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Microbial treatment of water containing the bacteria *Enterococcus faecalis* and *Escherichia coli* in microcosm using *Artemisia annua* plant extract: Hierarchical order of some abiotic factors affecting cell's cultivability

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Abstract

Phytotherapy is increasingly used against infections in many parts of the world. Plants of the genus *Artemisia*, *Asteraceae*, are among those most exploited and several of its species were thus described as having antibacterial and antifungal effects. The infusions of *Artemisia annua* has been indicated as having disinfecting capacity in bacteriological contaminated water. The cultivability of *Enterococcus faecalis* and *Escherichia coli* was carried out in different extract solutions of *A. annua* aimed at describing the order of importance of some abiotic factors on this process. The bacterial abundance undergoes temporal variation with respect to pH of extract, *A. annua* extract and glucose concentrations, light intensity. The hierarchical organization of these factors has been carried out by expressing percentage and ranking in descending order of the sum of squares of each MANOVA test factor. It showed that cultivability of *E. faecalis* in *A. annua* extract solutions was influenced by the incubation durations, followed by the type of dilutions, light intensity, pH of extract, concentration of *A. annua* extract, and glucose concentrations. On the contrary, the cultivability of *E. coli* in *A. annua* extract solutions was influenced by the incubation durations, pH of infusion, type of dilutions, concentrations of *A. annua* extract, glucose concentrations and light intensity. The increase of the incubation durations, light intensity and pH of extract solutions caused a significant ($P \leq 0.01$) decrease of the effect of *A. annua* on *E. faecalis* and *E. coli* cultivability.

Keywords: *A. annua* plant extract, water, microbial treatment, cell's cultivability, impacting factors

1. Introduction

Phytotherapy is increasingly used against infections in many parts of the world. Plants of the genus *Artemisia*, *Asteraceae*, are among those most exploited and several of its species were thus described as having antibacterial and antifungal effects. These included among others *Artemisia nilagirica*, *A. siberie*, *A. annua* and *A. salina*. *Artemisia nilagirica* is used against the bacteria *Escherichia coli*, *Yersinia enterocolitica*, *Salmonella typhi*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Shigella flaxneri* and it inhibits the metabolism of *Microsporium* ^[1, 2]. *Artemisia siberie* has a potential inhibitory effect on *P. aeruginosa*, *S. aureus* and *Escherichia coli*. *Artemisia annua* negatively affects the culture of *Vibrio fischeri* ^[3]. As for *A. salina*, it is known to act on *Cladosporium sphaerospermum*, *S. aureus*, *E. coli*, *Bacillus cereus* and *P. aeruginosa* ^[4]. *Artemisia annua* is widely distributed in the world especially in temperate, subtropical zones and Asia. This plant grows in the wild state in China, Korea, Taiwan and Vietnam ^[5]. However, its natural distribution seems much broader,

extending from southern Siberia to Vietnam and northern India ^[6]. In Europe, it covers the eastern, central and southern areas. It is also common in the Canary Islands and the southeast of Spain. It is present on millions of hectares in tropical regions of Africa. *Artemisia annua* is found among others in Kenya, Democratic Republic of Congo and Cameroon ^[7].

As all plants, *A. annua* produces classic primary and secondary metabolites ^[8]. Secondary metabolites are involved in the interactions between the plant and its environment ^[9]. Because of its antimalarial properties, *A. annua* has been the subject of many biochemical studies in the world. The major and minor secondary metabolites of the plant have therefore been isolated in order to explain the originality of this plant. They can be classified in three groups that are terpenoids, phenolic and aromatic compounds and alkaloids ^[10]. *A. annua* is grown on an industrial scale for the manufacture of therapeutic combinations based on artemisinin or *Artemisia* combination therapies (ACTs), used in the treatment of malaria ^[6]. Artemisinin, the bioactive molecules of the plant

proved effective against schistosomes and some viruses ^[11, 12]. This molecule inhibits the development of *Cryptosporidium*, *Giardia intestinalis*, *Entamoeba histolytica*, and various species of *Leishmania* ^[13, 14]. The effectiveness of this molecule and its synthetic derivatives against *E. coli*, *Enterococcus faecalis*, *Salmonella abony* has been indicated by Vikas *et al.* ^[15].

According to WHO ^[16], water and food can serve as routes of many bacterial transmissions of public-health concern mostly in developing countries. The diseases often reported include gastroenteritis, cutaneous infections, typhoid, colitis among others ^[17-19]. The use of infusions of *A. annua* showed its disinfecting capacity in the bacteriological contamination of water ^[3, 20]. The aquatic environments in which bacteria live consist of a set of biotic and abiotic factors that characterize them. However, the influence of intrinsic abiotic factors on water is still not clearly elucidated in this method of disinfection. Factors such as nutrient concentration, temperature, light intensity and pH are among those whose fluctuations have a significant impact on bacterial growth in aquatic medium ^[21-23]. Although many studies have been focused on the exposure of bacteria to *A. annua* extracts, light condition often leads to inactivation of the latter at varying degrees, and the combined effect of these factors on the survival of bacteria cells in aquatic environments ^[24-25], there is little information on hierarchical order of implication of biotic and abiotic factors on microbial treatment of water using plant extracts. The present study aimed at assessing the hierarchical importance of some abiotic factors impacting *Enterococcus faecalis* and *Escherichia coli* cultivability in aquatic microcosm.

