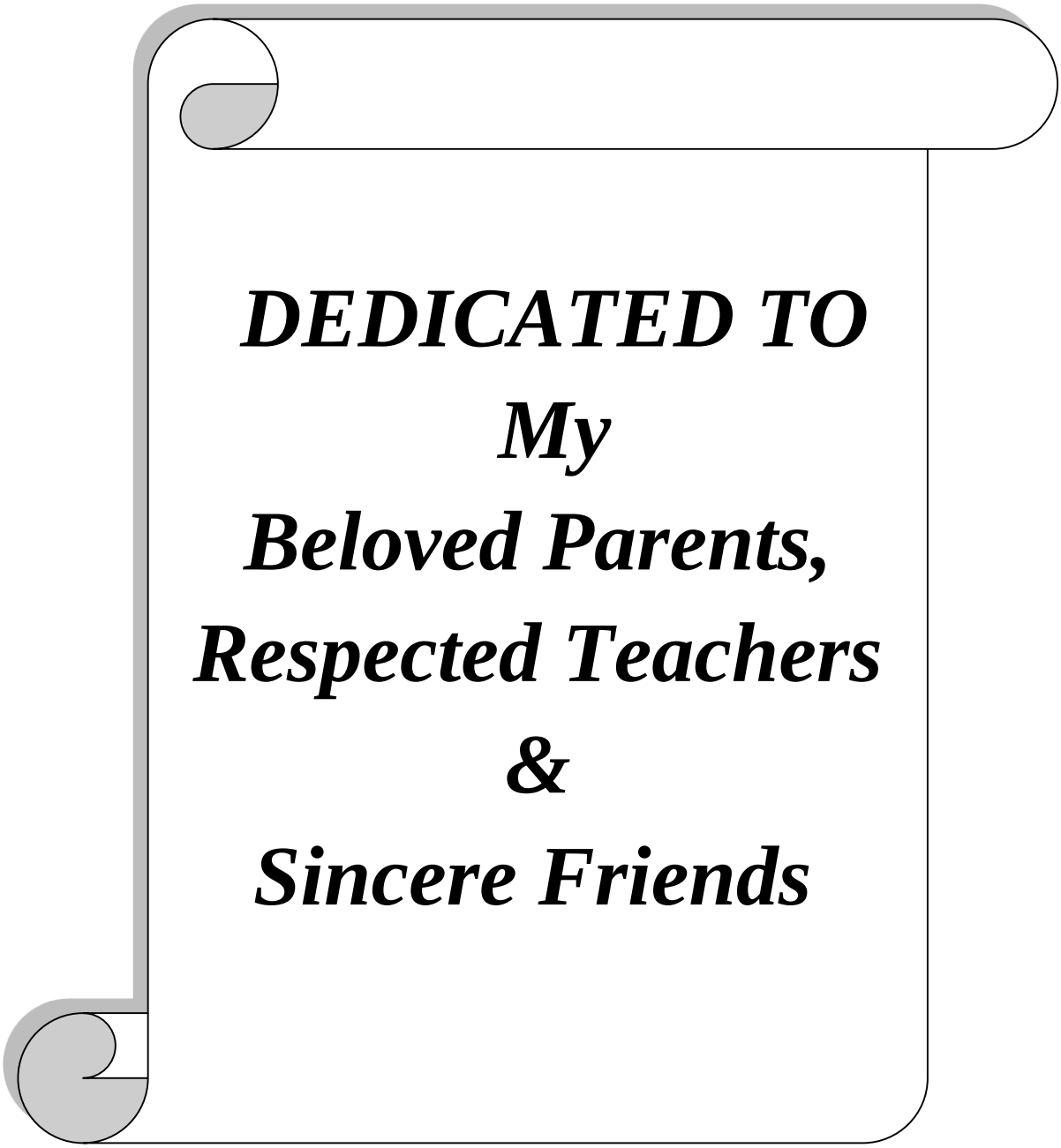
Effect of phytochemicals under variable disinfection conditions on heterotrophic bacteria in canal water

Submitted by: **Shaheera Pervaiz**
2016-MS-CH-32

Supervised by: **Dr. Nadeem Feroze**

DEPARTMENT OF CHEMICAL ENGINEERING
University of Engineering & Technology, Lahore


DEDICATED TO
My
Beloved Parents,
Respected Teachers
&
Sincere Friends

Acknowledgement

"He is Allah; there is no deity except Him. To Him is praise in the first [life] and the Hereafter. And His is the [final] decision, and to Him you will be returned" (Al Qasas: 70).

I'm grateful to **Almighty Allah** for giving me all the grace to pursue this study. Undoubtedly these are His unlimited blessings that made me complete this work. And peace and blessings be upon **Prophet Muhammad (PBUH)** who enlightened our minds to recognize our creator.

I thanks to my dear parents, for their encouragement in all respects of my study. I would also like to include a special note of thanks to my siblings who incited and stood by me.

My sincere thanks and gratitude to my advisor Prof. Dr. Nadeem Feroze for his critical comments on early drafts, kind behaviour, constructive suggestions and all the way helpful supervision. Without his tireless guidance and patience, this research would not have been completed.

Lastly, I feel pleasure and deep sense of indebtedness to all friends for being prompt and supportive at all times.

Contents

LIST OF FIGURES

ABSTRACT

Availability of decontaminated and clean drinking water determines the living standard of individuals. Provision of clean drinking water appearing as a major problem because of increased population level. In this work, cost effective and energy efficient solar disinfection technique was applied for the disinfection of Heterotrophic bacteria. Phytochemicals were added to enhance the disinfection efficiency because solar light is a variable source. Sample was taken from canal water and exposed to sunlight in the presence of phytochemicals and viable colonies were counted after incubation period. Study was carried out with various concentrations of different phytochemicals and variable environmental conditions. Almost 70-75% efficacy of bacterial disinfection was achieved.

CHAPTER 1

INTRODUCTION

Availability of clean drinking water is the basic necessity of human health. According to Stockholm International Water Institute, approximately half of all hospital beds are filled by people suffering from water borne diseases. Increasing urbanization has exacerbate the contamination level in drinking water and results in water borne diseases i.e. gastroenteritis, cholera, dysentery, typhoid, poliomyelitis hepatitis etc. Provision of safe water is necessary for improving the health and quality of life and for alleviating poverty [1].Contaminated drinking water is one of the major issue that results in a waterborne diseases at a high extent [2], [3].

As ever poor are badly affected, 50% population of developing countries are facing the problem of contaminated water sources. According to one estimation 884 million people has no access to meliorated water and others are compelled to use microbiologically contaminated water that results in the transmission of water related diseases i.e. polio, hepatitis, typhoid and cholera. Water is the basic requisite for human health and value of life, but unluckily water supplies are coming under pressure and this scarcity is due to increase in population, overuse and consumption [3], [4]

In Pakistan, 38.5 million people lack access to clean drinking water and its shortage is increasing rapidly. In remote areas safe water drinking supply is barely exist so disinfection by sunlight is one of the economical way to reduce the occurrence of waterborne diseases as well as it is easily available technique that reduces the microbial contamination load [5]. There are number of methods that are used for the processing of contaminated water at domestic level in developing countries i.e. boiling, flocculation, filtration, or chlorination. However each treatment is associated

with its own shortcomings such as taste, poor microbicide efficacy or high cost. [6]. Most of the chemical disinfectants produces several undesirable chemicals considered as disinfection by products (DBPS) in water which get mixed with naturally occurring organic matter and produces toxic compounds so that natural herbs works as a substitute to chemical treatment [1].

Several researchers are in a way to enhance the efficacy of SODIS by using different compounds such as phytochemicals, TiO_2, H_2O_2 and copper plus ascorbic acid [7].

Herbs such as Ocimum kilimandscharicum, Ocimum sanctum, Cuminum cyminum, Vetiveria zizanioides and Murraya Koenigii etc. have antibacterial properties against various bacteria like total coliform, faecal coliform, bacillus specie, Escherichia coli and serratia specie [8].

In that era, environmental friendly options such as ultra violet radiations (natural or artificial) plant extracts and combination of both can be an effective option for water disinfection at small level [9]. Natural source of ultra violet radiations is solar light and the disinfection by sunlight is one of the cost effective, energy efficient, robust and reliable household water treatment process used to mitigate the occurrence of waterborne illness especially to extenuate the frequency of diarrheal disease as it is suggested by United Nations Children's Fund (UNICEF) [10]–[12].

Solar disinfection is the most simplest and economical technique in which sample is placed in PET bottles which are then exposed to sunlight and that exposure reduces the pathogenic load significantly by solar radiations and temperature [13], [14] but the exposure time varies from 6-48 hrs. contingent on the sunlight intensity as well as microbial contamination [15] .

CHAPTER 2

LITERATURE REVIEW

Several billion diseases and up to 10 million deaths are only caused by waterborne pathogens. Many waterborne enteric bacteria can be killed by heating water at 80^0 C for 30 sec [16].

HETEROTROPHIC BACTERIA:

Heterotrophic bacteria are naturally present in human being and animal. Various types of heterotrophic bacteria are Aeromonas, Proteus, Enterobacter, Alcaligenese Pseudomonas, Citrobacter, Klebsiella, Moraxella, Flavo bacterium, Sratya and Acinetobacter (Gram negative) Bacillus and Micrococcus (Gram positive). Some heterotrophic bacteria are considered as pathogenic indicator i.e. pseudomonas which can cause severe infection to skin and lungs and Aeromonas cause gastrointestinal disorders [17].

BACTERIAL DISINFECTION METHODS:

- Boiling (heating upto 110^0 C)
- Filtration (passing through semi-permeable membrane)
- Flocculation (addition of chemical)
- Chlorination (sparge chlorine)
- Ozonation (pass ozone)

CHEMICAL WATER DISINFECTION:

Water disinfection by chemicals i.e. chlorine, chlorine dioxide, hypochlorous acid, hypochlorite, N-chloramine, hypochlorite, hydrogen per oxide, mercury, silver and copper etc., chlorine disinfection is a well-established technology and it is more cost effective than other chemicals but

chlorine residuals even at low concentration is toxic and corrosive. Similarly formation of toxic disinfection by-products (DBPs) associated with chlorine, mutagenic and carcinogenic effects of glutaraldehyde and high instability of per acetic acid have made doubts about the usage of chemical disinfectants [18].

CONVENTIONAL INTRUSIONS:

Conventional interventions on the distribution point have been used for long time but that are not proved to be fulfilling the desired level so household interventions need to be focused so that safe drinking water can be accessed and waterborne diseases can be mitigated. According to the report of Clasen and Haller 2008, solar disinfection has a big advantage on other household techniques because of its independency on chemical dispersion, as it is required in household chlorination method which is slightly effective than SODIS but it leads to the production of toxic disinfection by-products. Sunlight has a combined effect of infrared, UV and visible energies that can effectively deactivate pathogenic organisms in water [19].

INVESTIGATION REPORT:

According to one survey, 67 moderate income countries for HWT practices illustrated that 0.2% of surveyed homes used SODIS, compared with 5.7% adding bleach, 21.0% boiling, and 4.2% filtering their water [12].

SOLAR DISINFECTION:

In batch-process contaminated water is placed in plastic bags, glass/plastic bottles and then exposed to sunlight for about 6-8 h before consumption. Optical and thermal effect of sunlight

results in biological disinfection and synergistic influence happens only when temperature of water exceeds to 45^0 C [16], [20].

KEY OPERATIONAL PARAMETERS OF SODIS:

Experimental studies have revealed the key operating parameters that effect the SODIS process are:

(i) Light intensity

(ii) Wavelength

(iii) Turbidity

(iv) Temperature

(v) Solar exposure time

(vi) Availability of oxygen

ROLE OF ULTRAVIOLET RADIATIONS:

UV radiation (200–400 nm) can be categorized as UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (200–280 nm). Ozone layer absorbs UV-C along with a proportion of the UV-B; therefore UV-A consider as a key driver for SODIS. UV component as well as blue end of the visible spectrum is accountable for biocidal act during SODIS [13].

