Title: *Benefits of photo-Fenton at low concentrations for solar disinfection of distilled water.* A case study: *Phytophthora capsici.*

Authors: Polo-López M.I.[a], Oller I.[a], Fernández-Ibáñez P.[a]*

[a]Plataforma Solar de Almería –CIEMAT, P.O. Box 22, 04200 Tabernas, Almería, Spain (pilar.fernández@psa.es).

***CORRESPONDING AUTHOR:**

Dr. Pilar Fernández-Ibáñez

Plataforma Solar de Almería – CIEMAT, PO Box 22, 04200 Tabernas, Almería, Spain

Phone: + 34 950 387957

Fax: + 34 950 363015

Email: pilar.fernandez@psa.es

Abstract

Water phytopathogens may be a big issue in irrigation water. Huge efforts for controlling this problem have changed along the time, from traditional culturing to the use of chemical and biological methods like fungicides and antagonist microorganisms. Moreover, techniques to enhance water quality are still under investigation especially due to the increasing pressure over human intensive agriculture activities. Advanced Oxidation Processes (AOPs) have been demonstrated to be highly efficient on the removal of hazardous chemical compounds as well as microorganisms contained in water. This work reports on the capacity of photo-Fenton to remove *P. capsici* zoospores in distilled water at small scale (250 mL solar bottle reactor) under natural solar radiation. Photo-Fenton process efficiency was evaluated using two different iron sources, ferrous sulphate (Fe^{2+}) and ferric nitrate (Fe^{3+}), which led to different zoospores inactivation kinetics. The highest inactivation rate was measured with 5 mg/L of Fe^{3+} (89.5 μM) and 10 mg/L of H_2O_2 (294 μM), which required 2.5 kJ/L of solar UV-dose (only 60 min of solar exposure). Different results observed between both iron salts may be due to the nature of zoospores cell wall and the different role played by the iron speciation in cells. In addition, the separated effects of H_2O_2, Fe^{2+} and Fe^{3+} over *P. capsici* spores under natural solar radiation and in the dark were also evaluated. For all solar processes evaluated, we observed the following order of inactivation of *P. capsici* zoospores: Fe^{3+}-H_2O_2/Solar > H_2O_2/Solar > Fe^{2+}- H_2O_2/Solar ≥ Fe^{3+}/Solar > Fe^{2+}/Solar > Solar photolysis.

Keywords: *Phytophthora capsici*, zoospores, photo-Fenton, water disinfection, solar radiation.

1. Introduction

Agriculture is by far the largest consumer of water according to United Nations Food and Agriculture Organization FAO [1]. Many problems have arisen in the generation of alternative cultivation techniques like hydroponic cultivation. Hydroponics has been successfully applied in those areas with high solar radiation and sufficient underground water, and in salinization lands. Generally, hydroponic is a soilless method of horticultural operations for plants production in water. The liquid nutrient solutions used in soilless culture constitute a different growing environment compared with traditional crops, thus new difficulties in managing waterborne diseases may appear [2]. Water and nutrient sources allow pathogens spread easily through such system endangering the entire crop and therefore making essential the water disinfection for avoiding the plantation loses. The most phytopathogens occurrence in this system comes from *Fusarium* spp, *Pythium* sp, *Phytophthora* spp and *Olpidium* spp, as well as various bacteria and nematodes.

Phytophthora spp, a soilborne worldwide pathogen is one of the most occurring fungi species in irrigation water [3]. *P. capsici* is the causative agent of blight pepper disease and one of the main responsible of economic and production losses generated in agriculture. This pathogen can also infects solanaceous and cucurbitaceous hosts in intensive agriculture, including cucumber, eggplant, tomato, pumpkin, squash, melon, and zucchini [4]. Significant diseases on aerial plant tissues including rot of the fruit, steam, and crown and blighting of the foliage, in addition to root rot are produced by *Phytophthora* spp [3,5]. This genus belongs to Oomycota phylum, characterized by asexual swimming biflagellate spores called zoospores [6] which are produced in water from mature sporangia. It may be differentiated between 20-40 zoospores per sporangia under favorable conditions [7] and constitutes the main spread method exhibited by

Oomycetes in the field. This phytopathogen also generates thick-walled chlamydospores and oospores like survival structures under stress conditions [6].