2. Materials and methods

2.1 Collection of *Artemisia annua* leaves and extracts preparation

Artemisia annua leaves were harvested from Bangante, located in the western region of Cameroon (Central Africa). This region is located between latitude 5 and 16 north, and between longitude 10 and 11 west. Its climate is tropical and humid and has two main seasons: a dry season which last from October to March and a rainy season that runs from late March to October. The topography of the area is mountainous with plains and plateaus many of whose height fluctuated between 1000 and 1500 meters. Temperatures range from 15 to 27 °C with peaks in some areas of up to 37 °C. Nights are usually cool, especially between July to October. The soils of the western region are mostly lateritic, clay and volcanic in some area ^[26]. The leaves were harvested in July 2011 and then dried in the laboratory at room temperature (22 ± 2 °C) for 1 month. Thereafter, the leaves were ground into powder. The infusions were prepared from 10, 20 and 30 g of dried ground leaves and mixed in 1 liter of boiling distilled water. These infusions were then filtered and left at room temperature ^[27, 28]. The preparation of *A. annua* leaves in the form of infusion is customary to the ancient traditional Chinese medicinal method ^[29].

2.2 Collection and identification of bacteria

Escherichia coli strain used was isolated from surface waters of Yaoundé in Cameroon (Central Africa). *Escherichia coli* is Gram-negative, catalase positive, reduce nitrate, mobile, oxidase negative, capable of fermenting glucose, lactose,

mannitol and metabolize tryptophan to indole. The isolation was performed on Endo (Biokar Diagnostics) by membrane filter technique, followed by incubation for 24 hours at 44 °C ^[30, 31].

Enterococcus faecalis was provided by the Centre Pasteur of Cameroon (Central Africa). These cells are anaerobic facultative, Gram-positive and generally have white colonies on Plate Count Agar medium (PCA). They are oxidase positive, generally catalase negative and quickly reduce triphenyl tetrazolium chloride (TTC). *E. faecalis* cells were highlighted on Bile-Esculin Azide (BEA). On this medium, colonies are black and surrounded by a clear halo. The black color of the colonies reflects the production of H₂S and the hydrolysis of esculin that binds with iron ^[32].

Biochemical identification of the cells was done according to standard techniques described by Holt *et al.* ^[32]. Each pure strain was then cultured in tryptocasein broth (Bio-Rad) for 24 hours at 37 °C. The cells were then washed 3 times by centrifugation at 3600 rev/min for 15 minutes at 10 °C in NaCl (8.5 g/l). The pellet was then dissolved and 5 ml of each suspension was introduced into a test tube, and then preserved in cold glycerol.

2.3 Experimental protocol

Five hundred (500) ml capacity flasks each containing 250 ml of NaCl solution (8.5 g/l) were used in this study. They were organized into three series A, B and C of 7 round-bottom flasks (Erlenmeyer) each: f1, f2, f3, f4, f5, f6 and f7. For each series, the values of pH in flasks f1, f2, f3, f4, f5 and f6 were adjusted to 4, 5, 6, 7, 8 and 9 respectively. The pH was adjusted with HCl (0.1M) and NaOH (0.1M). Flasks f7 contained only NaCl (8.5 g/l), and served as a control. All seven flasks were sterilized in an autoclave.

Prior the experiment, each frozen vial containing *E. coli* or *E. faecalis* strain was thawed at room temperature. Then, 100 ml of the culture was transferred to a tube containing of 10 ml of nutrient broth (Oxford), and incubated at 37 °C for 24h. The cells were then harvested by centrifugation at 3600 rev/min for 15 minutes at 10 °C and washed twice with a solution of sterile NaCl (8.5 g/l). The pellets were then resuspended in 50 ml of sterile NaCl solution.

After a series of dilutions, 1 ml of the suspension of a single microbial strain was added to 250 ml of sterile NaCl in each of the 7 Erlenmeyer flasks (f1, f2, f3, f4, f5, f6 and f7) as described above. Based on our preliminary study, the cell concentration was adjusted to 2.83 units (log (CFU/ml)). Then, 10 ml of the extract of *Artemisia annua* prepared as described above was added to Erlenmeyer f1, f2, f3, f4, f5 and f6. The extract was not added to the Erlenmeyer flask f7 which was considered as the blank. These preparations were enriched with glucose at concentrations of 0.001, 0.01, 0.1 and 1 g/l. The Erlenmeyer flasks containing the sample with *A. annua* prepared as described above, alternately exposed to light intensities of 1400, 3600 and 5900 lux were then incubated for 10 hours at room temperature (23 ± 2 °C). Erlenmeyers flasks containing the blank were also incubated for 10 hours at room temperature (23 ± 2 °C) in the dark. The analyses were performed in triplicate using the three sets A, B and C.

2.4 Incubation of bacterial suspensions and analysis

Bacteriological analysis of the content of the Erlenmeyers

flasks was performed every 2 hours. The contents of each flask were first homogenized and then 100 µl was taken and analyzed by the technique of surface spreading on agar culture medium. Selective culture media were used. In this case, the culture media Bile-Esculin Azide (Bio-Rad) and Endo (Biokar Diagnostics) were respectively used for the isolation of *E. faecalis* and *E. coli* contained in Petri dishes. These Petri dishes were then incubated for 24 hours at 37 °C for *E. faecalis*, and 44 °C for *E. coli* [30, 31]. The numbers of colony forming units (CFU) were later determined.

2.5 Qualitative phytochemical screening

To identify the phytochemical derivatives in the extracts, standard phytochemical screening was performed [33]. Alkaloids test was performed by Dragendorff's and Meyer's tests, amino acids by ninhydrin, carbohydrates by Barfoed's and Fehling tests, flavonoids by FeCl₃, saponin by frothing test, tannins by FeCl₃ and lead acetate and terpenoids by Salkowski test [33, 34].

2.6 Data analysis

The mean abundance of each species observed in each experimental condition were calculated and illustrated with histograms. Degrees of correlations were calculated between cell abundance and diverse factors impacting water treatment with *A. annua* extracts. All data processing was performed using the program Statistical Package for Social Science (SPSS) version 16.0.

A hierarchical organization of abiotic factors influencing *E. coli* and *E. faecalis* cells cultivability was performed using the MANOVA test with the R software. This hierarchical organization was made by expressing percentage and ranking in descending order of the sum of squares of each MANOVA test factor.

