ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Comparative effect of different visible light energy on bacterial growth

Fouad Hussein Kamel[1], Chiman Hameed Saeed[2], Nawroz Ismael Hassan[1].

1. Medical Technical Institute, Erbil Polytechnic University, Erbil, Iraq.
2. Medical Research Centre, Hawler Medical University, Erbil, Iraq.

Manuscript Info

Manuscript History:

Received: 14 December 2015
Final Accepted: 25 January 2016
Published Online: February 2016

Key words:
Bacteria; growth; visible light.

Corresponding Author

Fouad Hussein Kamel.

Abstract

The purpose of this work was to examine the influence of non-coherent polarized light upon growth of bacteria (*Staph. aureus*, *E. coli*, *Pseudomonas aeruginosa*), and develop attractive approach by reduce incubation time in medical diagnosis laboratory. Experiments were designed to test the main hypothesis that this kind of polychromatic light can produce decimal reductions in numbers of bacteria. The survival of bacterial cells was monitored by Optical density (O.D.), cell number per milliter and colony forming units (CFU) before and after exposure of the suspended bacteria to visible light. Bacterial cultures were exposed to light for different periods (15, 30, 45, 60, 90 and 120 minutes). The source of non-coherent polarized light was NARVA-lamp (Germany) with the following technical characteristics: wavelength 400–2000 nm and light intensity 82.14 w/m^2.

Results for all strains showed decrease at f the first 15 min. more over with use of blue later red color filter. While slightly increase in bacterial count in response to an irradiation time of 30, 45, 60, 90, 120 min., moreover with cool white and green. Bacterial counts after treatments displayed a linear relationship with the total count of bacteria before treatments as well as the percentage surviving bacteria and irradiation time. In green and white color were found to get more bacterial cell or less phototoxic effect, while the cell number significantly higher than starting number. That's mean improve growth rate during the incubation of patient samples, exactly in case of low growing bacteria

Introduction:-

Solar radiation is one of the most important factors affecting the survival bacteria in aquatic environments [1]. Many studies have shown that hazardous effect of light on bacteria is further increased by light sensitive molecules known as photosensitizers [2]. Khaengraeng and Reed [3] in their study suggested that the sub-lethal damage to bacterial cells caused by light leads to an reactive oxygen species (ROS) sensitive state, since it imposes an additional stress on these bacteria. Idil and his Colleges [4] reported that red light has effect alone in seawater and it was found that cell population decreased by 99% in the effect of red light and he added that this finding revealed that red light was the most effect among visible wavelength and it was effect as UV. Few cases were reported that the activity of blue-light sensors could be correlated to infectivity and/or has been shown to be involved in the activation of specific genes, resulting in selective growth patterns [5, 6].

The clinical microbiology laboratory is tried to test specimens from patients for microorganisms that are, or may be, a cause of the illness and to provide information about the in vitro activity of antimicrobial drugs against the microorganisms identified after incubation of culture media 24 hours or more [7]. Thus, there is an urgent need to search alternative technique for combating pathogenic bacteria [8].

Aim of study: The purpose of this work was to examine the influence of non-coherent polarized light upon the growth of bacteria (*Staph. aureus, E. coli, Pseudomonas aeruginosa),* and develop attractive approach by reduce incubation time in medical diagnosis laboratory .

Materials and Methods:-

Non- coherent polarized light source with standard color glass filter of blue (470 nm), green (510nm), yellow (570 nm), red (650nm) wave length, directly light source (white) and without light as a control were used. All color glass filter were placed at equal distance (12 cm) from an illuminated light NARVA-lamp (cool white 220-240 Germany) parallel to the color filter inside the incubator.

Tryptic soya broth media (Oxide) were inoculated with overnight grown culture of standard strain of *E. coli* (ATCC 35218), *Staph.aureus* (ATCC25923*)* and *Pseudomonas aeruginosa* (ATCC 19615) that adjusted to 0.5 McFarland.

The experiments were carrying out in 5ml in glass bacterial tubes, every sample in experiments were prepared in three parallels. Tubes were incubating at 37 °C for 2 hrs under exposure of light source and without it. The growth of *E. coli, Staph.aureus, Pseudomonas aeruginosa* were quantify spectrophotometrically at 600 nm after (15, 30,45,60,90 and 120) min. The standard plate count method was used to determine the total number of viable cells of *E. coli*, *Staph.aureus* and *Pseudomonas aeruginosa* as the colony forming units (CFU) on nutrient agar media after incubation at 37°C for 24hrs.

