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Elimination of Plant Pathogenic Bacteria by Solar Ultraviolet Radiation in Hydroponic Systems

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ABSTRACT

Removing plant pathogens with the sun as a free, available, clean, and sustainable source of energy is interesting. However, there is no data for disinfecting major plant pathogenic bacteria such as *Pseudomonas syringae* and *Clavibacter michiganensis* subsp. *michiganensis* by solar ultraviolet radiation. To obtain the required time for killing these bacteria at different temperatures, a bacterial suspension of active growing cells (approximately 10^7 CFU mL^{-1}) was prepared and subjected to heat inside a water bath. The minimum required time for killing both of the bacteria was achieved 420, 45, and 15 min at 50, 55, and 60 °C, respectively. To examine the effect of solar ultraviolet radiation, the bacteria suspensions inside a quartz tube were exposed to the sun on a horizontal surface at the constant temperature of 50 °C within the water bath (water depth: 0.1 m). Both of the bacteria were killed after one hour by receiving 95.481 kJ m^{-2} ultraviolet and 2.79315 MJ m^{-2} solar radiation doses. The synergy of heat and solar UV could considerably reduce the killing time of the bacteria (7 to 1 hours) at 50 °C. The recommended solar UV dose is 95.481 kJ m^{-2} for this condition.

Keywords: Disinfection, Heat, Renewable energy, Soilless culture, UV, Water treatment

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1. Introduction

Worldwide predictions state that approximately 70,000 km^2 annually enter the classification of the desert Şen (2015). "Over the last 25 years, droughts covered more than 37% of the EU territory and affected more than 100 million people" (Andreu et al. 2015). Population growth and climate change will increase water shortage for different purposes such as drinking water and irrigation. Therefore, reusing reclaimed water is essential (Fatta-Kassinos et al. 2016). The hydroponic system is one of the proper methods in dry regions to increase water usage efficiency. Also, reusing hydroponic water drainage can reduce 20 to 30 percent of water consumption (Tripanagnostopoulos & Rocamora 2008). However, disease control is a key factor in hydroponic systems since plant pathogens rapidly spread in irrigation water. Thus, water disinfection is unavoidable.

Various methods have been applied for water disinfection. Heat and ultraviolet (UV) radiation are physical methods without a significant level of by-products versus chemical materials (Bolton & Cotton 2008). Hence, cleaner agricultural products can be supplied without chemical residues. Soil and water will not be contaminated by fungicides and other chemical materials.

One of the best disinfectants is the sun. The sun has thermal radiation, and its ultraviolet rays can kill pathogens (Aniruddha Bhalchandra & Jyoti Kishen 2013). Furthermore, solar energy is a renewable, clean, free, and sustainable resource. Therefore, using solar radiation for disinfection has all benefits together.

In this study, Pseudomonas sp. and Clavibacter michiganensis subsp. michiganensis as important plant pathogenic bacteria in hydroponic systems were considered for disinfection with heat, UV, and solar radiation.

Table 1 briefly shows previous researches in this field. Most studies in the field of ultraviolet have focused on UVC (100-280 nm) since 240-280 nm effectively inactivates microorganisms and irreparably damages nucleic acid (Aniruddha Bhalchandra & Jyoti Kishen 2013). Maximum relative ultraviolet absorption by DNA pertains to the wavelength of 260 nm. Since low-pressure lamps produce a narrow band of UV light peaking near 254 nm, these lamps are the most efficient source of germicidal UV light (Wolfe 1990).