3. Results

3.1 Temporal evolution of cell abundances with respect to different extract dilutions

In solutions containing *A. annua* extract E1 and diluted at 10, 20 and 30%, the abundances of *E. faecalis* ranged from 1.71 to 2.18, 1.93 to 2.24 and from 2.07 to 2.26 units (log (CFU/ml)) respectively. As for *E. coli* densities, it fluctuated between 1.62 and 2.02, 2.08 and 2.22, and between 1.57 and 2.01 units (log (CFU/ml)) in extract E1 diluted respectively at 10, 20 and 30%. The minimum value has been registered in the solution containing the plant extract E1 diluted at 10% after an incubation time of 2 hours for *E. faecalis*. In extract solutions E1 diluted at 30%, it was noted after 10 hours of incubation for *E. coli*. The maximum value was obtained after 2 hours of incubation in extract E1 diluted at 20% for *E. coli*. With *E. faecalis*, the maximum value has been recorded in the extract solution E1 diluted at 30% after 10 hours of incubation (Fig. 1).

In *A. annua* extract E2 diluted at 10, 20 and 30%, the abundances of *E. faecalis* oscillated between 1.83 and 2.22, 1.69 and 1.86 and between 1.60 and 1.99 unit (log (CFU/ml)) respectively. Under the same conditions, the densities of *E. coli* ranged from 1.71 to 2.14, 1.77 to 2.13 and from 1.87 to 2.20 units (log (CFU/ml)) respectively in solutions diluted at 10, 20 and 30% (Fig. 1). The lowest values were recorded in the extract solution E2 diluted at 30%, after 4 hours of incubation for *E. faecalis*. For *E. coli*, the lowest values were

registered after 8 hours of incubation in extract E2 diluted at 10% (Fig. 1). The highest values were observed in the extract solutions E2 diluted at 10% after 2 hours of incubation for both bacteria strains (Fig. 1). In the blank solutions, cell abundances ranged from 2.02 to 2.28 and from 2.17 to 2.29 units (log (CFU/ml)) respectively for *E. coli* and *E. faecalis* (Fig. 1).

In the plant extract E3 diluted at 10, 20 and 30%, the densities of *E. faecalis* fluctuated between 1.91 and 2.29, 1.84 and 2.30 and between 2.03 and 2.27 units (log (CFU/ml)) respectively. Similarly, the abundances of *E. coli* oscillated between 1.67 and 2.01, 1.87 and 2.16 and between 1.68 and 1.99 units (log (CFU/ml)) respectively in extract E3 diluted at 10, 20 and 30%.

The highest and the lowest abundances were recorded at different incubation periods depending on the dilution of extract solutions E3 and bacterial species (Fig. 1). The cell abundances fluctuated between 2.12 and 2.22 and between 2.10 and 2.19 units (log (CFU/ml)) respectively for *E. coli* and *E. faecalis* in blank solutions (Fig. 1).

3.2 Temporal evolution under light of the cell abundances

In the presence of *A. annua* extract, the densities of *E. faecalis* ranged from 2.26 to 2.63, 2.18 to 2.63, 2.26 to 2.73 and from 2.03 to 2.71 units (log (CFU/ml)) respectively in the solutions enriched with glucose at concentrations 0.001, 0.01, 0.1, and 1 g/l under a light intensity of 1400 lux. The minimum value was obtained in media containing 1 g/l of glucose after an incubation period of 10 hours. The maximum value was noted in plant extract enriched with 0.1 g/l of glucose after 2 hours of incubation. In the absence of *A. annua* extract, the abundances of *E. faecalis* oscillated between 2.88 and 3.03 units (log (CFU/ml)) (Fig. 2).

Under a light intensity of 1400 lux, *E. coli* densities fluctuated between 2.34 and 2.77, 2.12 and 2.62, 2.17 and 2.64, and between 2.06 and 2.61 units (log (CFU/ml)) in solutions containing the *A. annua* sample and glucose at concentration of 0.001, 0.01, 0.1 and 1 g/l respectively. The minimum value was registered in plant extract enriched with glucose at concentration of 1 g/l after an incubation period of 8 hours. The maximum value was obtained in extract solution enriched with 0.001 g/l of glucose after 2 hours of incubation. In the blank solutions, the abundance of *E. coli* ranged from 2.76 to 3.05 units (log (CFU/ml)) (Fig. 2).

The densities of *E. faecalis* fluctuated between 2.03 and 2.71, 2.34 and 2.92, 2.43 and 2.83 and between 2.30 and 2.75 units (log (CFU/ml)) in the presence of the extract and glucose at concentrations of 0.001, 0.01, 0.1, and 1 g/l respectively under a light intensity of 3600 lux. The minimum value was recorded in the plant extract containing 0.001 g/l of glucose after 10 hours of incubation. The maximum value was obtained in plant extract enriched with 0.01 g/l of glucose after 2 hours of incubation. In blank solutions, the abundances of *E. faecalis* fluctuated between 2.87 and 3.03 units (log (CFU / mL)) (Fig. 2).

Under a light intensity of 3600 lux, the densities of *E. coli* ranged from 1.92 to 2.72, from 2.07 to 2.70, from 2.26 to 2.71 and from 2.24 to 2.63 units (log (CFU/ml)) in *A. annua* extracts containing respectively 0.001, 0.01, 0.1, and 1 g/l of glucose. The minimum and maximum values were registered in plant extract enriched with glucose at concentration 0.001 g/l respectively after 2 and 8 hours of incubation.