Results:-

The survival of bacterial cells following irradiation was monitored by plate count before and after exposure of the suspended bacteria to light. This was performed by counting the number of colony forming units (CFU) on Nutrient agar plates and calculating their number per ml.

Table (1), Figures (1A&1B) appeared the effect of different wavelength of visible light on bacterial cell number and its colony forming unit of *S.aureus*. at a different time . Generally the bacterial cell number were decreased within the first 15mint of incubation since the higher decrease shown at Red Color ($2.32x10^7$) compare to starting number ($1.5x10^8$) later the blue color ($3.44x10^7$) and Green color ($4.64x10^7$). While the same effect presented in uses of Yellow, and White color ($4.56x10^7$), but the darkness condition showed the lower effect on bacterial cell number ($4.88x10^7$).

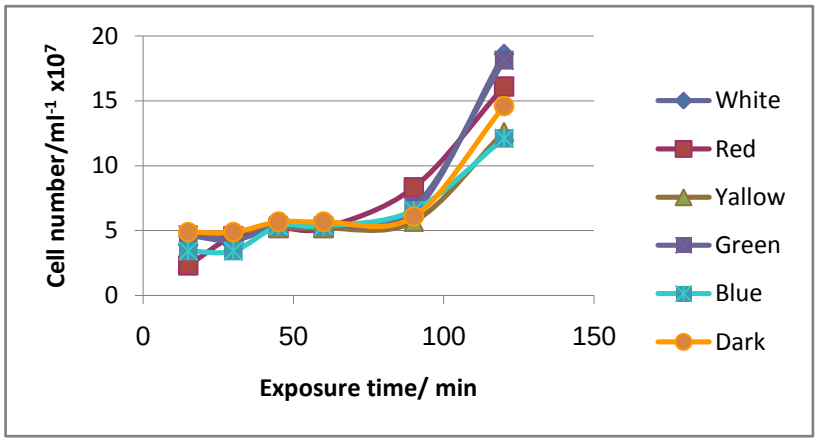
The same table also showed an increase in bacterial cell number with incubation for longer time (15, 30, 45, 60, 90,120 min.), comparing with bacterial number before exposure or incubation.

At the time (120 min) the Bacterial cell number raised more than the begging number only and using a green and white color comparing to other condition may be due to lower effect of both used color (green and white). Similar effect shown on colony forming unit and O.D. of *Staph. aureus* after exposure.

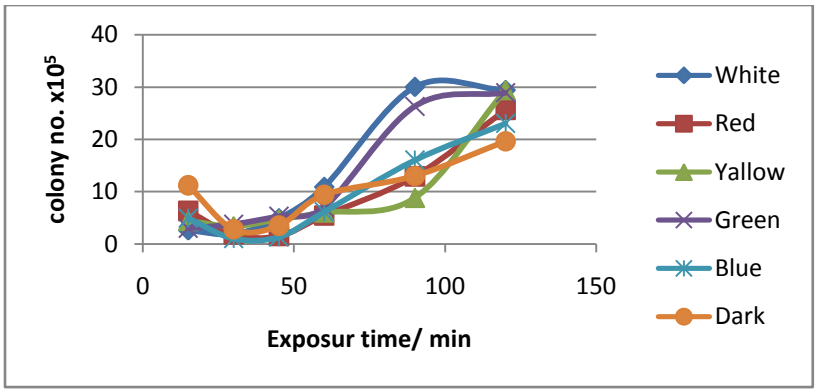
Table (1) Effects of different light wavelength on bacterial cell number and Colony forming unit of *Staphylococcus aureus* at different times.