Table 1- A research list about the disinfection of some plant pathogenic bacteria by heat and UV

Pathogens	*Conditions*	*Reference*
Clavibacter michiganensis subsp. *michiganensis*	30 min at 56 °C	(Fatmi et al. 1991)
Clavibacter michiganensis subsp. *michiganensis*	113 mJ cm^{-2}, UV (254 nm)	(Scarlett et al. 2016)
Pseudomonas aeruginosa (wild type)	5 min at 55 °C	(Spinks et al. 2006)
Pseudomonas chlororaphis	10 min at 60 °C	(Tu & Zhang 2000)
Pseudomonas corrugata Strains	30 min at 60 °C	(Bella et al. 2002)
Pseudomonas syringae	24h at 40 °C	(Hao et al. 2012)
Pseudomonas syringae	4 h sunlight at 25-27 °C	(Miller et al. 2001)
Xanthomonas campestris	24h at 48 °C	(Hao et al. 2012)
Xanthomonas campestris pv. *malvacearum*	20 min at 65 °C	(Honervogt & Lehmann-Danzinger 1992)
Xanthomonas fragariae	60, 15 min at 52, 56 °C	(Turechek & Peres 2009)
Xanthomonas sp.	Solar UV-B	(Gunasekera & Paul 2007)

Considered treatments in some studies relate to seeds, fruits, and leaves. However, these data are not applicable for water in hydroponic systems, surface water, and irrigation water, since the contamination form is a suspension in these cases.

Killing pathogens at lower temperatures is desirable for conventional solar collectors due to radiation limitations and heat losses. Moreover, the required energy for heating can be declined and much water will be disinfected when a goal temperature is low. Therefore, the effect of lower temperatures on pathogens is considered in this research.

The synergistic effect of heat and UV on some pathogens has been proved (Tyrrell 1976; Petin et al. 1997; Kim et al. 2001; Maktabi et al. 2011), but there are not data about some plant pathogens, particularly for solar UV.

This paper aims to obtain the required solar UV dose for killing two major plant pathogenic bacteria (Pseudomonas syringae and Clavibacter michiganensis subsp. michiganensis) at a low temperature in hydroponic systems using the sun as a clean and sustainable source of energy. Safe agricultural production and the reduction of chemical controls are the importance of this study.

2. Material and Methods

Pseudomonas syringae (Pss) and Clavibacter michiganensis subsp. michiganensis (Cmm) were selected as important plant pathogens, particularly in irrigation water (Lamichhane & Bartoli 2015; Scarlett et al. 2016). Their isolates were supplied from the Laboratory of Plant Pathology, Urmia University. Clavibacter michiganensis subsp. michiganensis was isolated from tomato plants with bacterial canker symptoms, a widespread and destructive disease of tomato plants in Urmia, West Azarbaijan, Iran, and its pathogenicity was confirmed on healthy tomato plants based on Koch's postulates. Pseudomonas sp. was isolated from tomato plants with tomato pith necrosis symptoms, a disease that is commonly found in tomato fields and causes economic losses and its pathogenicity was confirmed on tomato plants. The inoculum concentration used for all experiments was 10^7 CFU mL^{-1}. One pathogenic strain was used for the experiments for both pathogenic bacteria.

A suspension of active growing cells of the pathogens (approximately 10^7 CFU mL^{-1}) was prepared (Petin et al. 1997; Wolf & Beckhoven 2004; Berney et al. 2006) and subjected to heat treatments at different lapses of time to determine the minimum lethal time at each temperature and pathogen. The temperatures and time were selected according to pre-tests and the results of previous researches (Fatmi et al. 1991; Grondeau et al. 1992; Toben & Rudolph 1997; Hao et al. 2012). The pathogens inside a tube were submerged in a water bath (Figure 1). The water temperature was controlled by an electronic board (WX-101W, Shenzhen Eshinede Technology Co.), a heater, and a water pump. The maximum variation of water temperature was ±0.25 °C. As the temperature of suspensions before submerging inside the water was room temperature (25-30 °C), delay time, 2-3 min (at 50 to 60 °C), was added for temperature balance according to the temperature measurements of water in the tubes.