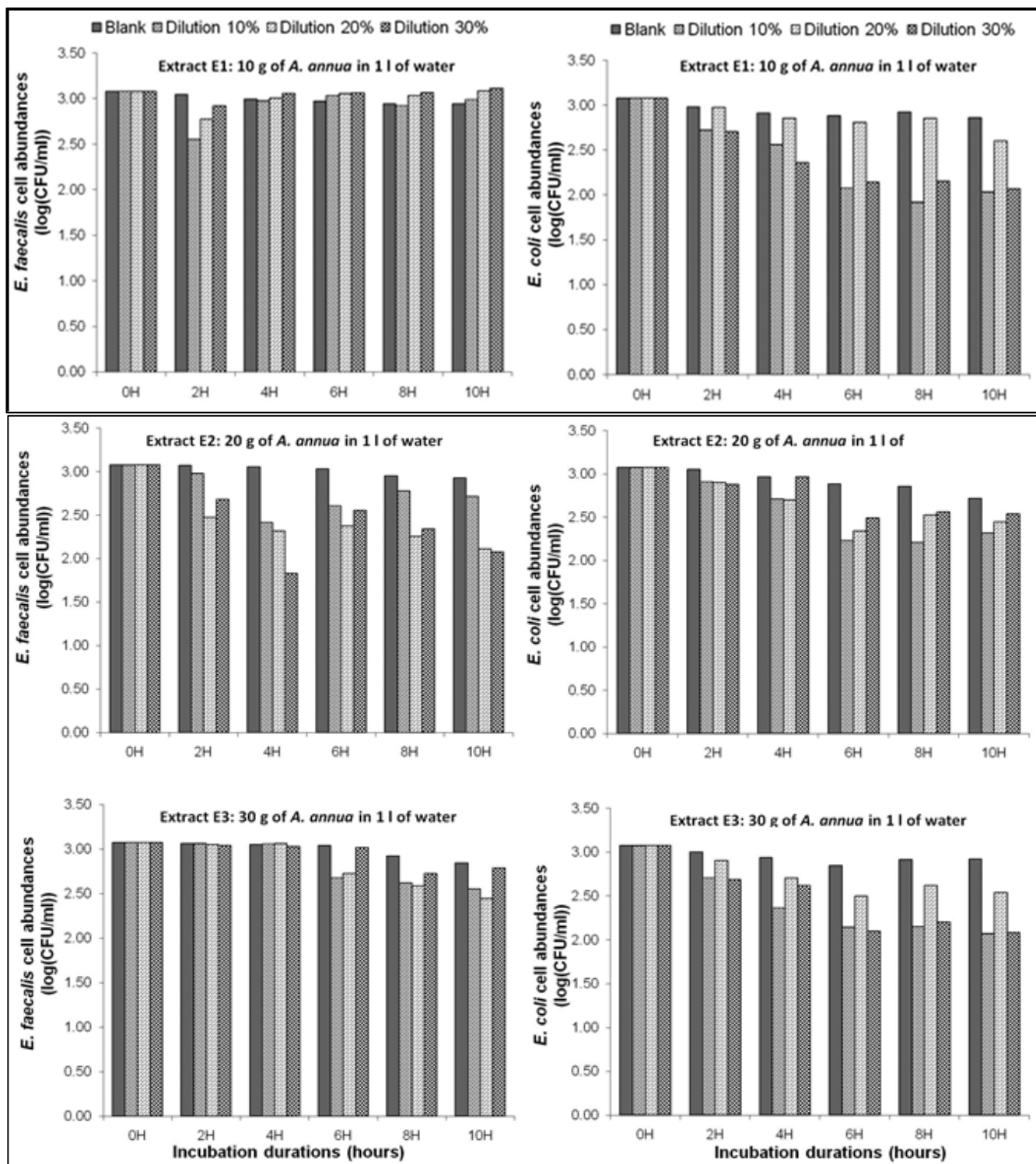


Fig 1: Temporal evolution of cell abundances at different infusion dilutions obtained from 10 g (E1), 20 g (E2) and 30 g (E3) of *A. annua*.

In blank solutions, the abundances of *E. coli* oscillated between 2.77 and 3.02 units (log (CFU/ml)) (Fig. 2). The densities of *E. faecalis* ranged from 2.43 to 2.87, 2.32 to 2.73, 2.25 to 2.91, and from 2.56 to 2.82 log units (CFU/ml) in *A. annua* aqueous extracts enriched with glucose at concentrations of 0.001, 0.01, 0.1 and 1 g/l respectively, under a light intensity of 5900 lux. The minimum and maximum values were observed in plant solutions enriched with 0.1 g/l glucose respectively after 8 and 2 hours of incubation. In

blank solutions, the abundances of *E. faecalis* fluctuated between 2.86 and 3.02 units (log (CFU/ml)) (Fig. 2). Under a light intensity of 5900 lux, the abundances of *E. coli* oscillated between 1.90 and 2.48, 2.08 and 2.68, 2.13 and 2.73 and between 2.19 and 2.81 units (log (CFU/ml)) in *A. annua* aqueous extracts enriched with glucose at concentrations of 0.001, 0.01, 0.1, and 1 g/l respectively. The minimum value was obtained in extract solution enriched with 0.001 g/l of glucose after 8 hours of incubation. The maximum value was

recorded in the solutions containing 1 g/l of glucose after 2 hours of incubation. In blank solutions, the abundances of *E.*

coli fluctuated between 2.86 and 2.98 units (log (CFU/ml)) (Fig. 2).