P. capsici control under real conditions includes cultural practices and the use of fungicides. However, this phytopathogen is not susceptible to most broad-spectrum fungicides, for example mefenoxam (phenylamide class of fungicides derives from metalaxyl). Its insensitivity on bell pepper has been previously reported, even the apparition of certain resistance [8]. Similar behavior was observed when chlorine is used as disinfectant. Besides, the trihalomethanes formation by chlorine reaction with the organic matter present in water is not desired. Other options to remove zoospores from water have been investigated although with limited use such as slow sand filtration [9], ozonation [10], ultraviolet irradiation [11], environmental modification like variation of calcium levels [12] and biological control agents like *Pseudomonas spp* to reduce zoospore taxes on roots [13].

Alternatives technologies to disinfect water have recently arisen which are sustainable because they reduce the environmental risks and operation costs. Among them, some Advanced Oxidation Processes (AOPs) have demonstrated to be highly efficient for water disinfection purposes [14]. The basics of AOPs success lies in their ability to produce hydroxyl radicals ($HO^{\bullet}$) which is the strongest oxidant after fluorine with the advantage of being non-selective. Among the AOPs currently under research, the most common treatments for this purpose are TiO_2/UV-Vis, H_2O_2/UV-Vis and photo-Fenton (Fe^{3+} or Fe^{2+}/H_2O_2/UV-Vis). The interest of using AOPs for disinfection has increased due to the high efficiency to inactivate different types of microorganisms. The oxidant effect of photo-Fenton for prions inactivation [15], TiO_2 and photo-Fenton to virus removal [16, 17, 18, 26], bacteria inactivation with TiO_2, H_2O_2 and photo-Fenton [19, 20, 21, 27], fungi spores inactivation with TiO_2, H_2O_2 and photo-Fenton

[22, 23, 24] and oocysts of *Criptosporium parvum* inactivation with TiO_2 [25] have been reported in the literature.

The main goal of the present work is the evaluation of *P. capsici* zoospores inactivation with the photo-assisted processes of H_2O_2/Solar, Fe^{2+} or Fe^{3+}/Solar and photo-Fenton treatments in distilled water. Moreover, similar experiments were carried out in darkness in order to establish the reagents effect on the zoospores viability.

2. Materials and Methods

2.1 Phytophthora capsici enumeration and quantification

P. capsici (CECT 126) strain was transfered onto V8-juice agar supplemented with $CaCO_3$ (2 g/L, MERCK, Germany) and exposed to fluorescent light at 25°C for a week. To induce sporangium production, mycelia plugs were removed to another plate and rinsed with potassium nitrate (1 g/L, MERCK, Germany) solution and then placed under fluorescent light for another 7 days at 25°C. After that, potassium nitrate solution was replaced with autoclaved distilled water and plates were exposed at 4°C for one hour. Then, zoospores were ejected from sporangia. Watman Nº1 lead was used to filter the suspension to separate zoospores from mycelium. Spore concentration was determined by direct counting with a Neubauer plate (Brand, Germany). An optical microscope (Eclipse 50i, Nikon, Japan) was used to enumerate the spores with a counting chamber (Neubauer, Germany). Initial spore concentration of each experiment was adjusted to 10^3 CFU/mL.

Zoospores' suspensions were shaken before counting and inoculating inside the bottle reactor (previously filled with 200 mL of distilled water with conductivity <10 μS/cm, Cl^- = 0.7–0.8 mg/L, NO_3^- = 0.5 mg/L, organic carbon <0.5 mg/L) to induce flagella

loosing. Therefore, the experiments were done with encysted zoospores as no difference in terms of viability between swimming and encysted zoospores were observed before (data not shown). Moreover, similar results for both types of zoospores have been reported in the literature [28]. Malt agar was used for *P. capsici* colonies counting. 50, 250, and 500 μL of each sample were plated out on malt agar (Sigma Aldrich, USA). Detection limit (DL) for each experiment was 2 CFU/mL. Sampling and colonies counting were done in triplicate. The plates were incubated at 26º C (optimal growing temperature) in dark for 1 day before counting. A one-way ANOVA ($P<0.05$, confidence >95%, Origin v7.03, OriginLab Corp., Northampton, USA) statistics of results reported a 95% confidence level for the average colony concentration and error.