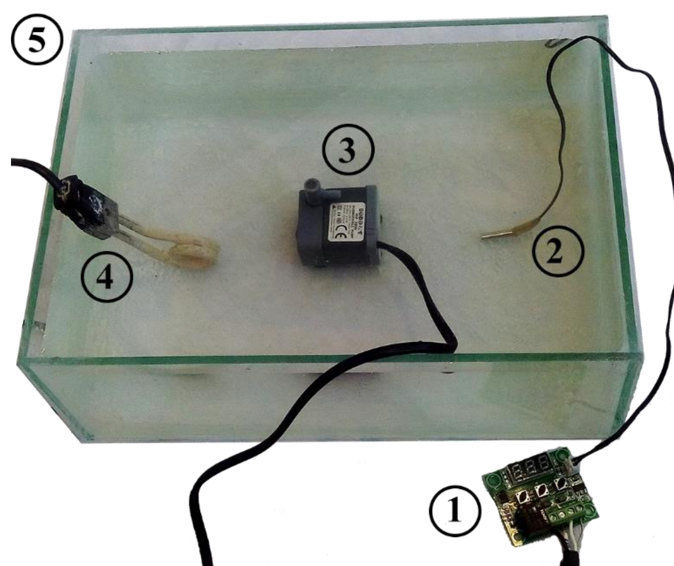

Figure 1- The water bath for temperature control, 1: Electronic board, 2: Temperature sensor, 3: Pump for water circulation, 4: Electric heater, 5: Glass container

The bacteria viability was tested at each temperature and exposure time (Table 2) with a completely randomized design in three repetitions. Temperatures lower than 50 °C were not considered because disinfection time was very long, for example, removing Pseudomonas syringae needs 24 h at 40 °C (Hao et al. 2012). The suspension of pathogens was cultured on Nutrient Agar (NA) medium after exposure to heat, and viable cells were counted after 72 h at 25 °C (Sholberg et al. 2005; Rai et al. 2006).

To examine the effect of solar ultraviolet radiation on disinfection, the pathogen suspension (approximately 107 CFU mL^{-1}) was spilled inside a quartz tube and was exposed to the sun on a horizontal surface. The quartz glass has a high transmittance of UV radiation (Gross et al. 2015). The mentioned water bath (water depth: 0.1 m) was used to control water temperature. Solar radiation was recorded on a horizontal surface during the experiment by a solar power meter, model ST-1307 (Standard, Hong Kong). Solar ultraviolet radiation was measured by the ML8511 UV sensor (LAPIS Semiconductor Co. 2013). This sensor is sensitive to UVA and UVB (Figure 2). Terrestrial solar ultraviolet radiations contain UVA and UVB (Caldwell et al. 2007). The frequency of data recording was 15 min.

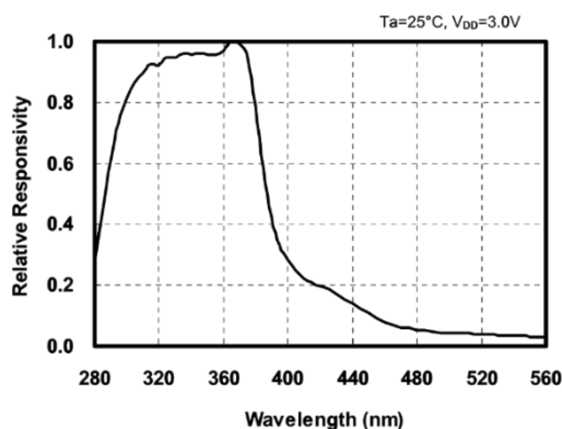

Figure 2- The spectral responsibility characteristics of the ML8511 UV sensor (LAPIS Semiconductor Co. 2013)

The fluence (UV dose) during the experiment was calculated by Equation 1 (Bolton & Cotton 2008).

$$UV dose\ (\mathrm{J\ m^{-2}}) = \int_{t_{start}}^{t_{end}} I_{UV}\, \mathrm{d}t \quad (1)$$

Where: I_{UV}, Ultraviolet radiation (W m^{-2}); t, time (s)

Since the suspension of pathogens was submerged in the water bath, the received dose had a lower value compared to Equation 1. On the other hand, the solar UV dose reported in this research is slightly higher than the actual value. However, to control the temperature, applying the water bath was necessary.

The effect of solar radiation on the bacteria was tested on three different days in August 2016 in Urmia University campus (37° 39' 30.33" N, 44° 58' 43.94" E), and the minimum lethal solar ultraviolet dose was reported. Another experiment (August 21, 2016) was conducted to examine shorter disinfection times (15, 30, and 45 minutes at 50 °C) for the solar ultraviolet test. The viable cell counting was used as described previously to evaluate solar radiation effects.