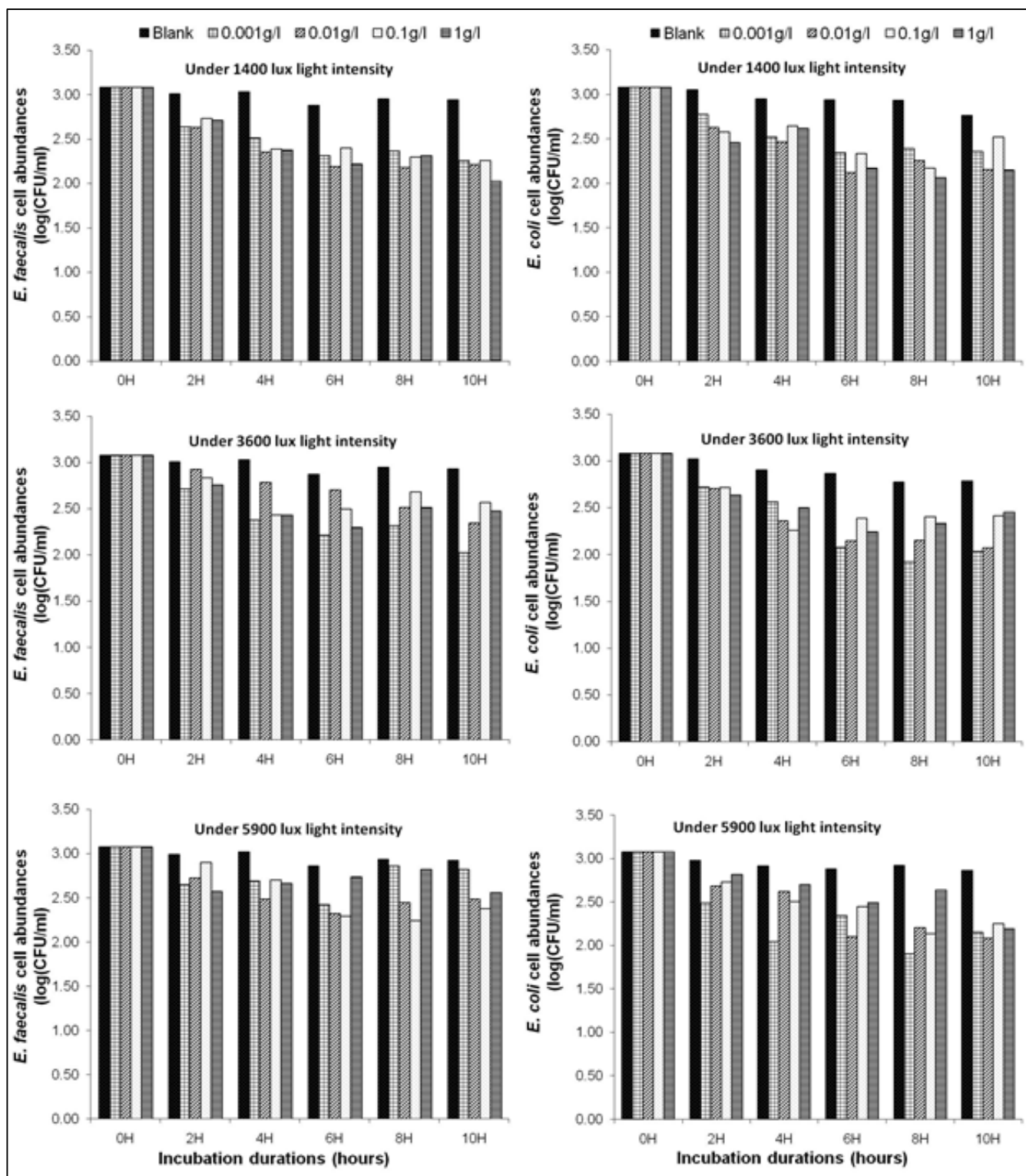


Fig 2: Temporal variations of cell abundances in *A. annua* infusion at different light intensities and concentrations of glucose.

3.3 Temporal variations of cell abundances in the presence of *A. annua* extract dilutions at different pH

The cells abundance of bacteria varied considerably with respect to the different pH values on one hand, and on the other hand the concentration of *A. annua* extract in the aquatic medium.

The abundances of *E. faecalis* fluctuated between 2.50 and 2.92, 2.65 and 3.16, 2.70 and 3.19, 2.71 and 3.26, 2.76 and 3.21, and between 2.84 and 3.18 units (log (CFU/ml)) in the presence of the extract solutions respectively at pH values 4, 5, 6, 7, 8 and 9. The lowest value was obtained at pH 4, after 2

hours of incubation, and the highest value was recorded at pH 7 after 10 hours of incubation. In the absence of the extract, the abundances of *E. faecalis* ranged from 2.51 to 2.62 units (log (CFU/ml)) (Fig. 3).

The densities of *E. coli* oscillated between 0.97 and 2.15, 1.78 and 3.13, 2.35 and 3.31, 1.99 and 3.43, 2.38 and 3.54 and between 2.64 and 3.54 units (log (CFU/ml)) in the presence of

the extract solutions at pH values 4, 5, 6, 7, 8 and 9 respectively. The highest values were observed at pH 8 and 9 after 10 hours of incubation, while the lowest value was recorded at pH 4, after 6 hours of incubation. In the absence of the extract, the abundances of *E. coli* ranged from 2.11 to 2.52 units (log (CFU/ml)) (Fig. 3).