The main driver of SODIS is UV-A (320-400nm) because a good amount of ultraviolet radiations reaches the earth surface (troposphere) although shorter wavelength UV-C has ability to direct damage nucleic acid by formation of thymine dimers but these wavelengths are completely absorbed in the earth stratosphere, along with the main stream of the sun UV-B radiation [5]

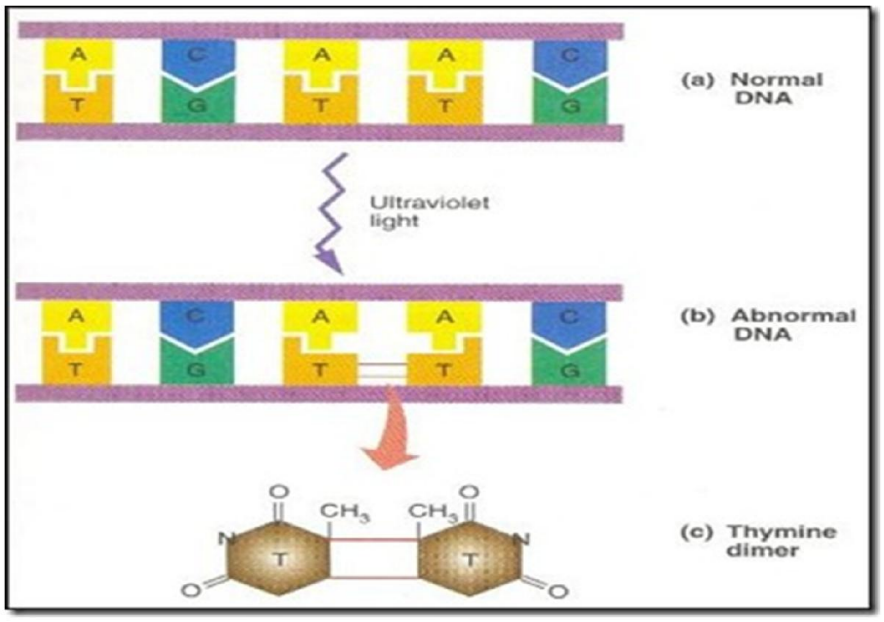


FIGURE 1: FORMATION OF THYMINE DIMER

Ultraviolet radiations of category A are not able to be absorbed by nucleic acid but it has ability to inactivate microorganisms by stimulating dissolved organic carbon (DOC) in water which leads to the creation of reactive oxygen species (ROS) [21]. Photosensitization of molecules and dissolved organic matter present in water helps in the absorption of photons having wavelengths from 320–400 nm which accelerate photochemical reactions [22]. Reactive species such as H_2O_2, $O_2^{\cdot}$ can cause lethal harm to microorganism by distraction of the cell membrane or by attack on DNA and RNA [23].

Solar disinfection treatment involves different biocidal paths based on UV-A radiations and thermal inactivation. Direct exposure of UV-A accelerates destruction of cellular membrane and halt bacterial growth [24] also UV-A act as catalyst in the formation of ROS which reduces the bacterial growth although it is least effective irradiation range to mutilate bacterial DNA but its efficacy is verified by internal and external ROS attacks, such as protein damage, destruction of

nucleic acid by forming bonds with other adjacent nitrogenous bases that ultimately leads toward cell inactivation [25], [26]. Major attack of that ultra violet radiations is on respiratory chain and the cell's ability to produce energy (ATP) [27] but disinfection by UV-A is more than 1,000-fold slower than by direct impairment of UV-C [5], [21]. Several researchers have looked for means to accelerate SODIS, using such compounds as phytochemicals, H_2O_2, TiO_2 and copper plus ascorbic acid [28]–[33]

ROLE OF TEMPERATURE:

Inactivation mechanism shows independency on thermal effect up to 40^{o} C but after that synergistic effect of solar and thermal energy has been observed because thermal energy assists in the captivation of red and infrared photons by water [22], [34]–[36].

Above 40^{0}C thermal stress applied to the cells that damage the cellular wall as well as protein and nucleic acid that leads to the bacterial death [37] this effect dominates as we increase temperature from 50 to 60^{o} C [38].

ROUTES OF PHOTOINACTIVATION:

Endogenous direct, endogenous indirect, and exogenous indirect are involved in the photo inactivation of microorganisms [39]. Endogenous direct photo inactivation causes a direct damage to genetic material such as microbial DNA that happens due to UV-B radiations (280-320nm) [40]. Indirect photo inactivation occurs either internally or externally to the cell and that involves electron or energy transfer to form reactive species that can cause cell death while exogenous indirect photo inactivation take in both the ultraviolet (UV) spectra and wavelengths extending to 550 nm [39], [41].

SODIS uses the synergistic effect of light and thermal energy when temperature rises to certain limit [34], [35], [42]. Water temperatures above 50 °C significantly increased the rate of bacterial inactivation [43] while sunlight that reaches the earth surface contains 4-6% UV domain spectrum [43].

WAYS TO ENHANCE SODIS:

Number of ways exist to meliorate the solar disinfection process which includes the following:

- Design of SODIS bags
- Use of dosimeter sensors
- Use of semiconductor photo catalysis [13].

CONS AND PROS:

SODIS has advantages over other conventional methods i.e. boiling, ozonation and chlorination (using sodium hypochlorite). Boiling is comparatively costly process while other chemical methods are likely to produce carcinogenic and mutagenic byproducts [44].

As SODIS has advantages over other methods it has disadvantages as it require long deactivation time as well as that time depends on solar irradiance, small sized bottles can be treated and it also depends on the quality of water that is to be treated [45] but researchers are trying to implement that disinfection method on large scale by using continuous flow reactors [46].

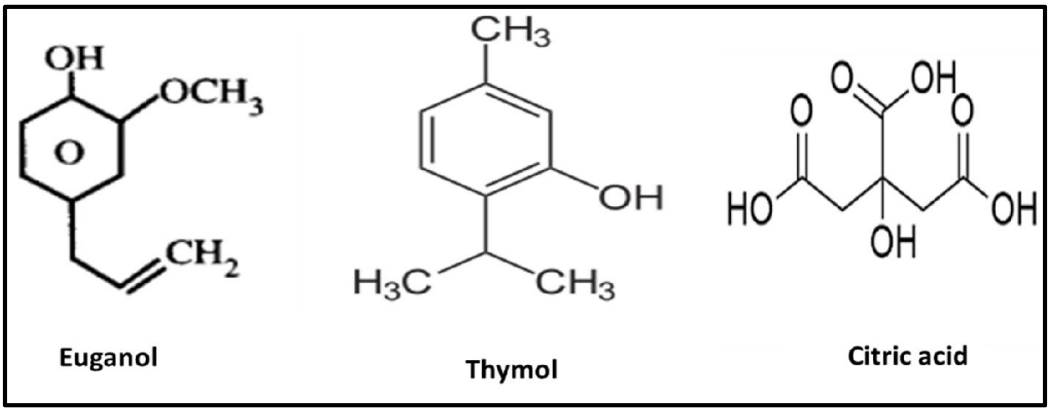
PHYTOCHEMICALS:

'Phyto' means plant so phytochemicals are plant extracts that have biologically active compounds and unique aroma due to which they are able to inhibit microbial growth of wide range of bacteria

[47]. Phytochemicals are useful to human health as they provide protection against various diseases through different modes of action. Thousands of phytochemicals have been discovered since now and it is anticipated that scientists will discover many more.

Phytochemicals such as carvacrol, citric acid, thymol and euganol containing phenolic group that causes disinfection mainly by disrupting cell membrane that causes cell content leakages and eventually cell death. Essential oils binds to active site in an efficient manner by hydrogen bonds and pi-pi stacking interactions due to its hydrophobic properties [9], [48].

CHEMICAL STRUCTURES:



Euganol Thymol Citric acid

TYPES 0F PHYTOCHEMICALS:

Different classes of phytochemicals are listed below: [9],[49]

- Essential oils
- Alkaloids

- Glycosides
- Flavonoid
- Phyto-estrogens
- phytosterols
- Phenolics
- Tannins
- Terpenes

Compounds	Class
Citric acid	Essential oils & Tannins
Euganol	Essential oils & Terpenoids
Thymol	Essential oils, Terpenoids, Phenolic alcohol, polyphenols & Flavones

MODE OF ACTION OF PHYTOCHEMICALS:

The elementary function of phytochemicals is to disrupt cellular structure by disturbing membrane configuration and permeability, which create hindrance in energy production, nutrient processing, formation of ATP, membrane transport phenomenon, synthesis of macromolecules, disruption of proton pumps and in other metabolic regulatory functions [50], [51]. Owing to their lipophilic nature, essential oils are able to penetrate through the cell wall and cytoplasmic membrane, which disturbs the arrangement of fatty acids , phospholipids bilayers, and polysaccharides molecules [47], [50], [51]. All

these events lead to the coagulation of inner cellular components in the cytoplasm and break down of the bonds between the lipid and protein layers [52].

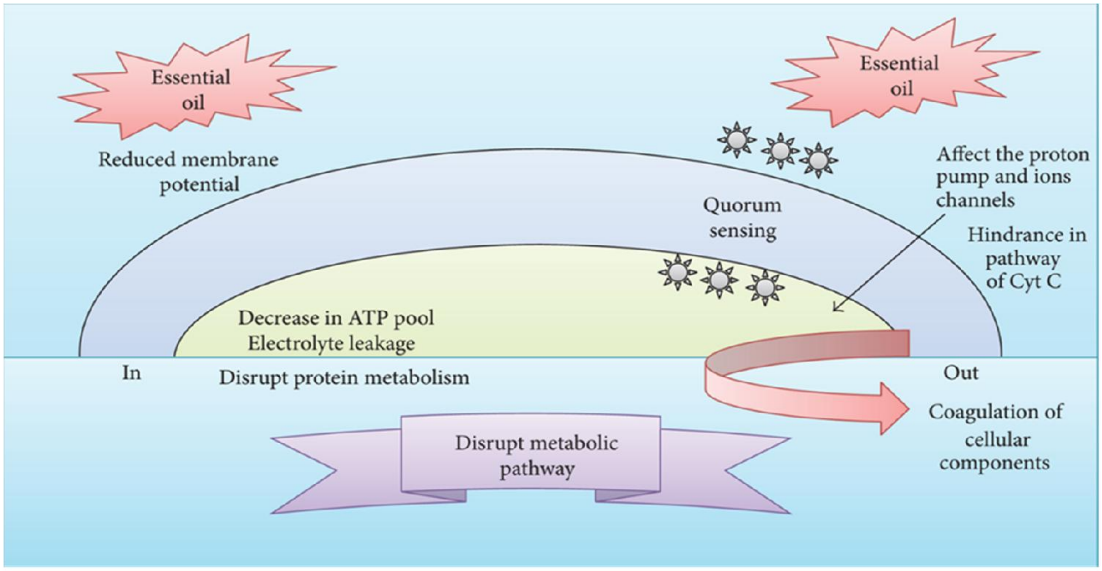


Figure 2: Antimicrobial mechanism of essential oil on microbes

CHAPTER 3

MATERIAL AND METHODS:

APPARATUS:

- **Autoclave**
- **Incubator**
- **PH meter**
- **Turbidity meter**
- **Solar meter**

CHEMICAL:

- R2A Agar
- Magnesium chloride
- Potassium hydrogen phosphate

Agar is basically a gelatinous substance provides nutrition to microbial culture derived from seaweed and it is recommended for enumeration of heterotrophic bacteria especially in potable water.

It consist of following components:

- Proteose peptone
- Yeast extract
- Glucose
- Starch

- Di-potassium phosphate
- Magnesium sulphate
- Casein hydrolysate
- Sodium Pyruvate
- Agar

PROCEDURE:

- All glass wares, solutions and distilled water that was used in experimentation were sterilized.
- Samples was collected from canal than it was placed in a container for the settlement of debris and other large particles for about one and half hour.
- Noted down its turbidity, pH, total dissolved solids and conductivity. If the turbidity lies naturally in a range of 10 to 20 NTU than it will be considered as it is but if that didn't lie in that range we had to be adjusted by dilution or by using sterilized clay that was also collected from nearby canal water passage.
- Performed serial dilutions to know that how much dilution is required.