Different wavelength of visible light		**Exposure time(min.)**					
		15	**30**	**45**	**60**	**90**	**120**
White	**O.D**	0.057	0.05	0.069	0.069	0.080	0.233
	Cell no./ml^{-1}	4.56 x10^{7}	4.56 x10^{7}	5.52 x10^{7}	5.52 x10^{7}	6.4 x10^{7}	1.86 x10^{8}
	Colony no.	2.6 x10^{5}	1.9 x10^{5}	4.9 x10^{5}	1.09 x10^{6}	3.0 x10^{6}	2.94 x10^{6}
Red	**O.D**	0.029	0.057	0.065	0.065	0.104	0.201
	Cell no./ml^{-1}	2.32x10^{7}	4.56x10^{7}	5.2x10^{7}	5.2x10^{7}	8.32x10^{7}	1.61 x10^{8}
	Colony no.	6.3x10^{5}	1.8x10^{5}	1.5 x10^{5}	5.4 x10^{5}	1.29 x10^{6}	2.56 x10^{6}
Yellow	**O.D**	0.057	0.061	0.065	0.065	0.071	0.157
	Cell no./ml^{-1}	4.56 x10^{7}	4.88 x10^{7}	5.2 x10^{7}	5.2 x10^{7}	5.69 x10^{7}	1.26 x10^{8}
	Colony no.	4.4 x10^{5}	3.4 x10^{5}	4.7 x10^{5}	6.0 x10^{5}	8.8 x10^{5}	2.94 x10^{6}
Green	**O.D**	0.058	0.053	0.067	0.067	0.085	0.226
	Cell no./ml^{-1}	4.64 x10^{7}	4.24 x10^{7}	5.36 x10^{7}	5.36 x10^{7}	6.8 x10^{7}	1.81x10^{8}
	Colony no.	3.0 x10^{5}	3.8 x10^{5}	5.3 x10^{5}	7.0 x10^{5}	2.63 x10^{6}	2.89 x10^{6}
Blue	**O.D**	0.043	0.043	0.067	0.067	0.082	0.161
	Cell no./ml^{-1}	3.44 x10^{7}	3.44 x10^{7}	5.36 x10^{7}	5.36 x10^{7}	6.56 x10^{7}	1.21 x10^{8}
	Colony no.	4.9 x10^{5}	1.0 x10^{5}	1.3 x10^{5}	6.1 x10^{5}	1.6 x10^{6}	2.3 x10^{6}
Dark	**O.D**	0.060	0.061	0.071	0.071	0.076	0.183
	Cell no./ml^{-1}	4.88 x10^{7}	4.88 x10^{7}	5.68 x10^{7}	5.68 x10^{7}	6.08 x10^{7}	1.46 x10^{8}
	Colony no.	1.12 x10^{6}	2.8 x10^{5}	3.5 x10^{5}	9.4 x10^{5}	1.3 x10^{6}	1.96 x10^{6}

O.D& bacterial cell no. /ml *and CFU of Staphylococcus aureus before exposure to the lights: 0.132 (1.5x10^{8}) and (3.96x10^{7}CFU/ml)*



Figures (1A) concluded *Staph. aureus* cell number variation rate with exposure time at different wavelength.



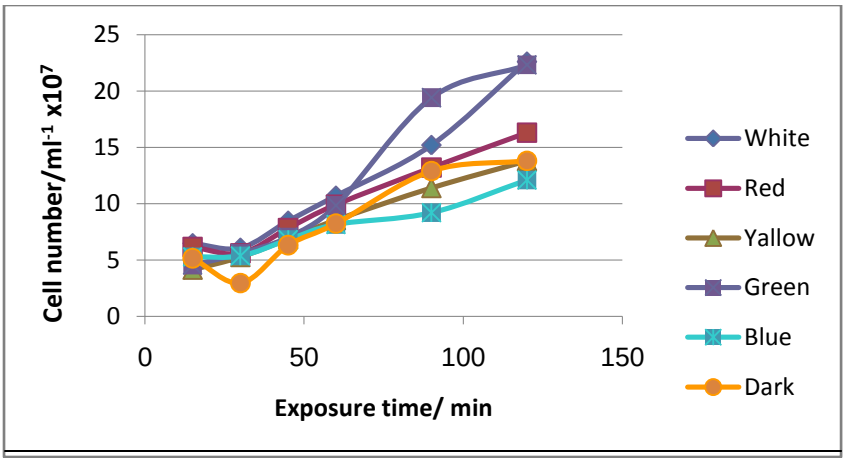
Figures (1 B) concluded *Staph.aureus* colony number variation rate with exposure time at different

Table (2) and Figures (2A&2B) shows the different light source and dark condition on bacterial cell number and colony forming unit of *E.coli* at different times. It's noted the effect of light source where different comparing with *S.aureus* bacterial cell and O.D. since arrange of the effect begin with higher effect at yellow color, Green, Darkness, Blue and Red color and that's mean difference in bacterial sensitivity to the light color. Also the number and O.D. of bacteria E.coli were a rinsed faster than ST. to begging number before exposure at (90min) in all color except in Blue. Colony forming unit and O.D. of E. coli has similar effect as it's in cell count.