2.2 Solar bottle reactor

Experiments were performed in 250 mL DURAN-glass (Schott, Germany) bottle stirred reactors [19, 22, 23]. The total volume of water irradiated was 200 mL with 0.0095 m^2 of irradiated surface. *P. capsici* spore suspensions and reagents added to different bottle reactors were stirred at 100 rpm. Bottles reactors were covered with a glass cap to allow the solar radiation enter from all directions, and were exposed to solar radiation for 5 hours on completely sunny days. All experiments were performed in triplicate. Control bottles (with and without additives of each treatment) were maintained in the dark. The first sample of each experiment was kept in the dark at room temperature and analysed at the end of the experiment to exclude any negative effect of the reagents. Last two samples of each experiment were maintained at room temperature (25º C) and plated again after two days for re-growth evaluation. No spore re-growth was observed for all samples that reached DL. Temperature (T), dissolved

oxygen (DO) and pH were monitored directly in the reactor bottle. Temperature was measured with a thermometer (Checktemp, Hanna instruments, Spain), dissolved oxygen and pH were measured with a pH-meter (multi720, WTW, Germany).

2.3 Analytical determination of iron and hydrogen peroxide

Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$, PANREAC, Spain) and ferric nitrate ($Fe(NO_3)_3 \cdot 9H_2O$, PANREAC, Spain) were used like source of Fe^{2+} and Fe^{3+} respectively. 1, 2.5 and 5 mg/L (17.9, 44.8, and 89.5 μM) of Fe^{2+} or Fe^{3+} were used to carry out photocatalytic treatments. Fe^{2+}, Fe^{3+} and total iron concentration were measured according to ISO 6332. This analytical method was used to determine iron concentrations between 0.01 and 5 mg/L; above 5 mg/L the samples were diluted accordingly. Water samples were filtered with NY 0.20 μm CHROMAFIL® Xtra PET-20/25 (PANREAC, Spain). Samples' absorbance was measured with a spectrophotometer (PG Instruments Ltd T-60-U) at 510 nm in glass cuvettes with a 1 cm path length. Fe^{2+} and Fe^{tot} concentrations were determined using corresponding calibration curves. Fe^{3+} concentration is obtained by difference between Fe^{tot} and Fe^{2+}. The concentration ratio of iron to H_2O_2 used in this experimental work was 1:2. Hydrogen peroxide (Riedel-de Haën, Germany, 30 % (w/v)) was used as received and added directly into the reactor. H_2O_2 concentration was measured with a spectrophotometer (PG Instruments Ltd T-60-U) at 410 nm in glass cuvettes with a 1 cm of path length based on the formation of a yellow complex from the reaction of titanium (IV) oxysulfate (Riedel de Haën, Germany, used as received) with H_2O_2 following DIN 38409 H15 . Absorbance was read after 5 min incubation time against a H_2O_2 standard curve linear in the 0.1–10 mg/L concentration range.

2.4 Solar Radiation

All experiments were conducted at Plataforma Solar de Almeria (PSA), Spain, located at 37º84' N and 2º34' W, under natural solar radiation. UV radiation was monitored with a global UVA radiometer (300-400 nm, Model CUV4, Kipp & Zonen, Netherlands). The radiometer provides data in terms of incident irradiation (W/m^2), which is defined as the solar radiant energy rate incident on a surface per unit area.

Q_{UV} is a parameter used to compare inactivation results under different experimental conditions. However, in this work, the experiment time (t) is also included in the graphs. Q_{UV} is the accumulative energy per unit of volume (kJ/L) received in a photo-reactor and is calculated by equation 1:

$$Q_{uv,n} = Q_{uv,n-1} + \Delta t_n \overline{UV}_{G,n} \; A_r / V_t ; \Delta t_n = t_n - t_{n-1} \qquad \text{(Eq. 1)}$$

where $Q_{UV,n}$ and $Q_{UV,n-1}$ is the UV energy accumulated per litre (kJ/L) at times n and n-1; $UV_{G,n}$ is the average incident radiation on the irradiated area (W/m^2); Δt_n is the experimental time of the sample; A_r is the illuminated area of collector (m^2); and V_t is the total volume of water treated (L).