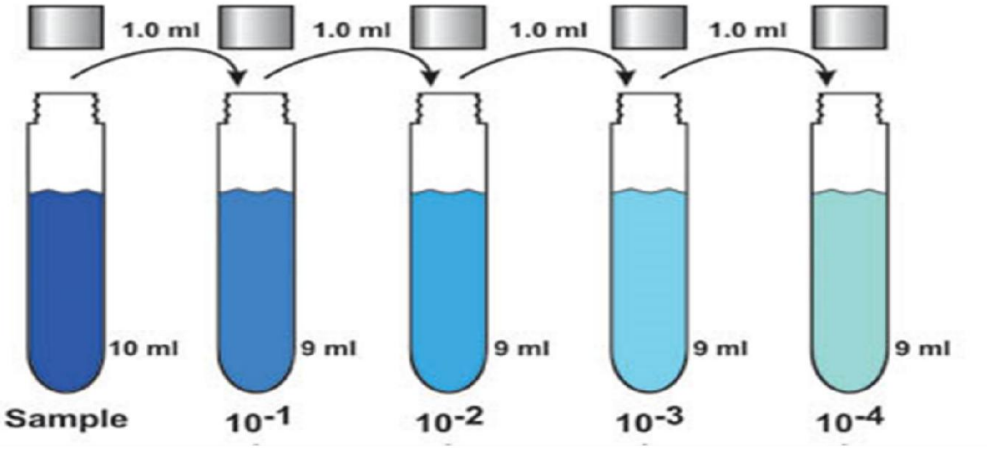


FIGURE 3: SERIAL DILUTION

- Three different types of phytochemicals (citric acid, euganol and thymol) were used at different constant concentrations whose stoke solutions were already prepared and stored in refrigerator.
- After specified dilution shake all polyethylene tetra ethylate (PET) bottles vigorously that contain phytochemicals for about 60-80 seconds.
- Prepared two set of samples each set containing four bottles three bottles with three different kind of phytochemicals while other one without any phytochemical.
- Placed one set of bottles in sunlight while other one in dark.
- Samples were taken on hourly basis with the help sterilized pipettes and placed in test tubes or veils that was filled with 9 ml solution of $MgCl_2$ and KH_2PO_4 in distilled water.
- Grabbed 0.1 ml of sample and pour it on prepared R2A agar petri dish and spread it by sterilized hockey stick (spread plate method).

- Incubate that inoculation at 20^0C for about seven days after that colonies were counted with the help of colony counter

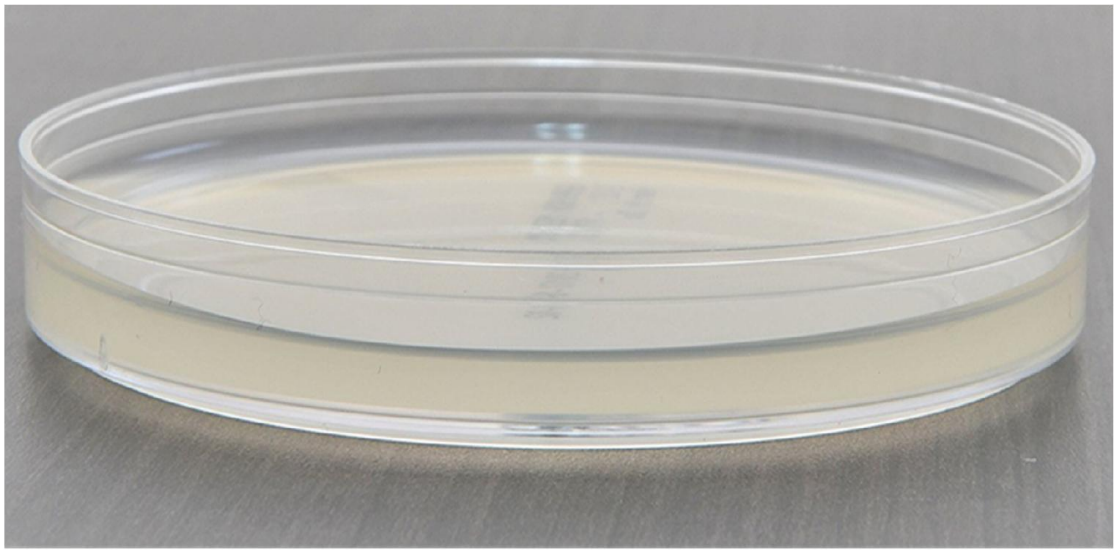
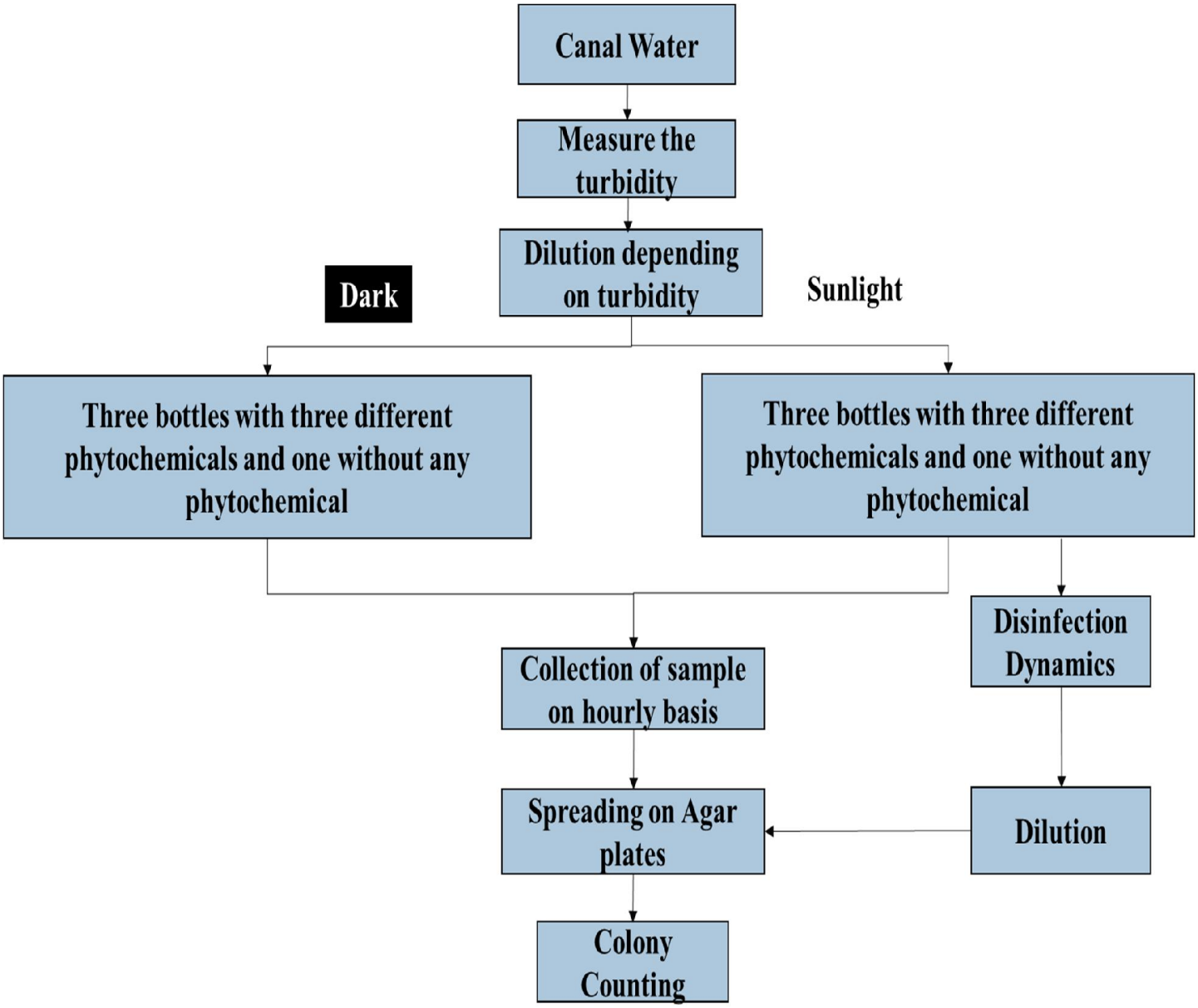


FIGURE 4: PREPARED PETRI DISH

METHODOLOGY:



CHAPTER 4

RESULTS AND DISCUSSIONS

EFFECT OF TIME ON DISINFECTION:

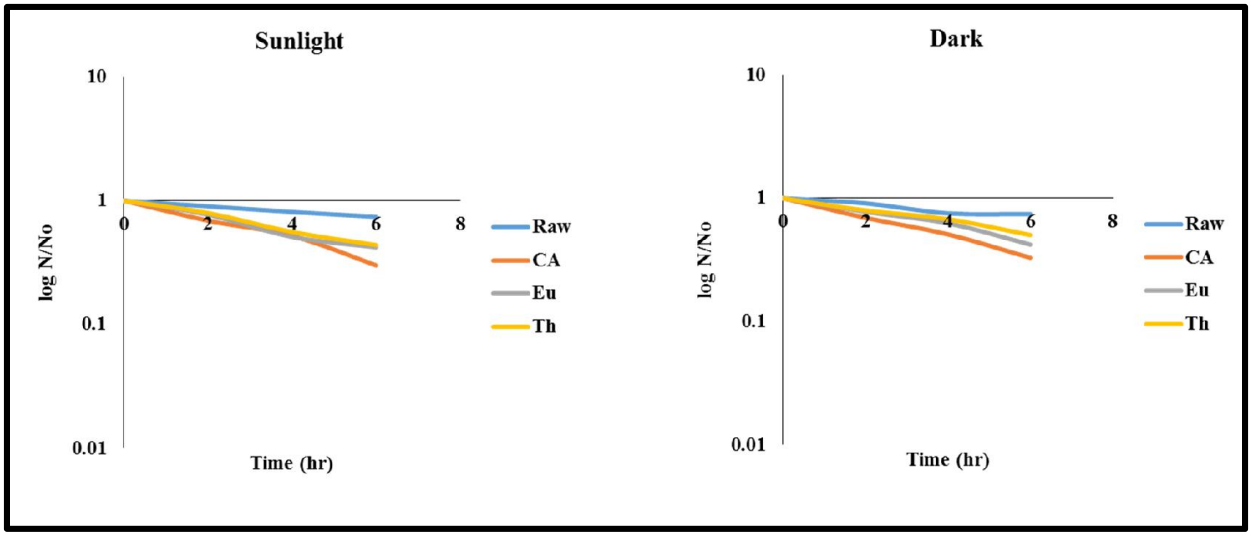


Figure 5: Effect of time on disinfection at chemical conc. of 100 ppm

Behavior of three phytochemicals i.e. Citric acid, euganol and thymol were studied which shows that citric acid has more pronounced disinfecting effect than others. Efficiency of disinfection almost double in the presence of sunlight as the intensity of ultraviolet radiations increases disinfection also increases. As the time of contact increases contamination load decreases.

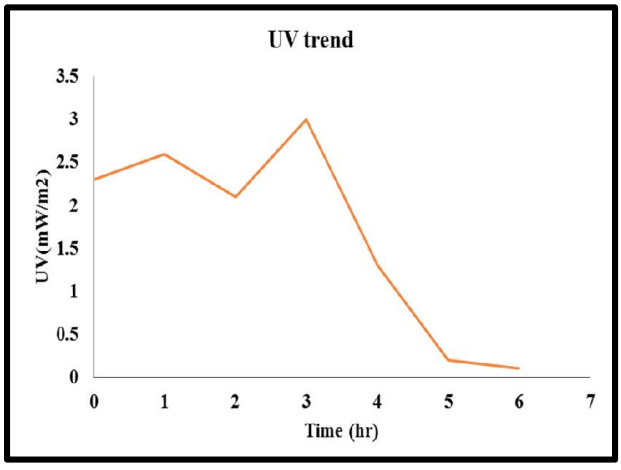


Figure 6: Intensity of light over a day

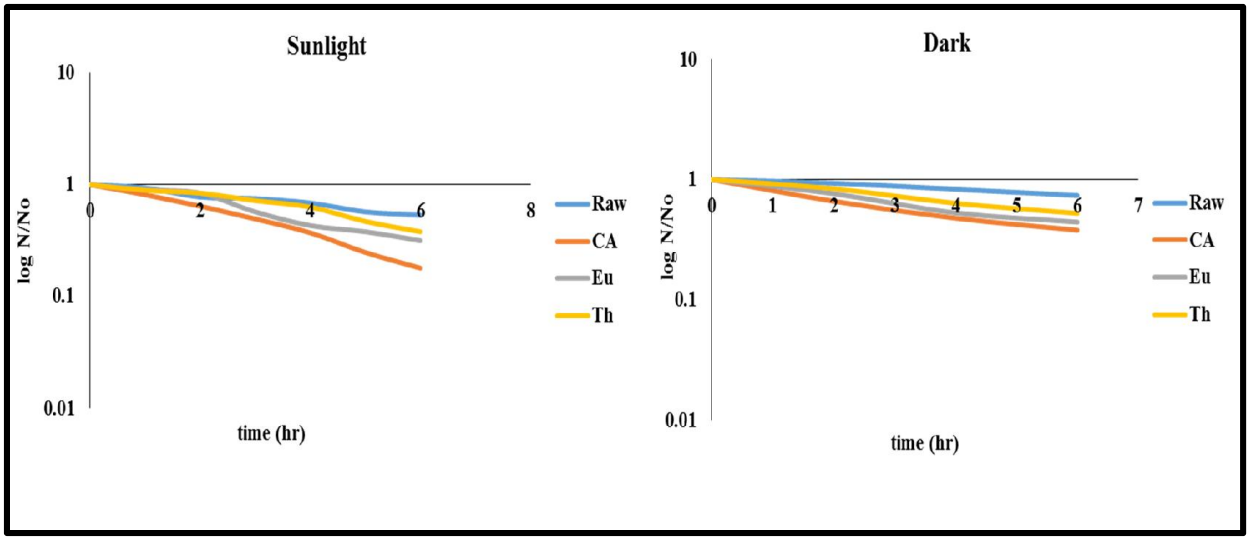


Figure 7: Effect of time on disinfection at chemical conc. of 75 ppm

As the concentration of phytochemicals decreases bacterial disinfection also decreases but we can get more pronounced results at low concentration in sunny day that has more ultraviolet radiations that are main source of solar disinfection. Almost same solar disinfection occurs because the intensity of ultraviolet radiations were almost same in both days.