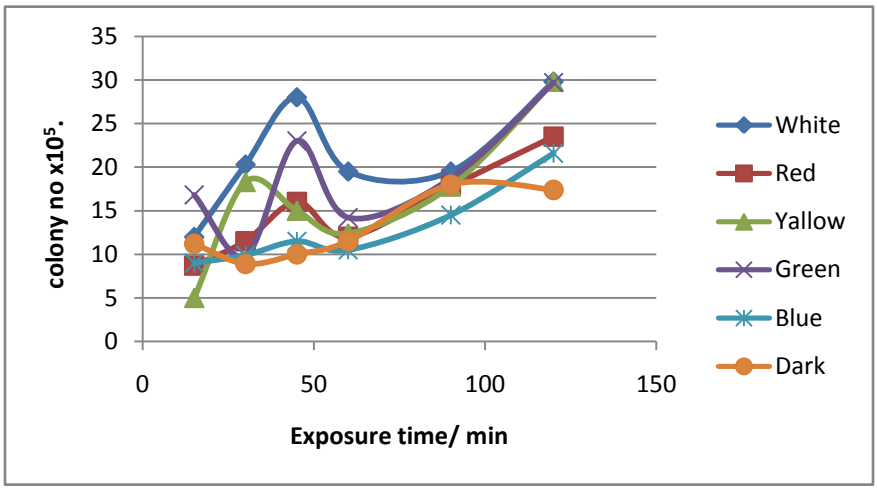
Table (2) Effects of different light wavelength on bacterial cell number and Colony forming unit of *E. coli at different times*

Different wavelength of visible light		**Exposure time(min.)**					
		15	**30**	**45**	**60**	**90**	**120**
White	O.D	0.081	0.076	0.106	0.134	0.190	0.282
	Cell no./ml^{-1}	6.48 x10^{7}	6.08 x10^{7}	8.48 x10^{7}	1.07 x10^{8}	1.52 x10^{8}	2.26 x10^{8}
	Colony no.	1.2 x10^{6}	2.03 x10^{6}	2.8 x10^{6}	1.95 x10^{6}	1.95 x10^{6}	2.98 x10^{6}
Red	O.D	0.077	0.070	0.098	0.124	0.165	0.204
	Cell no./ml^{-1}	6.16x10^{7}	5.6x10^{7}	7.84x10^{7}	9.92x10^{7}	1.32x10^{8}	1.63 x10^{8}
	Colony no.	8.7 x10^{5}	1.15 x10^{6}	1.6 x10^{6}	1.2 x10^{6}	1.78 x10^{6}	2.35 x10^{6}
Yellow	O.D	0.052	0.066	0.086	0.107	0.142	0.195
	Cell no./ml^{-1}	4.16 x10^{7}	5.28 x10^{7}	6.88 x10^{7}	8.56 x10^{7}	1.14 x10^{8}	1.38 x10^{8}
	Colony no.	5.0 x10^{5}	1.83 x10^{6}	1.5 x10^{6}	1.24 x10^{6}	1.78 x10^{6}	2.98 x10^{6}
Green	O.D	0.057	0.070	0.088	0.123	0.243	0.279
	Cell no./ml^{-1}	4.56 x10^{7}	5.6 x10^{7}	7.04 x10^{7}	9.84 x10^{7}	1.94 x10^{8}	2.23x10^{8}
	Colony no.	1.68 x10^{6}	9.8 x10^{5}	2.3 x10^{6}	1.42 x10^{6}	1.87 x10^{6}	2.97 x10^{6}
Blue	O.D	0.066	0.067	0.085	0.102	0.115	0.221
	Cell no./ml^{-1}	5.28 x10^{7}	5.36 x10^{7}	6.8 x10^{7}	8.16 x10^{7}	9.2 x10^{7}	1.21 x10^{8}
	Colony no.	9.0 x10^{5}	9.9 x10^{5}	1.15 x10^{5}	1.05 x10^{6}	1.45 x10^{6}	2.16 x10^{6}
Dark	O.D	0.072	0.037	0.079	0.103	0.167	0.173
	Cell no./ml^{-1}	5.16 x10^{7}	2.96 x10^{7}	6.32 x10^{7}	8.24 x10^{7}	1.29 x10^{8}	1.38 x10^{8}
	Colony no.	1.12 x10^{6}	8.9 x10^{5}	1.0 x10^{6}	1.15 x10^{6}	1.8 x10^{6}	1.74 x10^{6}

