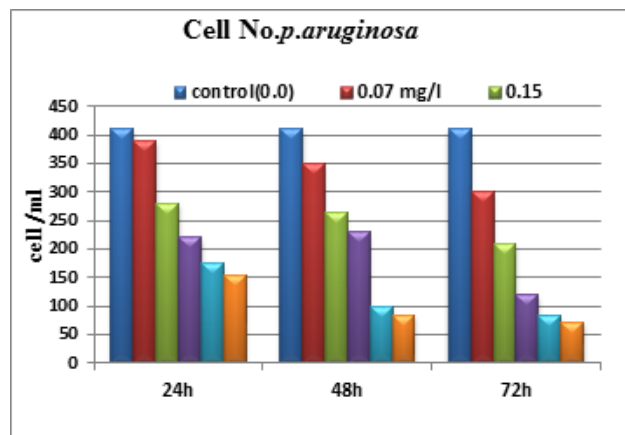


Fig. (2) Effect of Algal extractions on *P. aeruginosa* Growth

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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