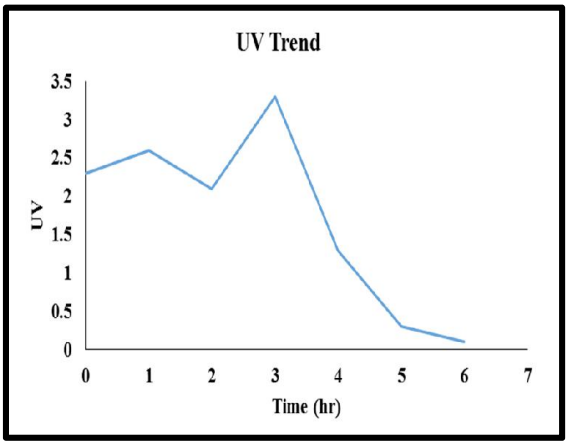


Figure 8: Intensity of light over a day

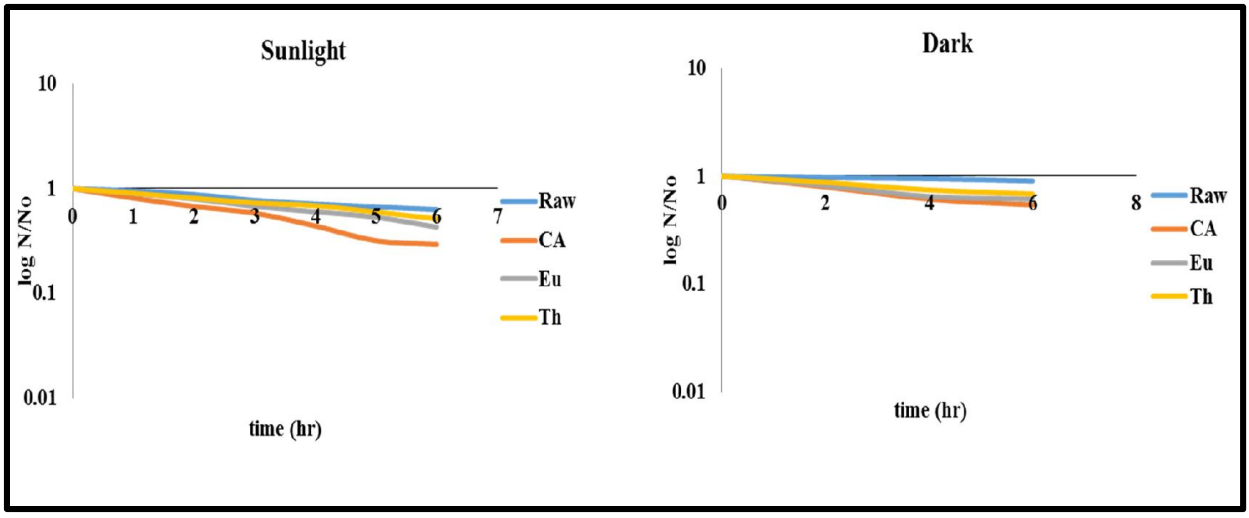


Figure 9: Effect of light on disinfection at chemical conc. of 50 ppm

In first two hours thymol and euganol had shown competitive behavior later on euganol had little bit more disinfection. Lower log reductions was achieved because intensity of sunlight was low when that experiment was performed.

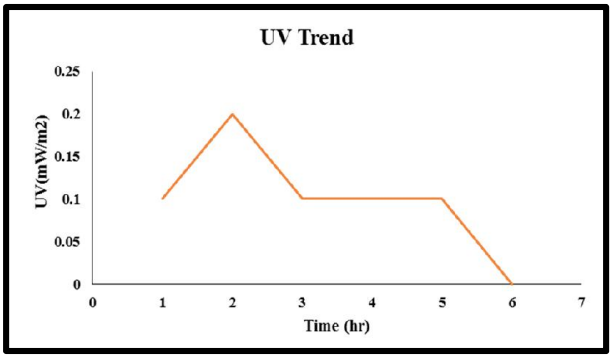


Figure 10: Intensity of light over a day

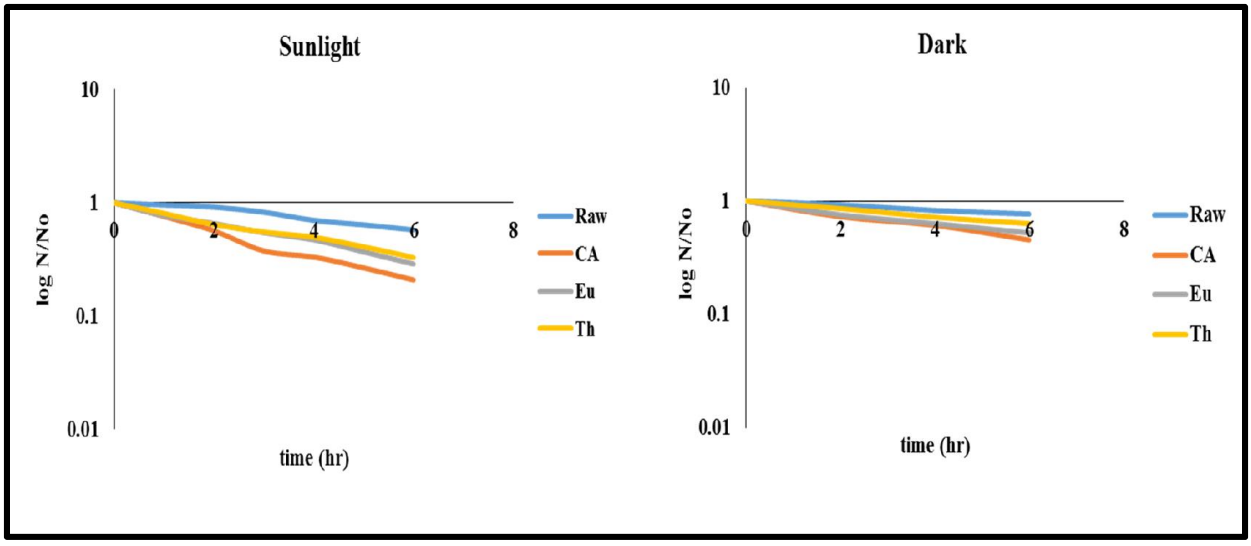


Figure 11: Effect of light on disinfection at chemical conc. of 25 ppm

At reduced concentration of phytochemicals euganol and thymol had shown approximately identical bacterial inactivation reason behind it is only uncontrolled factor that is sunlight. Concentration of phytochemicals were constant in all experimental runs but the thing which can only vary the disinfection results is solar light.

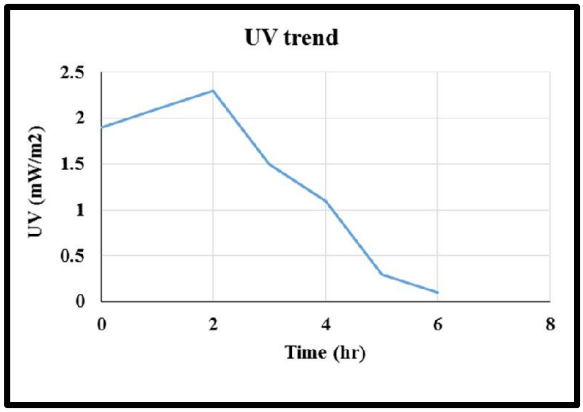


Figure 12: Intensity of Light over a day

COMPARISON OF DISINFECTION EFFICIENCY OF PHYTOCHEMICALS OVER A VARIOUS CONCENTRATION:

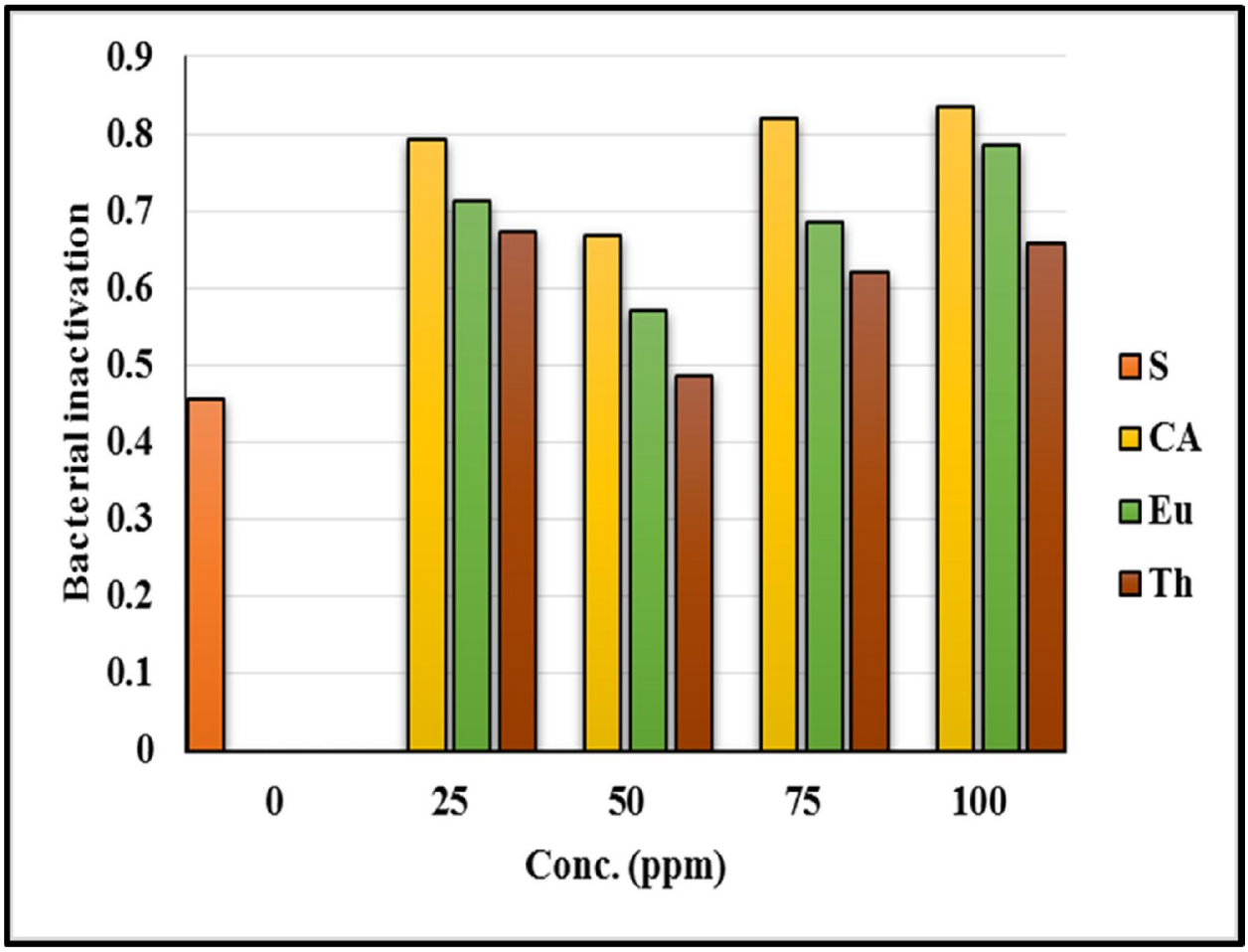


Figure 13: Comparison of disinfection efficacy in sunlight

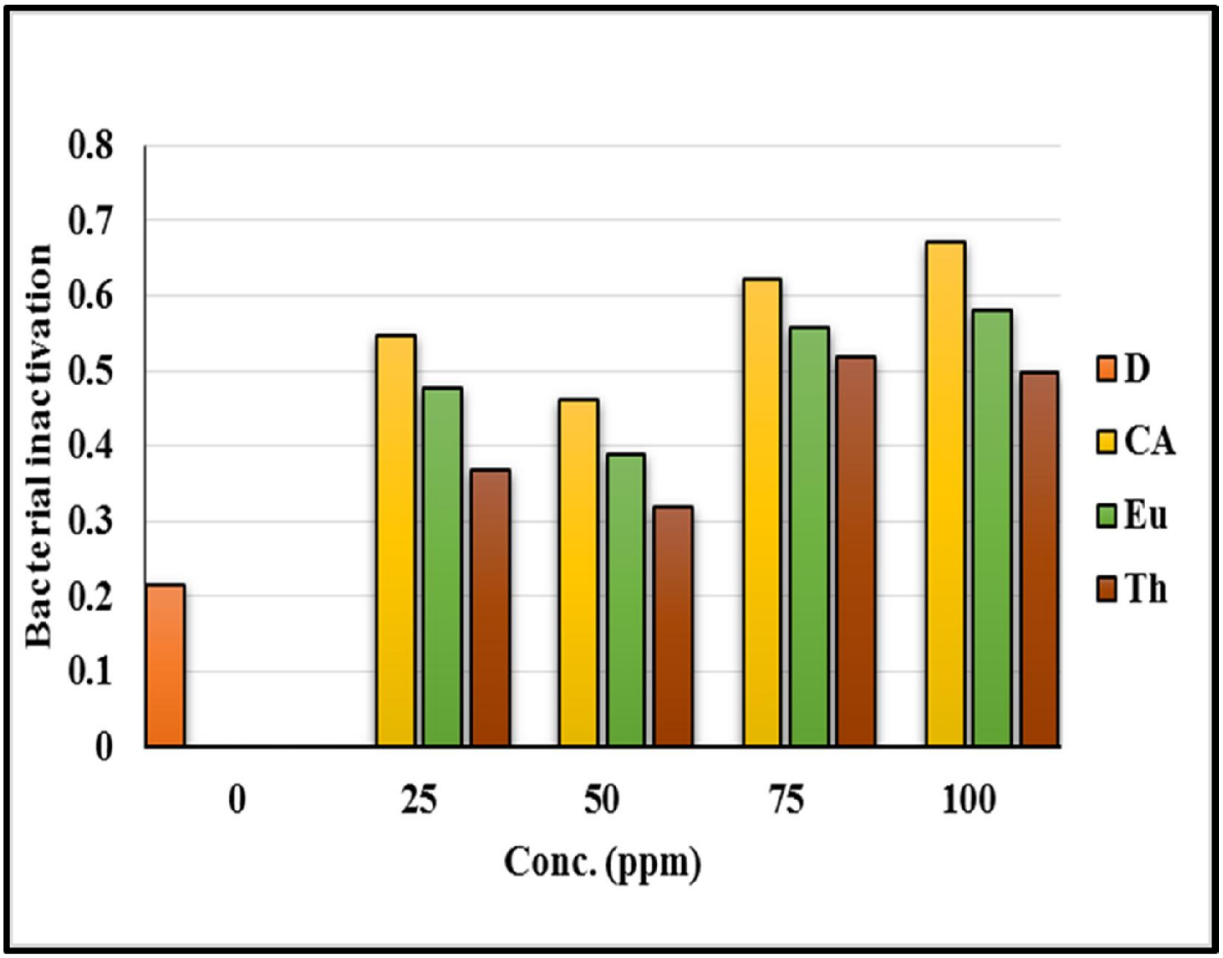


Figure 14: Comparison of disinfection efficacy

Nomenclature: CA = Citric acid, Eu = Euganol, Th = Thymol, D = Dark, S = Sunlight

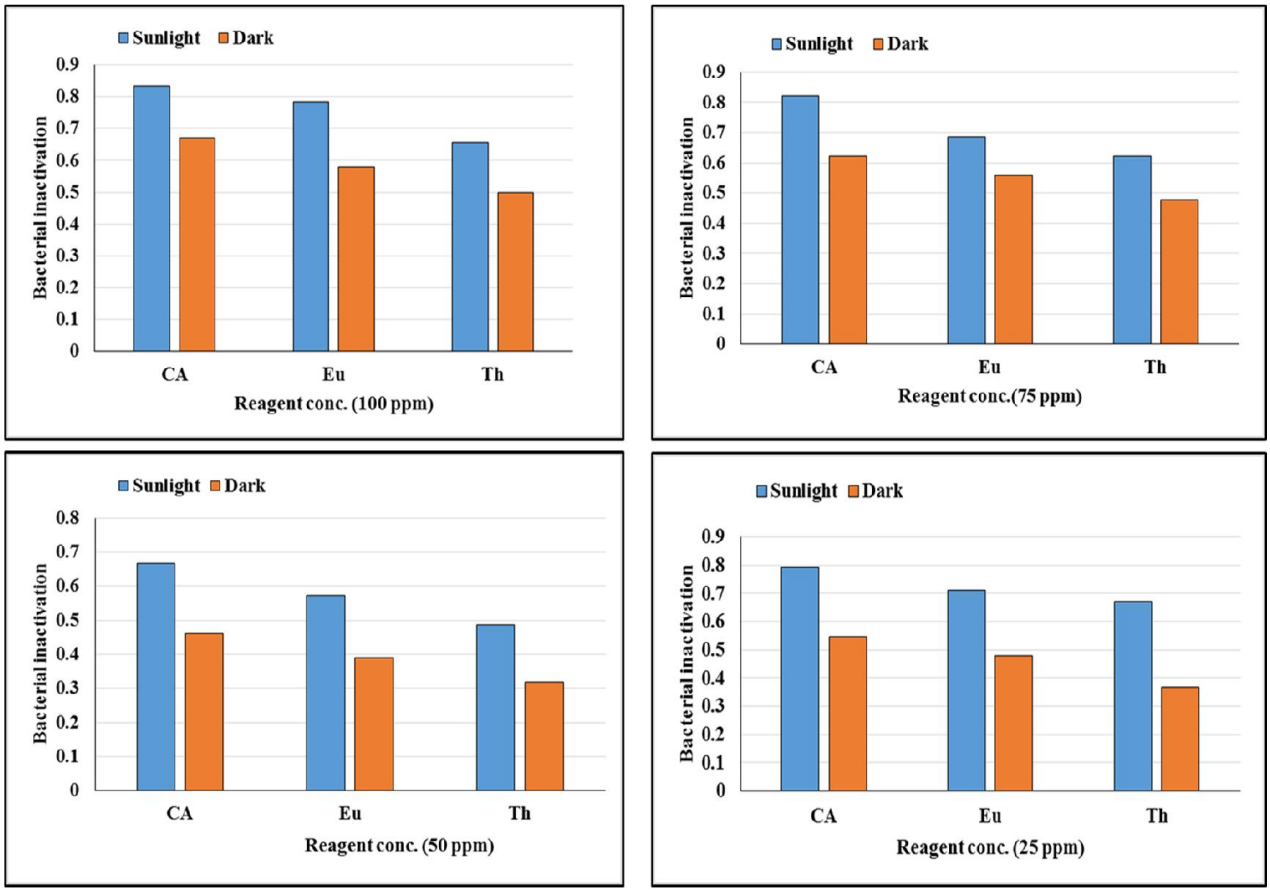


Figure 15: Effect of light on activity of phytochemical

*** V = 100 ml, C_{To} = 270 x 10^3 CFU**

MICROBIAL INACTIVATION KINETICS:

Kinetics of decontaminated water was studied by using following mathematical expression:

$$dNt/dt = -kNt \longrightarrow (1)$$

Where

dN_t/dt = rate of change in concentration of organisms with time

K = inactivation rate constant

N_t = number of organisms at time

By integrating eq (1)

$$lnNt/No = -kt \longrightarrow (2)$$

Where

N_t = Number of cells at zero time

N_o= Number of cells at time (t)

By rearranging equation (2), we get

$$K = 1/t\ (ln\ Nt/No)$$

Sunlight					Dark			
Specific rate constant for disinfection process								
Raw	conc.	CA	Eu	Th	Raw	CA	Eu	Th
0.09	25	0.17	0.2	0.19	0.05	0.13	0.11	0.08
0.07	50	0.27	0.13	0.11	0.02	0.11	0.09	0.07
0.11	75	0.27	0.19	0.14	0.05	0.17	0.14	0.12
0.14	100	0.28	0.23	0.19	0.06	0.18	0.13	0.11

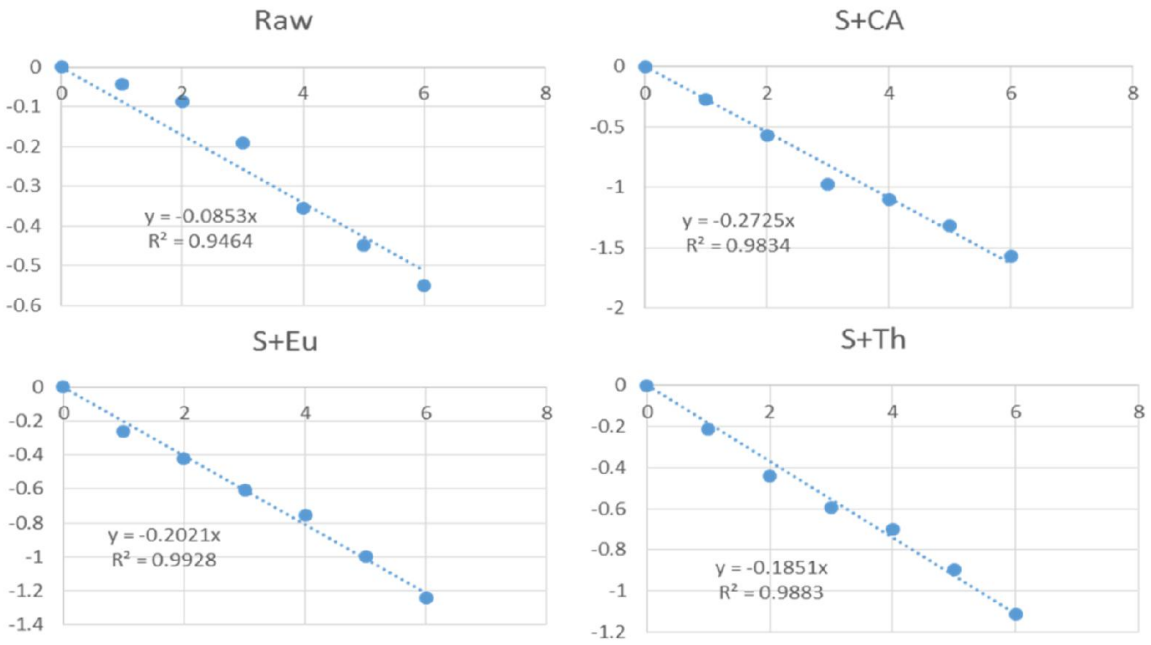


Figure 16: Microbial inactivation kinetics at phytochemical conc. of 25 ppm in sunlight