O.D& bacterial cell no. /ml and CFU of E.coli before exposure to the lights: 0.133 (1.5x10^{8}) and (3.99x10^{7}CFU/ml)



Figures (2A) concluded *E. coli* cell number variation rate with exposure time at different wavelength.



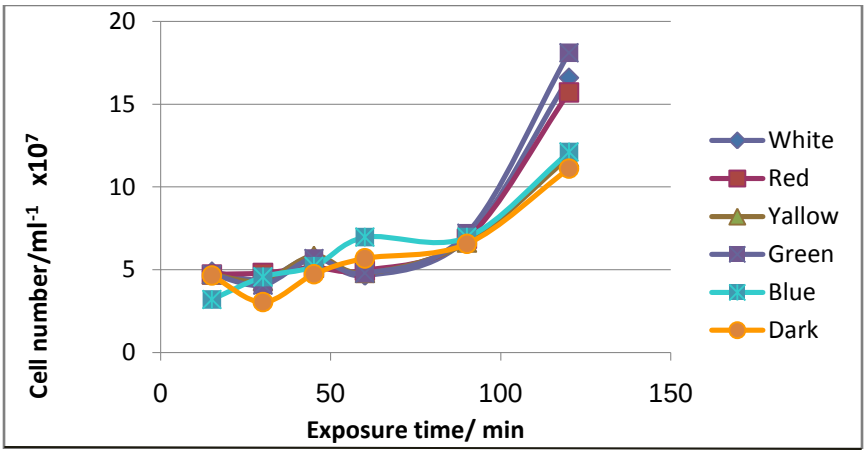
Figures (2B) concluded *E. coli* colony number variation rate with exposure time at different wavelength

In Table (3) and Figures (3A&3B) president the effect of different light and darkness on bacterial cell and colony forming unit of PS. At different times since decrease were occurred within the first incubation time (15min) in all wavelength condition but with different rate of bacterial cell number comparing to starting bacterial number ($1.5x10^8$) before exposure to light. The lower number and higher effect occurred in Blue ($3.2x10^7$), later similar result of Red, Green, and Yellow ($4.72x10^7$) after that dark ($4.64x10^7$) while lower effect in white color ($4.88x10^7$). The total bacterial cell number of *Pseudomonas* during the incubation using different wavelength seen to be arise to begging number at (120min) like *S.aureus* bacteria. The result investigated the same effect on colony forming unit and O.D. of *Pseudomonas* bacteria in different wavelength.

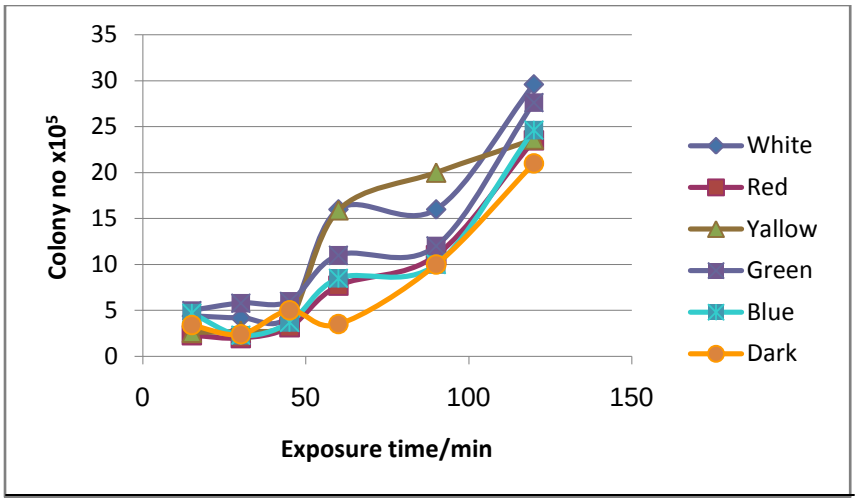
Table (3) Effects of different light wavelength on bacterial cell number and Colony forming unit of *Pseudomonas aeruginosa at different times*