Burch & Thomas (1998) reported that disinfection time changed exponentially with temperature. Hence, a regression equation can be in the form of Equation 2.

$$time = a_1 \times e^{(b_1 \times temperature)}, \quad (2)$$

Where: a1 and b1, are constant. By taking natural logarithm of Equation 2, the following expression could be derived:

$$temperature = a + b \times Ln(time), \quad (3)$$

Where: a and b, are constant; If let $x = Ln(time)$, Equation 3 is converted to the following equation:

$$temperature = a + b \times x \quad (4)$$

Equation 4 is related to a straight line. Therefore, Pearson's correlation coefficient test can be used to certify the statistical significance of temperature and time dependency. IBM SPSS Statistics software was used for statistical analysis.

3. Results and Discussion

Table 2 shows the results of experiments for different temperatures. Pss and Cmm had the same response. Cases with 100% survival were completely similar to control treatment, and zero survival cases had very clear surfaces without any colony (Figure 3).

Table 2- Disinfection by only heat for *Pseudomonas syringae* (Pss) and *Clavibacter michiganensis* subsp. *michiganensis* (Cmm)

Temperature (°C)	*Time (min)*	*Survival (%) Pss and Cmm*	*Temperature (°C)*	*Time (min)*	*Survival (%) Pss and Cmm*	*Temperature (°C)*	*Time (min)*	*Survival (%) Pss and Cmm*
50	45	100	**55**	15	100	**60**	10	100
	60	100		20	100		15	0
	75	100		30	100		20	0
	90	100		45	0		30	0
	120	100		60	0		45	0
	180	100		120	0		60	0
	240	100		180	0		90	0
	300	100		240	0			
	360	100						
	420	0						
	480	0						
	540	0						
	600	0						
	660	0						

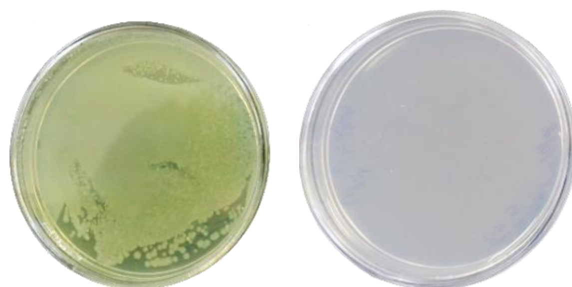

Figure 3- Cmm control (left), completely clear without any bacterial colony (right)

Tu & Zhang (2000) reported that Pseudomonas chlororaphis needed 10 min at 60 °C to be killed. This condition is close to the results of Pss (15 min at 60 °C).

A logarithmic regression between the lethal temperature and time was fitted (Figure 4) according to the results presented in Table 2. The logarithmic regression was used since a linear relation was reported between the logarithm of time and disinfection temperature (Burch & Thomas 1998; Tang 2007). Root mean square error (RMSE) and coefficient of determination (R^2) were obtained 0.8932 and 0.9628 for the regression, respectively. This certifies that the logarithmic regression presented in Figure 4 is proper, and it is consistent with the results of Burch & Thomas (1998), and Tang (2007). Therefore, it was confirmed that the results showed a linear relation between disinfection temperature and the logarithm of time. Hence, Pearson's correlation coefficient can determine the statistical significance of this relation. Table 3 presents the result of Pearson's correlation coefficient test. The correlation coefficient was obtained 0.981 showing there is a statistically significant relation between disinfection temperature and the logarithm of time. The minus sign in Table 3 shows that increasing temperature reduces the killing time.