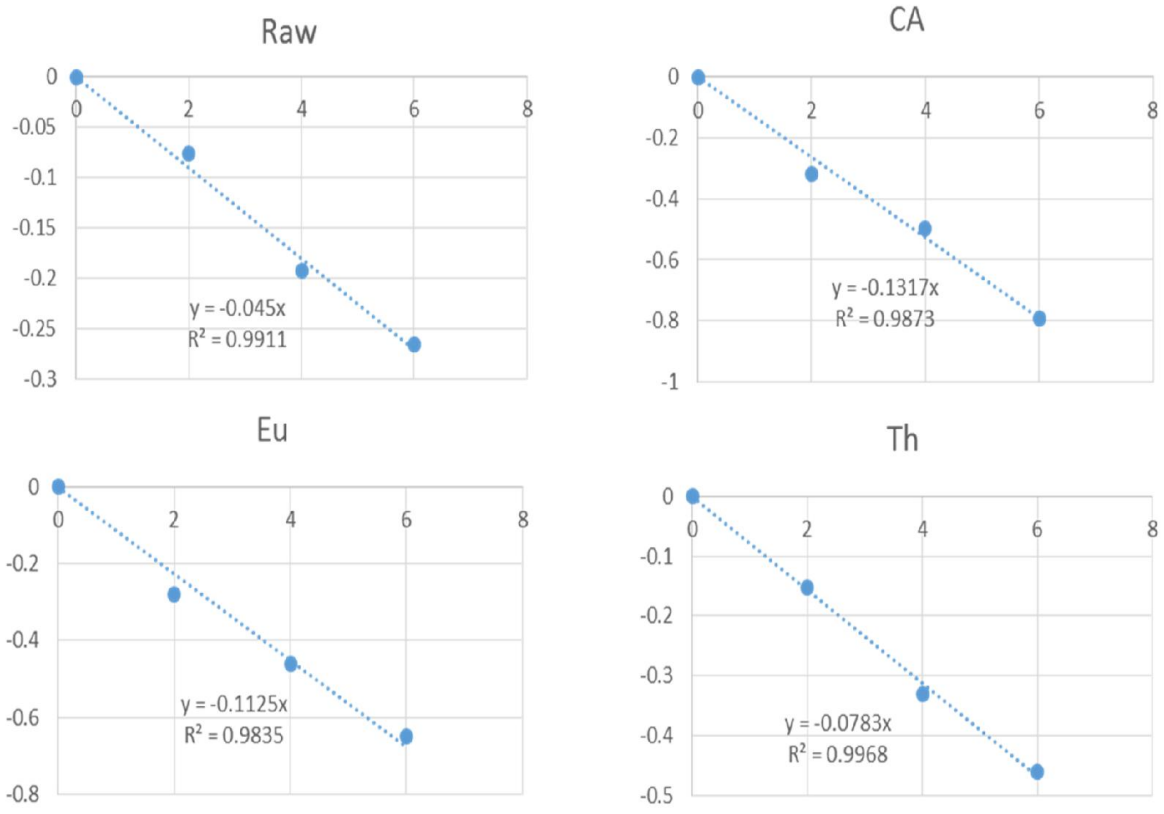


Figure 17: Microbial inactivation kinetics at phytochemical conc. of 25 ppm in dark

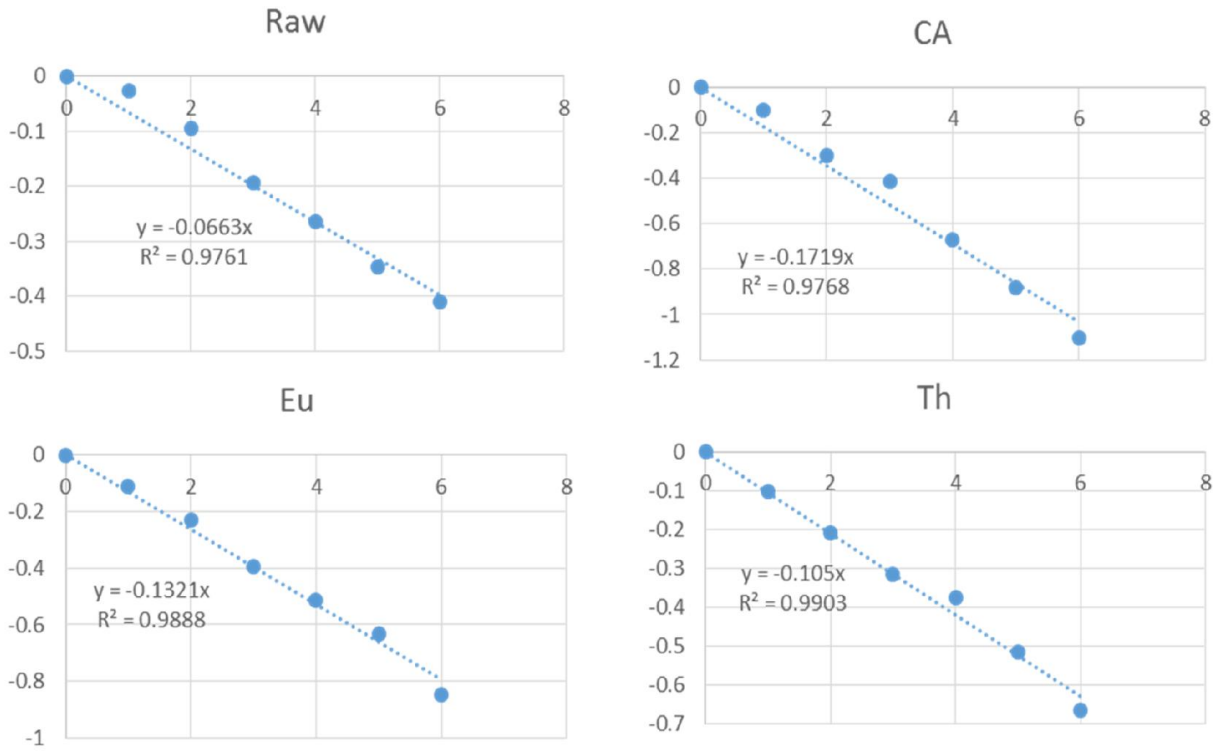


Figure 18: Microbial inactivation kinetics at phytochemical conc. of 50 ppm in sunlight

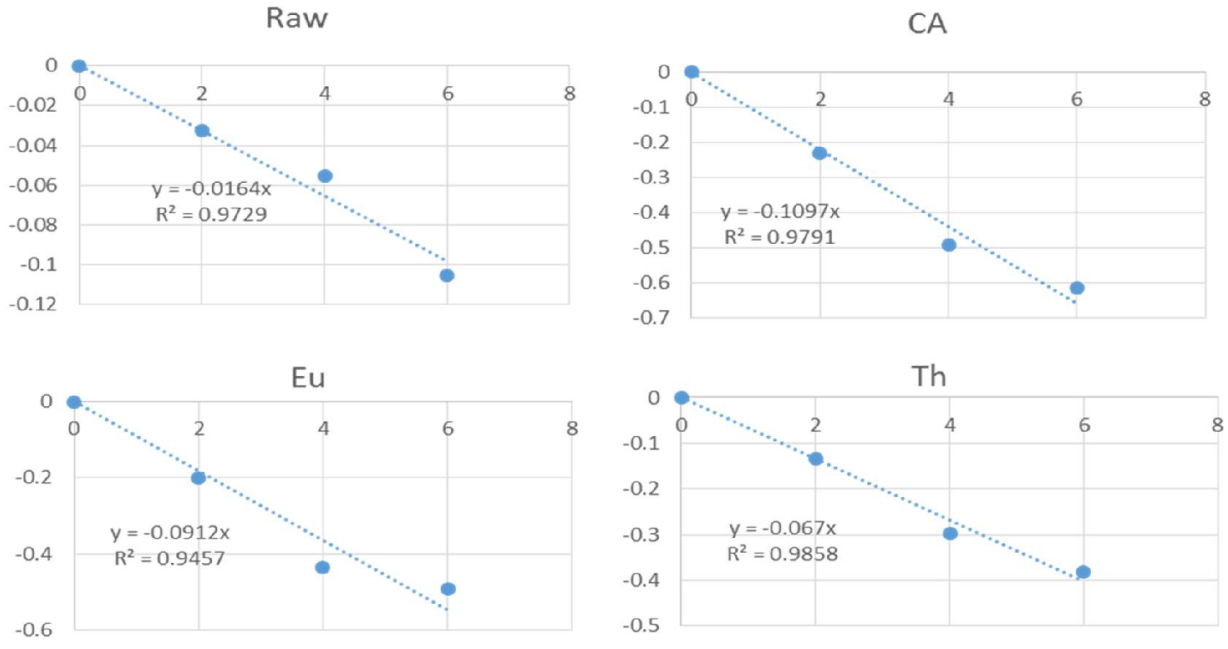


Figure 19: Microbial inactivation kinetics at phytochemical conc. of 50 ppm in dark

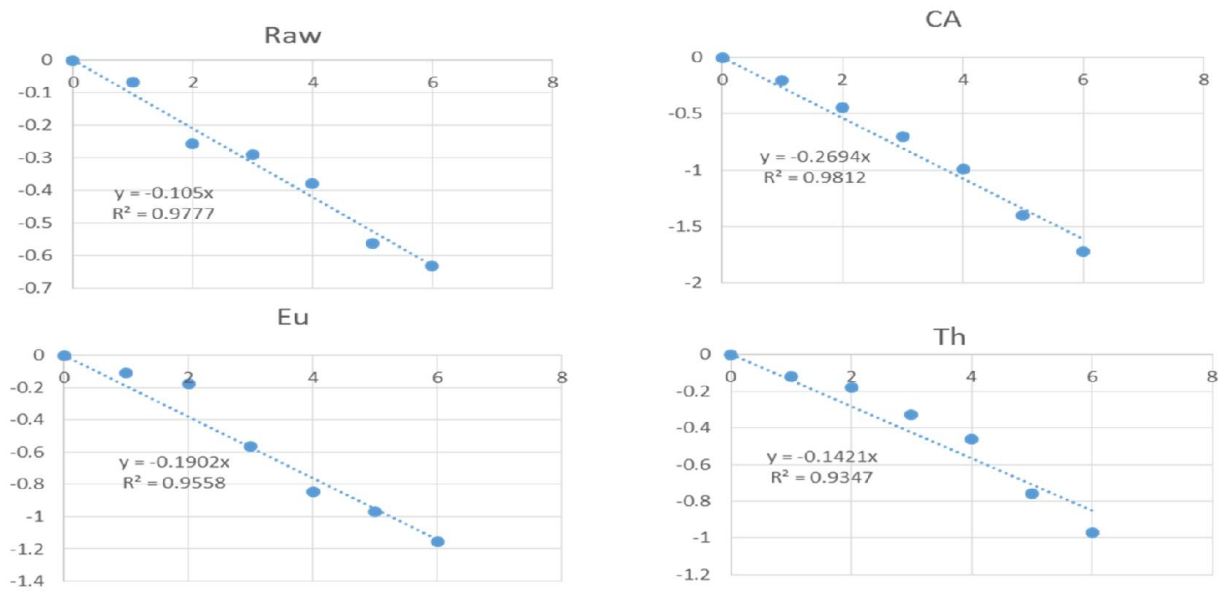


Figure 20: Microbial inactivation kinetics at phytochemical conc. of 75 ppm in sunlight

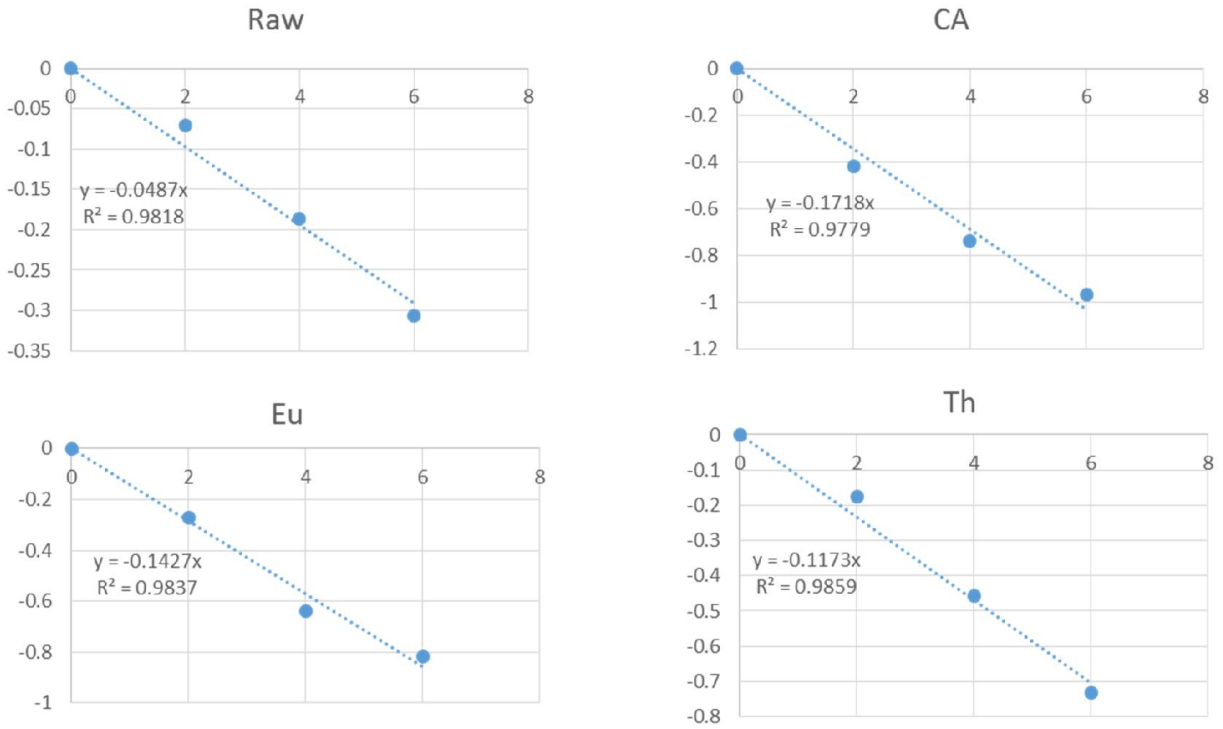


Figure 21: Microbial inactivation kinetics at phytochemical conc. of 75 ppm in dark