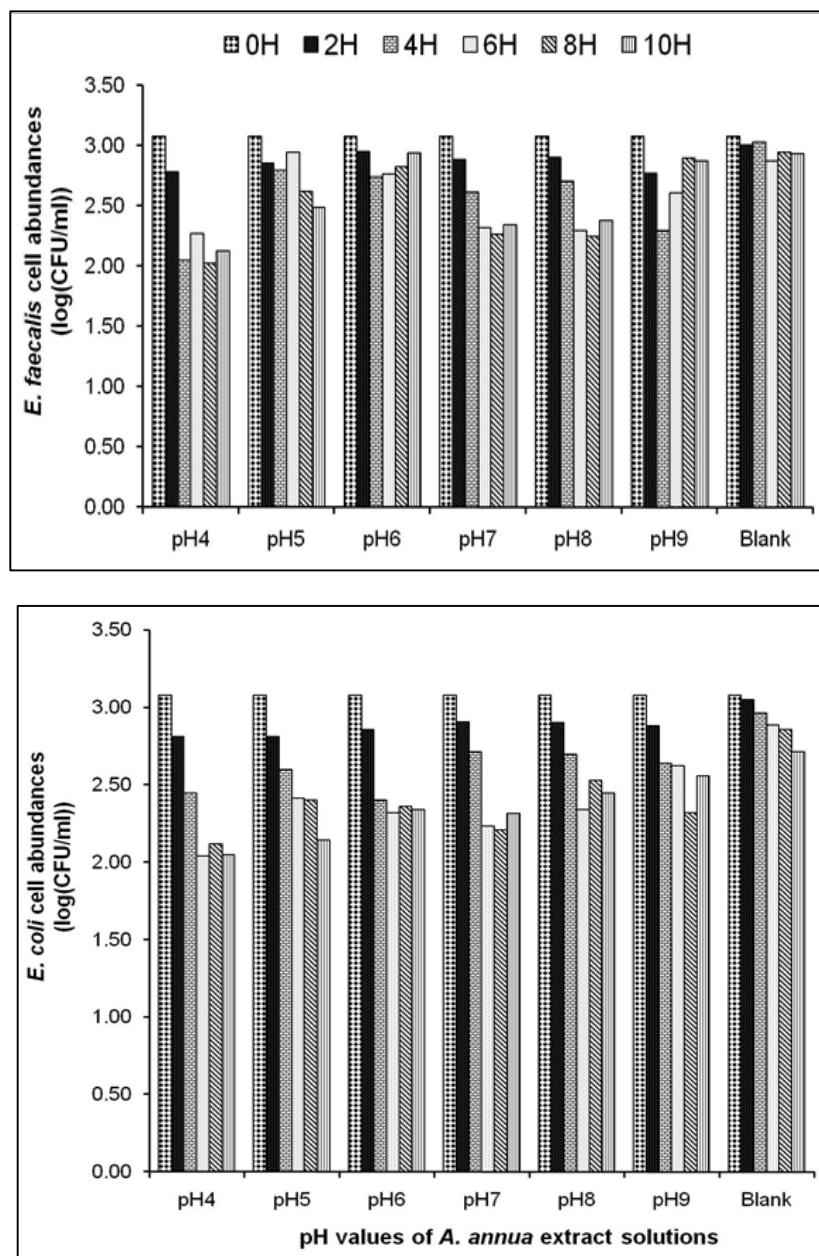


Fig 3: Temporal variation of cell abundances in the presence of *A. annua* extract at different pH.

3.4 Correlation between cells abundance and considered factors influencing bacteria cells cultivability in *A. annua* extract solutions

Spearman "r" correlation coefficients between cells abundance and diverse considered factors impacting bacteria cells cultivability in plant extract solutions were assessed and are presented in Table 1. It is noted that the increase of the incubation durations, light intensity and pH of extract solutions caused a significant ($P \leq 0.01$) decrease of the effect

of *A. annua* on both *E. faecalis* and *E. coli* cultivability. On the contrary, there is not significant ($P > 0.05$) correlation between bacteria abundance and type of infusion, concentration of extract and glucose concentrations.

Table 1: Spearman “r” correlation coefficient between cells abundance and abiotic factors considered

Bacteria species	Abiotic factors considered and “r” value					
	Conc. Infus.	Typ. Infus.	Incub. dur.	Light int.	Glu. conc.	pH infus.
<i>E. faecalis</i>	0.049	-0.158	-0.450**	-0.348**	-0.110	-0.450**
<i>E. coli</i>	0.067	-0.036	-0.806**	-0.283*	-0.117	-0.806**

*: P≤0.05; **:P≤0.001

3.5 Hierarchical importance of considered factors on bacteria cells cultivability in *A. annua* extract solutions

It appears in the hierarchical influence of abiotic factors that the cultivability of *E. faecalis* in *A. annua* extract solutions was influenced by the incubation durations, type of dilutions, light intensity, pH of infusion, concentrations of *A. annua*

extract, and glucose concentrations. On the contrary, the cultivability of *E. coli* in *A. annua* extract solutions was influenced by the incubation durations, pH of infusion, type of dilutions, concentrations of *A. annua* extract, glucose concentrations and light intensity (Fig. 4).