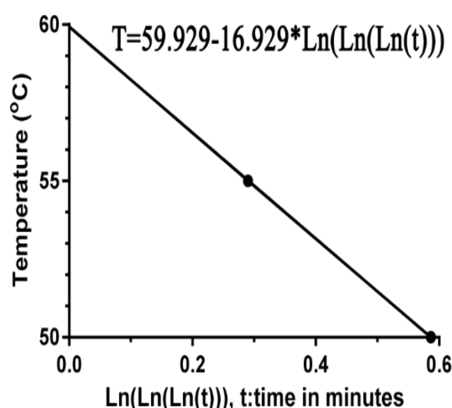

Figure 5- Linear regression for temperature and time by a natural logarithm, (R^2=1, RMSE=0.0106)

Table 3- Pearson's correlation coefficient test for the lethal temperature and the natural logarithm of time (each temperature had three repeats)

Factors		*Temperature*	*Ln(time)*
Temperature	Pearson Correlation	1	-0.981**
	Sig. (2-tailed)		0.000
	N	9	9
Ln(time)	Pearson Correlation	-0.981**	1
	Sig. (2-tailed)	0.000	
	N	9	9

**: The correlation is significant at the 0.01 level (2-tailed)

If a natural logarithm is reused for Figure 4 data, regression errors will be lower, and the estimation will be better (Figure 5).

The equation between temperature and time (Figure 4) can be rearranged so that time becomes an independent variable and temperature becomes a dependent variable (Equation 5).

$$time = e^{\left(\frac{67.092\text{-}temperature}{2.889}\right)} \quad (5)$$

This equation can predict the required disinfection time for a specific temperature.
The derivative of the time respect to the temperature is:

$$\mathrm{d}(time)=\frac{-1}{2.889}\mathrm{e}^{\left(\frac{67.092\text{-}temperature}{2.889}\right)}\mathrm{d}(temperature) \tag{6}$$

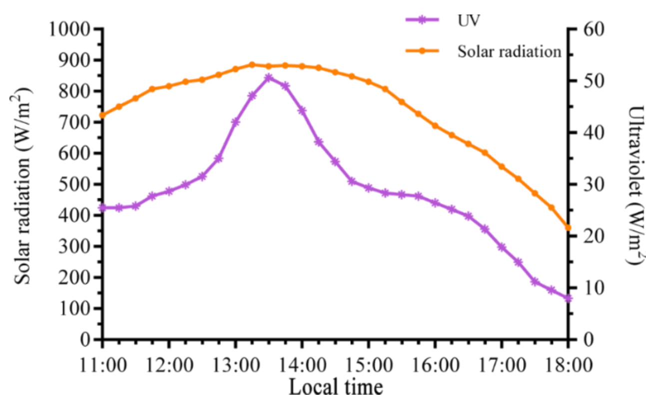

Figure 6- Solar and UV radiation on 17 August 2016 in Urmia University campus.

This equation determines the sensitivity of disinfection time to temperature variations. On the other hand, if the temperature varies one-Celsius degree, Table 4 shows variations in time, based on Equation 6. The minus sign in Table 4 indicates that the time decreases with increasing the temperature. The time variations of lower temperatures are considerably higher than the temperature of 60 °C. This issue is confirmed by comparing the result of Hao et al. (2012) for Pss (24h at 40 °C, Table 1) with the disinfection time of 50 °C (7 h, Table 2). Changing 10 °C in temperature led to the difference of 17 h in disinfection time.

Table 4- The time variation when the temperature increased by 1 degree Celsius at each temperature

Temperature (°C)	*d(time)(min)*
50	-128.4
55	-22.8
60	-4.0

When temperature increases from 55 to 56 °C, the disinfection time decreases 22.8 minutes (Table 4). Therefore, the disinfection time is estimated 22.2 min (45-22.8=22.2) at 56 °C for Cmm (Table 2) approaching Fatmi et al. (1991) report (30 min at 56 °C, Table 1).

Killing Pathogens are not always proportional to temperature-time product (Tang 2007). As Table 5 shows, the temperature-time product is considerably variable at each temperature, and it shows that temperature-time product cannot be a valid criterion for killing Cmm and Pss.