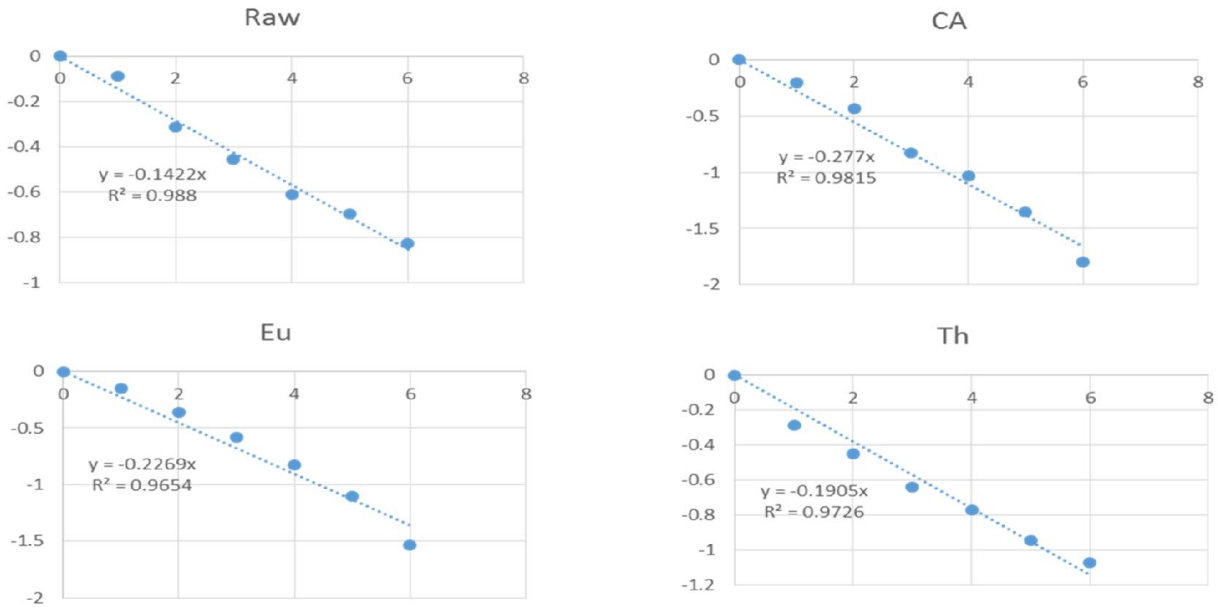


Figure 22: Microbial inactivation kinetics at phytochemical conc. of 100 ppm in sunlight

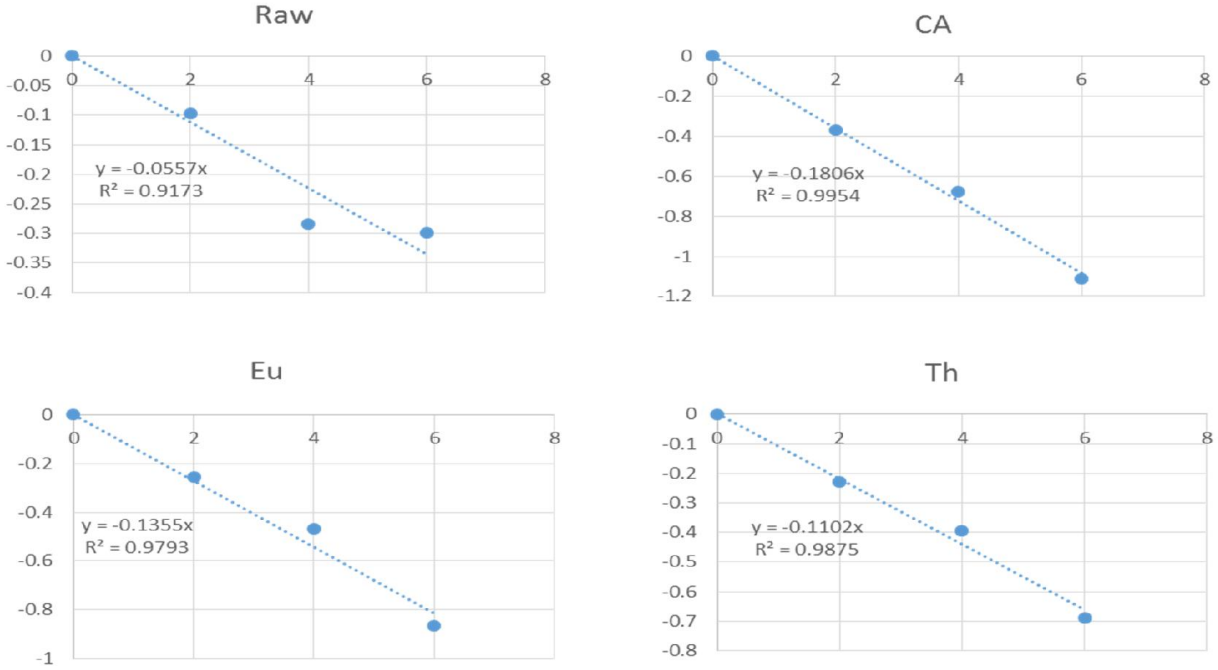
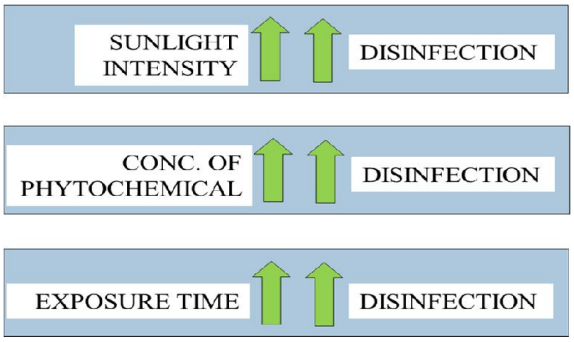


Figure 23: Microbial inactivation kinetics at phytochemical conc. of 100 ppm in dark

CHAPTER 5

CONCLUSIONS

- ✓ Efficacy of phytochemicals was pronounced in the presence of sunlight.
- ✓ Viability of heterotrophic bacteria declined with increase in exposure time whether the dark or sunlight.
- ✓ A direct relationship between the disinfection of heterotrophic bacteria and concentration of phytochemical was observed.
- ✓ Citric acid has showed more pronounced effect than other phytochemicals tested.
- ✓ Least disinfection effects was observed in case of Thymol.
- ✓ Process of disinfection is sophisticated in the presence of sunlight than in the dark.
- ✓ For all tested concentrations the citric acid is most superior to all others irrespective of UV light presence.
- ✓ UV light enhanced the disinfection efficiency
- ✓ Disruption of cell membrane (composed of peptidoglycan) enhanced as the concentration of phytochemicals increases
- ✓ Euganol is superior in its activity than thymol because it binds the active sites in most efficient manner.



APPENDIX

Disinfecting chemical concentration= 25 ppm

Time (hr)	Temperature(℃)	Dark	N/No	D+CA	N/No	D+EU	N/No	D+TH	N/No
0	18	274	1	274	1	274	1	274	1
2	25	254	0.92701	199	0.72628	207	0.75547	235	0.85766
4	27	226	0.82482	167	0.60949	173	0.63139	197	0.71898
6	27	210	0.76642	124	0.45255	143	0.5219	173	0.63139

Exposure time (hr)	Temperature(℃)	UV(mW/m2)	Sunlight	N/No	S+CA	N/No	S+Eu	N/No	S+Th	N/No
0	18	1.9	274	1	274	1	274	1	274	1
1	21	2.1	262	0.9562	208	0.75912	211	0.77007	221	0.80657
2	30	2.3	251	0.91606	155	0.56569	180	0.65693	176	0.64234
3	33	1.5	226	0.82482	103	0.37591	149	0.5438	151	0.55109
4	34	1.1	192	0.70073	91	0.33212	129	0.4708	136	0.49635
5	33	0.3	175	0.63869	73	0.26642	101	0.36861	112	0.40876
6	29	0.1	158	0.57664	57	0.20803	79	0.28832	90	0.32847

Disinfecting chemical concentration= 50 ppm

Exposure time (hr)	Temperature(℃)	UV(mW/m2)	Sunlight	N/No	S+CA	N/No	S+Eu	N/No	S+Th	N/No
0	25	0.1	280	1	280	1	280	1	280	1
1	26	0.1	273	0.975	253	0.903571	251	0.896429	253	0.903571
2	27	0.2	255	0.910714	208	0.742857	223	0.796429	227	0.810714
3	28	0.1	231	0.825	185	0.660714	189	0.675	204	0.728571
4	29	0.1	215	0.767857	143	0.510714	168	0.6	192	0.685714
5	29	0.1	198	0.707143	116	0.414286	149	0.532143	167	0.596429
6	28	0	186	0.664286	93	0.332143	120	0.428571	144	0.514286

Time (hr)	Temperature(℃)	Dark	N/No	D+CA	N/No	D+EU	N/No	D+TH	N/No
0	25	280	1	280	1	280	1	280	1
2	26	271	0.967857	222	0.792857	229	0.817857	245	0.875
4	27	265	0.946429	171	0.610714	181	0.646429	208	0.742857
6	27	252	0.9	151	0.539286	171	0.610714	191	0.682143

Disinfecting chemical concentration= 75 ppm

Exposure time (hr)	Temperature(℃)	UV(mW/m 2)	Sunlight	N/No	S+CA	N/No	S+Eu	N/No	S+Th	N/No
0	31	2.3	235	1	235	1	235	1	235	1
1	37	2.6	220	0.93617	191	0.812766	211	0.897872	209	0.889362
2	41	2.1	182	0.774468	151	0.642553	197	0.838298	197	0.838298
3	43	3.3	176	0.748936	116	0.493617	134	0.570213	169	0.719149
4	43	1.3	161	0.685106	87	0.370213	101	0.429787	148	0.629787
5	42	0.3	134	0.570213	58	0.246809	89	0.378723	110	0.468085
6	39	0.1	125	0.531915	42	0.178723	74	0.314894	89	0.378723

Time	Dark	N/No	D+CA	N/No	D+EU	N/No	D+TH	N/No
0	235	1	235	1	235	1	235	1
2	219	0.931915	155	0.659574468	179	0.761702	197	0.838298
4	195	0.829787	112	0.476595745	124	0.52766	149	0.634043
6	173	0.73617	89	0.378723404	104	0.442553	123	0.523404

Disinfecting chemical concentration = 100 ppm

Exposure time (hr)	Temperature(˚C)	UV(mW/m2)	Sunlight	N/No	S+CA	N/No	S+Eu	N/No	S+Th	N/No
0	31	2.3	283	1	283	1	283	1	283	1
1	37	2.6	259	0.915194	232	0.819788	243	0.858657	213	0.75265
2	41	2.1	207	0.731449	184	0.650177	197	0.696113	181	0.639576
3	43	3	180	0.636042	124	0.438163	158	0.558304	149	0.526502
4	43	1.3	154	0.54417	101	0.35689	124	0.438163	131	0.462898
5	42	0.2	141	0.498233	73	0.257951	94	0.332155	110	0.388693
6	39	0.1	124	0.438163	47	0.166078	61	0.215548	97	0.342756

Time (hr)	Temperature(˚C)	Dark	N/No	D+CA	N/No	D+EU	N/No	D+TH	N/No
0	31	283	1	283	1	283	1	283	1
2	36	257	0.908127	196	0.69258	219	0.773852	225	0.795053
4	39	213	0.75265	144	0.508834	177	0.625442	191	0.674912
6	39	210	0.742049	93	0.328622	119	0.420495	142	0.501767

REFRENCES:

[1] S. P. Pathak and K. Gopal, “Evaluation of bactericidal efficacy of silver ions on Escherichia coli for drinking water disinfection,” *Environ. Sci. Pollut. Res.*, vol. 19, no. 6, pp. 2285–2290, 2012.