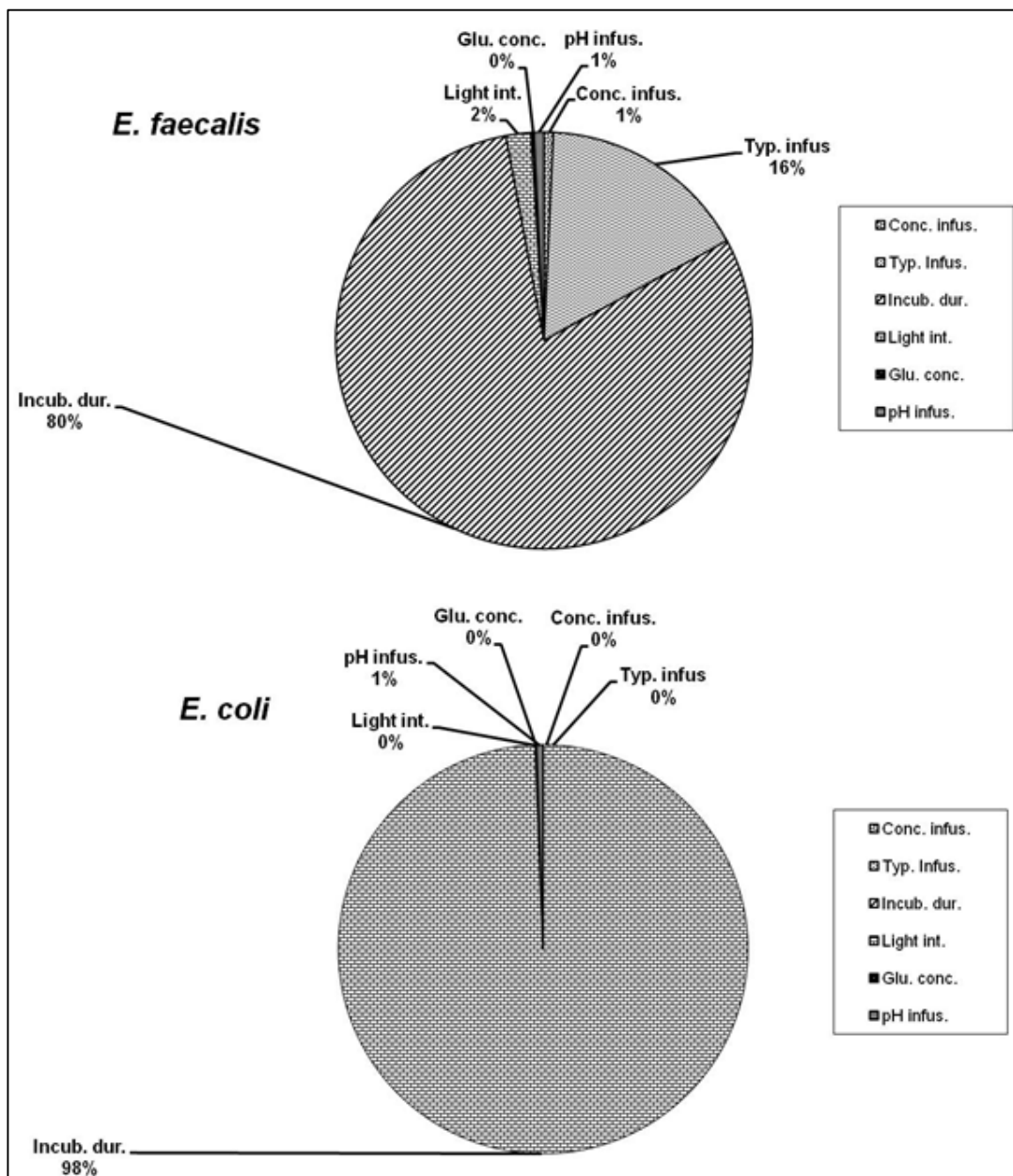


Fig 4: Representation of the hierarchical importance of biotic and abiotic factors on the bacteria cells cultivability in *A. annua* extract dilutions (Conc. Inf: concentration of infusion; Typ. Infus.: type of infusion; Incub. Dur.: incubation duration; Light int.: light intensity; Glu. Conc.: glucose concentration; pH infus.: pH of infusion).

4. Discussion

4.1 Chemical compounds of *A. annua* aqueous extract

Chemical compounds identified in *A. annua* extracts are alkaloids, free flavonoids, tannins, triterpenes and sterols, anthocyanins, reducing compounds, mucilage and coumarins. The triterpenes and sterols can be classified as terpenoids. Anthocyanins are flavonoids. They are polyphenols as tannins and coumarins. The presence of these compounds was also observed by Pourcel *et al.* [35], in the identification of the compounds of the plant. Similarly, previous works have also revealed in addition to the presence of flavonoids, coumarins, steroids, other compounds such as phenolic compounds, purines, lipids, aliphatic, monoterpenoids, sesquiterpenoids and triterpenoids [6, 36].

These plant extracts host a variety of phenolic compounds. *A. annua* has sometimes been called a source of antioxidants because of its richness in flavonoids and coumarin [37]. The diversity of phenolic compounds and eventually their high concentration would be partly responsible for the antimicrobial activity of the plant [38].

4.2 Impact of the concentrations of *A. annua* extract on bacterial cultivability

It has been observed that the effect of different concentrations of *A. annua* extract on bacterial cultivability fluctuates. These fluctuations depend on the concentrations of extract and the bacterial strains. Indeed, there is a significant difference when comparing the evolution of *E. faecalis* abundances in *A. annua* aqueous extracts diluted at 20 and 30%. These results can be explained by the fact that different extract concentrations allow to variations in concentrations of bioactive compounds. Previous studies have revealed the presence of artemisinin in *A. annua* extracts at different concentrations with respect to the quantity of leaves used [27, 39].

Flavones present in the plant, seem to improve the solubility of artemisinin in an infusion [40]. The effectiveness of artemisinin and its synthetic derivatives against *Escherichia coli*, *Enterococcus faecalis*, and *Salmonella abony* was indicated by Vikas *et al.* [41]. However, the study of the effect of different concentrations of *A. annua* essential oil on *S. aureus*, *E. coli* and *Salmonella sp.*, among others, has revealed mitigated results. Some cell species were sensitive even at concentrations of 0.3 mg/ml, while others tolerate concentrations of around 80 mg/ml [7, 36]. Furthermore, Natarajan and Schmittl [42] showed that the more the temperature is high or the more the ratio of water mass on mass of leaves of *Camellia sinensis* increases, the more the extraction of the compounds from the plant is important.

4.3 Impact of the light intensity and the organic nutriment

On the whole, the results showed that after the light exposure time, cell abundances are relatively higher in solutions containing more of the biodegradable organic matter than those that contain lower quantities. The abundances of *E. coli* relatively increase with the concentration of glucose and light intensities. Similar results were observed by Jenkins *et al.* [43]. These authors showed that the increase in nutrient concentration would more expose microorganisms to the light effects. To this would be added the oxidative stress undergo by the bacteria under irradiation. Oxygen was identified as an important parameter of the photo-disinfection by photo-oxidation. According to Klungland *et al.* [44], the combined