Different wavelength of visible light		**Exposure time(min.)**					
		15	**30**	**45**	**60**	**90**	**120**
White	**O.D**	0.061	0.055	0.072	0.058	0.086	0.208
	Cell no./ml^{-1}	4.88x10^{7}	4.4 x10^{7}	5.76 x10^{7}	4.64 x10^{7}	6.88 x10^{7}	1.66 x10^{8}
	Colony no.	4.4 x10^{5}	4.2 x10^{5}	4.5 x10^{5}	1.6 x10^{6}	1.6 x10^{6}	2.96 x10^{6}
Red	**O.D**	0.059	0.064	0.064	0.062	0.086	0.196
	Cell no./ml^{-1}	4.72x10^{7}	4.8x10^{7}	5.12x10^{7}	4.96x10^{7}	6.88x10^{7}	1.57 x10^{8}
	Colony no.	2.3 x10^{5}	2.0 x10^{5}	3.2 x10^{5}	7.7 x10^{5}	1.1 x10^{6}	2.35 x10^{6}
Yellow	**O.D**	0.059	0.054	0.073	0.060	0.083	0.148
	Cell no./ml^{-1}	4.72x10^{7}	4.32 x10^{7}	5.84x10^{7}	4.8 x10^{7}	6.64 x10^{7}	1.18 x10^{8}
	Colony no.	2.6 x10^{5}	2.8 x10^{5}	4.0 x10^{5}	1.59 x10^{6}	2.0 x10^{6}	2.36 x10^{6}
Green	**O.D**	0.059	0.051	0.071	0.060	0.090	0.230
	Cell no./ml^{-1}	4.72x10^{7}	4.08 x10^{7}	5.68 x10^{7}	4.8 x10^{7}	7.2 x10^{7}	1.81x10^{8}
	Colony no.	5.0 x10^{5}	5.8 x10^{5}	6.0 x10^{5}	1.1 x10^{6}	1.2 x10^{6}	2.76 x10^{6}
Blue	**O.D**	0.040	0.057	0.067	0.061	0.087	0.159
	Cell no./ml^{-1}	3.2 x10^{7}	4.56 x10^{7}	5.2 x10^{7}	6.96 x10^{7}	6.96 x10^{7}	1.21 x10^{8}
	Colony no.	4.7 x10^{5}	2.3 x10^{5}	3.7 x10^{5}	8.5 x10^{5}	1.0 x10^{6}	2.46 x10^{6}
Dark	**O.D**	0.058	0.038	0.059	0.071	0.082	0.139
	Cell no./ml^{-1}	4.64x10^{7}	3.04 x10^{7}	4.72 x10^{7}	5.68 x10^{7}	6.56 x10^{7}	1.11 x10^{8}
	Colony no.	3.4 x10^{5}	2.4 x10^{5}	5.5 x10^{5}	3.5 x10^{5}	1.0 x10^{6}	2.1 x10^{6}

O.D& bacterial cell no. /ml and CFU of *Pseudomonas aeruginosa* before exposure to the lights: 0.133 (1.5x10^{8}) *and (4.41x10^{7}CFU/ml)*



Figures (3A) concluded *P. aeruginosa* cell number variation rate with exposure time at different wavelength.



Figures (3B) concluded *P. aeruginosa* colony number variation rate with exposure time at different wavelength.

Discussion and Conclusion:-

Thus porphyrymacro cycles are highly conjugated systems and consequently they typically have very intense absorption bands in the visible region and may be deeply colored.

The phototoxic effect was found to involve induction of reactive oxygen species (ROS) production by the bacteria. (Energy H-C, C-C, C-N bond) ROS production following blue (400–500 nm) light illumination was found to be higher than that of longer wavelength (500–800 nm). Whatever this phenomena of killing bacterial cell critically happened during the first 15min., while decreased after that according to the exposure time because of mutation occurred so the bacterial resistance.