[2] J. Colreavy *et al.*, “Applied Catalysis B : Environmental Nitrogen and copper doped solar light active TiO 2 photocatalysts for water decontamination,” vol. 131, pp. 8–13, 2013.

[3] T. Clasen and P. Edmondson, “Sodium dichloroisocyanurate (NaDCC) tablets as an alternative to sodium hypochlorite for the routine treatment of drinking water at the household level,” vol. 209, pp. 173–181, 2006.

[4] P. Health and E. Water, “Public Health and Environment Water, Sanitation and Health.”

[5] A. S. Harding and K. J. Schwab, “Using Limes and Synthetic Psoralens to Enhance Solar Disinfection of Water (SODIS): A Laboratory Evaluation with Norovirus , Escherichia coli , and MS2,” vol. 86, no. 4, pp. 566–572, 2012.

[6] M. B. Keogh *et al.*, “Capability of 19-L polycarbonate plastic water cooler containers for efficient solar water disinfection (SODIS): Field case studies in India, Bahrain and Spain,” *Sol. Energy*, vol. 116, pp. 1–11, 2015.

[7] W. Heaselgrave and S. Kilvington, “Antimicrobial activity of simulated solar disinfection against bacterial, fungal, and protozoan pathogens and its enhancement by riboflavin,” *Appl. Environ. Microbiol.*, vol. 76, no. 17, pp. 6010–6012, 2010.

[8] P. S. Harikumar and C. M. Manjusha, “Study on the Antibacterial activity of selected Natural Herbs and its application in Water Treatment,” pp. 1–14.

[9] G. P. Winward, L. M. Avery, T. Stephenson, and B. Jefferson, “Essential oils for the disinfection of grey water,” *Water Res.*, vol. 42, no. 8–9, pp. 2260–2268, 2008.

[10] P. O. F. Household, W. Treatment, S. In, and U. Wash, “No Title,” no. January, 2008.

[11] T. Clasen, L. Haller, D. Walker, J. Bartram, and S. Cairncross, “Cost-effectiveness of water quality interventions for preventing diarrhoeal disease in developing countries,” pp. 599–609, 2007.

[12] G. Rosa and T. Clasen, “Estimating the Scope of Household Water Treatment in Low- and Medium-Income Countries,” vol. 82, no. 2, pp. 289–300, 2010.

[13] J. A. Byrne, P. A. Fernandez-iba, P. S. M. Dunlop, D. M. A. Alrousan, and J. W. J. Hamilton, “Photocatalytic Enhancement for Solar Disinfection of Water : A Review,” vol. 2011, 2015.

[14] P. Schmid, M. Kohler, R. Meierhofer, S. Luzi, and M. Wegelin, “Does the reuse of PET

bottles during solar water disinfection pose a health risk due to the migration of plasticisers and other chemicals into the water ?," *Water Res.*, vol. 42, no. 20, pp. 5054–5060, 2008.

[15] K. G. McGuigan, R. M. Conroy, H. J. Mosler, M. du Preez, E. Ubomba-Jaswa, and P. Fernandez-Ibañez, "Solar water disinfection (SODIS): A review from bench-top to roof-top," *J. Hazard. Mater.*, vol. 235–236, pp. 29–46, 2012.

[16] W. S. Duff and D. A. Hodgson, "A simple high efficiency solar water purification system," vol. 79, pp. 25–32, 2005.

[17] N. Amanidaz, A. Zafarzadeh, and A. H. Mahvi, "The Interaction between Heterotrophic Bacteria and Coliform, Fecal Coliform, Fecal Streptococci Bacteria in the Water Supply Networks," *Iran J Public Heal.*, vol. 44, no. 12, pp. 1685–1692, 2015.

[18] M. . Davoudi, T. . Vakili, A. . Absalan, M. H. . Ehrampoush, and M. T. . Ghaneian, "Antibacterial effects of Hydrogen peroxide and silver composition on selected pathogenic enterobacteria," *Middle East J. Sci. Res.*, vol. 13, no. 6, pp. 710–715, 2013.

[19] T. Medicine, "Water Quality Interventions to Prevent Diarrhoea : Cost and Cost-Effectiveness."

[20] M. Boyle, C. Sichel, and P. Ferna, "Bactericidal Effect of Solar Water Disinfection under Real Sunlight Conditions ," vol. 74, no. 10, pp. 2997–3001, 2008.

[21] R. H. Reed, "Solar inactivation of faecal bacteria in water : the critical role of oxygen," pp. 276–280, 1997.

[22] P. M. Oates, P. Shanahan, and M. F. Polz, "Solar disinfection (SODIS): simulation of solar radiation for global assessment and application for point-of-use water treatment in Haiti," vol. 37, pp. 47–54, 2003.

[23] J. Herrmann, "Heterogeneous photocatalysis : fundamentals and applications to the removal of various types of aqueous pollutants," vol. 53, pp. 115–129, 1999.

[24] A. Hamamoto *et al.*, "New water disinfection system using UVA light-emitting diodes," vol. 103, pp. 2291–2298, 2007.

[25] J. M. C. Robertson, P. K. J. Robertson, and L. A. Lawton, "A comparison of the effectiveness of TiO2 photocatalysis and UVA photolysis for the destruction of three pathogenic micro-organisms," *J. Photochem. Photobiol. A Chem.*, vol. 175, no. 1, pp. 51–56, 2005.

[26] S. Pigeot-R??my, F. Simonet, D. Atlan, J. C. Lazzaroni, and C. Guillard, "Bactericidal efficiency and mode of action: A comparative study of photochemistry and photocatalysis," *Water Res.*, vol. 46, no. 10, pp. 3208–3218, 2012.

[27] F. Bosshard, M. Bucheli, Y. Meur, and T. Egli, "The respiratory chain is the cell's

Achilles' heel during UVA inactivation in Escherichia coli," *Microbiology*, vol. 156, no. 7, pp. 2006–2015, 2010.

[28] M. B. Fisher, C. R. Keenan, K. L. Nelson, and B. M. Voelker, "Speeding up solar disinfection (SODIS): effects of hydrogen peroxide , temperature , pH , and copper plus ascorbate on the photoinactivation of E . coli," 2008.

[29] D. Spuhler, J. A. Rengifo-herrera, D. Spuhler, and J. Andre, "The effect of Fe2 + , Fe3 + , H2O2 and the photo- Fenton reagent at near neutral pH on the solar disinfection (SODIS) at low temperatures of water containing Escherichia coli K12 Applied Catalysis B : Environmental," no. January 2016, 2010.

[30] F. Sciacca, J. A. Rengifo-herrera, J. Wéthé, and C. Pulgarin, "Chemosphere Dramatic enhancement of solar disinfection (SODIS) of wild Salmonella sp . in PET bottles by H 2 O 2 addition on natural water of Burkina Faso containing dissolved iron," *Chemosphere*, vol. 78, no. 9, pp. 1186–1191, 2010.

[31] K. Reyes, M. T. Leal, S. G. Ã, and L. A. Go, "A practical demonstration of water disinfection using TiO 2 films and sunlight," vol. 40, pp. 3274–3280, 2006.

[32] S. Dukan, "TiO 2 Photocatalysis Causes DNA Damage via Fenton Reaction-Generated Hydroxyl Radicals during the Recovery Period ," vol. 73, no. 23, pp. 7740–7743, 2007.

[33] W. Heaselgrave and S. Kilvington, "Antimicrobial Activity of Simulated Solar Disinfection against Bacterial , Fungal , and Protozoan Pathogens and Its Enhancement by Riboflavin ," vol. 76, no. 17, pp. 6010–6012, 2010.

[34] M. Berney, H. Weilenmann, A. Simonetti, and T. Egli, "Efficacy of solar disinfection of Escherichia coli , Shigella flexneri , Salmonella Typhimurium and Vibrio cholerae," vol. 101, pp. 828–836, 2006.

[35] K. G. Mcguigan, T. M. Joyce, R. M. Conroy, and J. B. Gillespie, "Solar disinfection of drinking water contained in transparent plastic bottles : characterizing the bacterial inactivation process," pp. 1138–1148, 1998.

[36] D. Alarco, J. Blanco, S. Malato, and P. Ferna, "Renewable and Sustainable Energy Reviews," vol. 13, pp. 1437–1445, 2009.

[37] R. A. Blaustein, Y. Pachepsky, R. L. Hill, D. R. Shelton, and G. Whelan, "Escherichia coli survival in waters : Temperature dependence," *Water Res.*, vol. 47, no. 2, pp. 569–578, 2012.

[38] C. Pulgarin, "Journal of Photochemistry and Photobiology A : Chemistry The antagonistic and synergistic effects of temperature during solar disinfection of synthetic secondary effluent Adjusted Sum of Squares," vol. 280, pp. 14–26, 2014.

[39] N. W. Dahl, P. L. Woodfield, C. J. Lemckert, H. Stratton, and A. Roiko, "A practical model for sunlight disinfection of a subtropical maturation pond," *Water Res.*, vol. 108,

pp. 151–159, 2017.

[40] A. P. Schuch, C. Frederico, and M. Menck, “Journal of Photochemistry and Photobiology B : Biology The genotoxic effects of DNA lesions induced by artificial UV-radiation and sunlight,” *J. Photochem. Photobiol. B Biol.*, vol. 99, no. 3, pp. 111–116, 2010.

[41] K. Kadir and K. L. Nelson, “ScienceDirect Sunlight mediated inactivation mechanisms of Enterococcus faecalis and Escherichia coli in clear water versus waste stabilization pond water,” *Water Res.*, vol. 50, pp. 307–317, 2013.

[42] B. Sciences, “The Inactivation of Microbes by Sunlight : Solar Disinfection as a Water Treatment Process,” vol. 54, 2004.

[43] S. Waste, *SODIS manual*. .

[44] J. M. Monteagudo, A. Durán, I. S. Martín, and A. M. Acevedo, “A novel combined solar pasteurizer/TiO2continuous-flow reactor for decontamination and disinfection of drinking water,” *Chemosphere*, vol. 168, pp. 1447–1456, 2017.

[45] D. A. Keane *et al.*, “Solar photocatalysis for water disinfection: materials and reactor design,” *Catal. Sci. Technol.*, vol. 4, no. 5, pp. 1211–1226, 2014.

[46] S. Malato, P. Fernández-Ibáñez, M. I. Maldonado, J. Blanco, and W. Gernjak, “Decontamination and disinfection of water by solar photocatalysis: Recent overview and trends,” *Catal. Today*, vol. 147, no. 1, pp. 1–59, 2009.

[47] M. K. Swamy, M. S. Akhtar, and U. R. Sinniah, “Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review,” vol. 2016, 2016.

[48] J. Sikkema, J. A. de Bont, and B. Poolman, “Mechanisms of membrane toxicity of hydrocarbons,” *Microbiol Rev*, vol. 59, no. 2, pp. 201–222, 1995.

[49] M. M. Cowan, “Plant Products as Antimicrobial Agents,” vol. 12, no. 4, pp. 564–582, 1999.

[50] J. S. Raut and S. M. Karuppayil, “A status review on the medicinal properties of essential oils,” *Ind. Crops Prod.*, vol. 62, pp. 250–264, 2014.

[51] N. Y. Saad, C. D. Muller, and A. Lobstein, “Major bioactivities and mechanism of action of essential oils and their components,” *Flavour Fragr. J.*, vol. 28, no. 5, pp. 269–279, 2013.

[52] M. H. Gracia-Valenzuela, C. Orozco-Medina, and C. Molina-Maldonado, “Efecto antibacteriano del aceite esencial de orégano (Lippia berlandieri) en bacterias patógenas de camarón Litopenaeus vannamei,” *Hidrobiologica*, vol. 22, no. 3, pp. 201–206, 2012.