Table 5- Temperature-time product for Cmm and Pss disinfection

Temperature (°C)	*Time (min)*	*Time*temperature (min*°C)*
50	420	21000
55	45	2475
60	15	900

Figure 6 and Table 6 present solar and ultraviolet radiation data on 17 August 2016. This day had a minimum lethal UV dose among three different days. As Table 6 shows, a shorter time and lower UV dose may exist to kill the pathogens. However, the result of the experiment on August 21, 2016 showed that Cmm and Pss were survived at 45 min with 84.665 kJ m^{-2} and 2.3510 MJ m^{-2} ultraviolet and solar radiation, respectively. Therefore, lethal UV dose and time at 50 °C were obtained 95.481 kJ m^{-2} and 1 h, respectively.

Table 6- Bacteria survival after exposing to solar ultraviolet radiation on a horizontal surface on 17 August 2016 in Urmia University campus

Temperature (°C)	*Time (min)*	*UV dose (kJ m^{-2})*	*Solar radiation (MJ m^{-2})*	*Survival (%)*
				PSS, CMM
50	Control	0	0	100
	60	95.481	2.79315	0
	120	214.151	5.8203	0
	180	385.106	8.99145	0
	240	511.092	12.087	0
	300	611.834	14.83965	0
	360	695.142	17.10225	0
	420	738.914	18.78795	0

Comparing Table 2 (temperature: 50 °C, heat only) with Table 6 (heat + UV) shows that the required time for killing bacteria was decreased from 7 h to 1 h by adding the solar ultraviolet radiation (UV dose: 95.481 kJ m^{-2}). It confirms that there is a synergistic effect between solar UV and heat at 50 °C for Cmm and Pss.

Since no research was found that directly matched with the conditions of this research in solar radiation tests, similar cases were investigated.

In a study, the culturability reduction of Pss was reported at least 1000-fold when it was exposed to sunlight for 4 h at the temperature range of 25 to 27 °C (Miller et al. 2001). Results presented in Table 6 shows that 1 h was enough to remove Pss. However, the treatment temperature was 50 °C. This certifies again that there is a synergistic effect between solar UV and heat. Gunasekera & Paul (2007) estimated 24.8 MJ m-2 solar radiation to reduce Xanthomonas sp. population on tea leaves (LOG10(CFUs cm-2 leaf) ≈0). Table 6 shows that 2.79 MJ m-2 is required to eliminate Pss and Cmm. Apart from the bacterium type, this difference can be due to a synergistic effect. Moreover, Xanthomonas sp. bacteria attended in an environment with 18-19 °C temperature, while Cmm and Pss were exposed to heat at 50 °C.

In another research, inactivation time for Pseudomonas aeruginosa was reported 180 min when it was exposed to natural sunlight (Forte Giacobone & Oppezzo 2015). This time was higher than the inactivation time for Pss (60 min) due to the temperature difference. Pss was exposed to heat at 50 °C while the sample temperature for Pseudomonas aeruginosa was 29-31 °C.

A tilted surface with a proper angle can receive more solar radiation than a horizontal surface (Duffie & Beckman 2013). Thus, the reported effect of solar UV can be higher on a tilted surface and solar collectors. Also, Additives (Casado et al. 2021) and photocatalysts (Roshith et al. 2021) are other methods to enhance disinfection power and decrease disinfection time and solar UV dose.

4. Conclusions

Killing active growing cells of Pseudomonas syringae and Clavibacter michiganensis subsp. michiganensis by heat (50, 55, and 60 °C) and solar UV showed:

1) Solar UV and its synergy with heat could considerably reduce disinfection time (7 to 1h) at 50 °C. Thus, it can nicely recoup increasing time at lower temperatures,

2) The required solar UV dose was 95.481 kJ m^{-2} for killing Pss and Cmm at 50 °C temperature,

3) Governing equations between heat and time were logarithmic. Therefore, treatment time had lower sensitivity to temperature variations at higher temperatures,

4) Temperature-time product was not a valid criterion for killing Cmm and Pss.

Heat treatment at a lower temperature is desirable for solar collectors since more water will be disinfected with constant energy when the intended temperature is lower. Therefore, applying solar UV beside heat can enhance disinfection capability.

Future studies can precisely estimate the reported solar UV dose because it was overestimated due to the absorption of tube glass and water height (0.1 m). Moreover, considering the cumulative effects of solar UV and preheat will improve disinfection capacity.

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