action of light and oxygen causes bacterial lesions. The oxygen toxicity vis-a-vis some bacteria including enterobacteria is due to superoxide ion (O_2^-), Hydroxyl radicals and hydrogen peroxide formed during the oxidation reactions. All these molecules can destroy the DNA of bacteria by their oxidizing power. The action of these oxidants on the bacterial membrane results in a disturbance of the different cell process, and the inactivation of the bacterium and possible death. However, certain bacteria have developed a protective mechanism in which the superoxide dismutase enzyme is produced. It quickly converts superoxide anion into hydrogen peroxide which is then decomposed by catalase into water and oxygen gas. These enzymes are produced using information from DNA. Thus, if the DNA is destroyed or inactivated by irradiation, the protection mechanism will therefore be inactivated [45]. Similarly, Nola *et al.* [46] by their works, aim at performing the photo-inactivation of *S. aureus* and *Vibrio parahaemolyticus* in aquatic microcosm, revealed a significant impact of light on bacterial inhibition. This inhibition which is significantly greater in solutions enriched with organic matter. Other experiments showed that the percentage of inactivation of bacteria after irradiation is directly related to the intensity of solar radiation and varied with the nature of the bacteria. This could be explained by the relationship between the intrinsic susceptibility of bacteria to different wavelengths and the differential penetration of these wavelengths in water [47].

4.4 Impact of the chemical nature of the plant extract

Solar exposition of bacterial-contaminated solutions after adding alkaloid extracted from *A. annua*, leads to complete inactivation of cultivable cells. Alkaloids are hydrophobic cations with antibacterial properties on bacterial cells especially on DNA [48]. This inhibitory effect is characterized by a reduced incubation period under the influence of light conditions. The incubation period appears to be a major factor influencing the effect of extracts of *A. annua* on bacteria. The studies conducted by Allahdin *et al.* [20] indicate that the addition of an infusion of *A. annua* in bacterial-contaminated water causes a complete inactivation of the cultivable faecal coliforms after an incubation period of one hour.

With the addition of non-alkaloid plant extract, the presence of cultivable bacteria was observed throughout the experiments. Non alkaloid fraction is devoid of alkaloid and terpene compounds. These compounds have been washed during extraction. The persistence of bacteria observed could be explained either by a distortion of molecules with potential biocides after exposure to sunlight with high intensity, either through a limited action of the alkaloid extract not leading to sub-lethal damage of bacteria. The action of alkaloid molecules only would be insufficient to complete inactivation of bacteria. Furthermore, the synergistic effect of the various components of the plant would be in favor of its antibacterial activity. Indeed, crude *A. annua* extracts obtained by cold extraction have been studied for their antibacterial activity against Gram-positive and Gram-negative bacteria by many authors. The *A. annua* solutions extracted with methanol and chloroform showed activity against Gram-positive and Gram-negative bacteria tested. The extracts obtained with hexane and petroleum ether revealed activity against Gram-positive bacteria and no activity against Gram-negative bacteria [49, 50].

The antibacterial activity of *A. annua* extracts is also related to the quality of the extract and thus the method of extraction.

4.5 Hierarchical importance of considered factors in cells cultivability in *A. annua* aqueous extract

Abiotic parameters seem to influence at diverse degree the effect of *A. annua* extract on bacteria. First, the incubation period, which represents the contact time of the various dilutions extract with bacteria, would have a considerable impact on the effect of plant extract in cell cultivability. This is consistent with the results of many studies which showed that the effectiveness of a low level disinfectant to microorganisms is considerably high if we adjust the exposure time [51].

Then the pH of the solutions will be a determinative parameter which determines the effect of *A. annua* extract on different bacterial species. Laboratory tests have shown that the majority of microorganisms grow best in neutral or slightly alkaline to acidic pH [52]. Indeed, the presence of ionic species can alter the surface properties of microorganisms [53]. Furthermore, the concentration of the extract influences diversely its effect on the growth of bacteria, as well as the concentration of glucose. Other studies indicate that the antimicrobial activity of *A. annua* extracts against Gram-positive bacteria is more pronounced than against Gram-negative bacteria [54, 55]. These differences were attributed to the fact that the cell wall of Gram-positive bacteria consists of a single layer, whereas Gram-negative bacteria is a multilayer and rather complex structure [56, 57]. Intrinsic factors microorganisms led to considerable variation in their resistance to disinfectants and their different concentrations. Indeed, Gram-negative bacteria have an outer membrane which acts as a barrier to the absorption of disinfectants [58]. In addition, the cell wall of Gram-positive bacteria such as *E. faecalis* contains teichoic and lipoteichoic acids. In the aquatic environment, for the same bacterial strain, these characters changed according to the physicochemical properties of the medium [59, 60].

For light intensity, it causes an increase in water temperature. The change in water temperature would influence the effect of *A. annua* extract on the metabolism of bacteria, especially as temperatures fluctuated between 31.9 and 37.9°C. The temperature variation affects the metabolism and enzymatic activity of microorganisms, influencing some physicochemical parameters such as pH, ion activity, thermal agitation, the solubility of gases and the surface properties of microorganisms [61]. According to Wong *et al.* [62], the portion of UV-B of the white light spectrum is the most bactericidal, inducing DNA damage. The impact of these mutations can affect protein synthesis, DNA replication and the plasma membrane.

5. Conclusion

This study revealed that in an aquatic environment there was a relative increase of *E. faecalis* and *E. coli* cells cultivability in the presence of *A. annua* extract. Under diverse light intensities, there is a relative decrease of the cultivability of these two bacteria cells considered at different dilutions of *A. annua* extract enriched with glucose. *A. annua* extract favors cultivability of *E. faecalis* and *E. coli* in the dark, especially at slightly acidic pH. It is also noted that the increase of the incubation durations, light intensity and pH of extract

solutions caused a significant ($P \leq 0.01$) decrease of the effect of *A. annua* on *E. faecalis* and *E. coli* cultivability. The diversity of phenolic compounds and their high concentration would be responsible for the antimicrobial activity of *A. annua*. Incubation durations, light intensity, pH of extract solutions were among other abiotic factors impacting at various degrees water treatment process with *A. annua* extracts.

6. References

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