In previous studies [9, 10], we found that high-intensity broadband visible light (400–800 nm), can reduce viability of bacterial strains known for their predominance, in the absence of exogenous photo-sensitizers. The phototoxic effect was found to depend on oxy radical production; the amount of produced ROS correlates with the degree of phototoxic effect. Moreover, the phototoxicity is dependent on the cellular content of endogenous porphyrins and antioxidants expressed by each bacterial strain [9]. In addition, bacterial killing induced by blue light was studied by several groups [11–16]. In green and white color were found to get more bacterial cell or less phototoxic effect, while the cell number significantly higher than starting number. That's mean improve growth rate during the incubation of patient samples, exactly in case of low growing bacteria.

References:-

1. Siton LW, Hall CH, LynchPA, Davies-Collen RJ (2002). Sunlight inactivation of fecal indicator bacteria and stabilization pond effluent in fresh and saline waters. App. Environ. Microbiol. 68(3):1122-31.
2. Idil O, Ozkanca R, Darcan C, Flint KP (2010). *Escherichia coli*: dominance of red light ever other visible light sources in establishing viable but non culturable state. Photochem.Photobiol.86(1):104-9
3. Khaengraeng R, Reed RH (2005). Oxygen and photo inactivation of *Escherichia coli* in UVA and sunlight: implications for solar water treat- ment. J Appl. Microbiol. 99: 39–50.
4. Idil O, Darcan C, Ozan T, Ozkanca R (2013).The effect of UVA and varies visible light wavelengths radiation on expression level of *Escherichia coli* oxidative enzyme in seawater. Jundishapur Micrbiol.6 (3); 226-32.
5. Losi A**,** CMandalari C, Gartner W(2014). From Plant Infectivity to Growth Patterns: The Role of Blue-Light Sensing in the Prokaryotic World.Plants.3: 70-94.
6. Anat Lipovsky, Yeshayahu Nitzan, Aharon Gedanken,and Rachel Lubart (2010).Visible Light-Induced Killing of Bacteria as a Function of Wavelength: Implication for Wound Healing. Lasers in Surgery and Medicine 42:467–47

7. Kamel F.H. and Jarjes S.F (2015).Essentials of Bacteriology and Immunology, Hawler polytechnic un.Iraq.182.
8. JiangY, Leung W, Qingjuan T, Hongwei Z, Chuanshan X (2014). Effect of Light-Activated Hypocrellin B on the Growth and Membrane Permeability of Gram-Negative *Escherichia coli* Cells. International Journal of Photoenergy.1-6
9. Lipovsky A, Nitzan Y, Friedmann H, Lubart R (2009). Sensitivity of Staphylococcus aureus strains to broadband visible light. Photochem Photobiol.85 (1):255–260.
10. Lipovsky A, Nitzan Y, Lubart R.(2008). A possible mechanism for visible light-induced wound healing. Lasers Surg Med .40(7):509–514.
11. Maclean M, MacGregor SJ, Anderson JG, Woolsey G.(2008)High intensity narrow-spectrum light inactivation and wavelength sensitivity of *Staphylococcus aureus*. FEMS Microbial Lett .285(2):227–232.
12. Feuerstein O, Ginsburg I, Dayan E, Veler D, Weiss EI.(2005). Mechanism of visible light phototoxicity on Porphyromonas gingivalis and Fusobacterium nucleatum. Photochem Photobiol .81(5):1186–1189.
13. Enwemeka CS, Williams D, Enwemeka SK, Hollosi S, Yens D.(2009). Blue 470-nm light kills methicillin-resistant Staphylococcus aureus (MRSA) in vitro. Photomed Laser Surg .27(2):221–226.
14. Enwemeka CS, Deborah W, Steve H, David Y, Sombiri KE.(2008). Visible 405nm SLD light photo-destroys methicillin-resistant *Staphylococcus aureus* (MRSA) in vitro. Lasers Surg Med .40(10):734–737.
15. Feuerstein O, Persman N, Weiss EI. (2004). Phototoxic effect of visible light on Porphyromonas gingivalis and Fusobacterium nucleatum: An in vitro study. Photochem Photobiol. 80(3):412–415.
16. Lipovsky A. (2007). Phototoxic effect of visible light illumination on pathogenic microorganisms. The role of reactive oxygen species production: Bar-Ilan.