3. Results and discussion

3.1 Zoospores inactivation by H_2O_2/Solar radiation

Figure 1 shows the evaluation of zoospores inactivation by several H_2O_2 concentrations (2.5, 5 and 10 mg/L; 73.5, 147 and 294 μM)) after 4 hours of solar exposure. Best inactivation results were achieved with 10 mg/L of H_2O_2 which required only 1 hour of solar exposure (4 kJ/L of Q_{UV}) to attain the detection limit (2 CFU/mL) from an initial concentration of 315 (±85) CFU/mL. In the case of 2.5 and 5 mg/L of

H_2O_2, and solar photolysis complete inactivation was achieved from 288 (±103) CFU/mL, 512 (±81) CFU/mL and 209 (±60) CFU/mL, respectively within 3 hours of solar exposure with a Q_{UV} of 14 kJ/L. All the results reached the DL; nevertheless to assess the effectiveness of these treatments, the re-growth of spores in water samples was evaluated for all cases and no spore re-growth was observed.

Moreover, the effect of H_2O_2 concentrations on zoospore viability was evaluated in darkness. No negative effect was observed in any case since zoospore concentration remained constant during the experiment (Figure 1).

Hydrogen peroxide consumed under solar light for 2.5, 5 and 10 mg/L of H_2O_2 was very similar in all cases, 0.41 mg/L, 0.31 mg/L and 0.52 mg/L, respectively. Due to the absence of organic or inorganic compounds in the water matrix (distilled water), the degradation of H_2O_2 is due to the auto-decomposition into water and oxygen; influenced mainly by the temperature. Due to the absence of organic or inorganic compounds in the water matrix (distilled water), the degradation of H_2O_2 is due to the auto-decomposition in water and oxygen; influenced mainly by the temperature. It is well known that the H_2O_2 auto-decomposition rate is temperature dependent, increasing 2.3-fold with a rise of 10 ºC [29]. Maximum temperature achieved in these experiments was 26º C, which doesn't affect the H_2O_2 auto-decomposition. Although the presence of zoospores could affect the H_2O_2 decrease, this is not expected as other contributions showed that the H_2O_2 decrease was not affected by the presence of *Fusarium* spores at similar spore concentrations [23]. The pH slightly increased from approximate 6.3 to 6.5.

The inactivation pathway in this solar treatment (H_2O_2/UV-Vis) could be explained by the $HO^{\bullet}$ generation inside cells via photo-Fenton reactions (Eq. 2-3) [21, 23]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^{\bullet} \quad (k = 70\ M^{-1}s^{-1}) \qquad \text{(Eq. 2)}$$

$$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + HO^{\bullet} \qquad \text{(Eq. 3)}$$

This effect is produced by the possibility of H_2O_2 influx inside the cells provoked by its relatively high stability and no charge. Thus, H_2O_2 can cross membranes freely and react with intracellular or bond iron. The $HO^{\bullet}$ generated inside the cells can oxidize internal structures and finally produce the cell death. The critical factor in these reactions seems to be the availability of the cellular labile iron pool (LIP), which may also be favoured by UV light irradiation of cells [30]. On the other hand, under UV irradiation the main enzymatic cells defence system against oxidative stress (catalase and superoxide dismutase), could be inactivated, thus the internal overload of H_2O_2 favoured the cell death [31].

In our previous work, spore inactivation was reported once the spore germination had been initiated, as before germination the spore is a robust and hermetic survival structure [32]. During the step called “swelling”, the water is absorbed by the spore from the aquatic environment to re-hydrate its core, so that the metabolism activity was initiated. Along with water, H_2O_2 could be also up taken generating $HO^{\bullet}$ by internal Fenton and Haber-Weiss reactions which enhance the spore inactivation [23].

The germination mechanism of zoospores is different from fungi spores due to the role played by zoospores in the life cycle of Oomycetes. Zoospores are characterized by cell walls absence and present two flagella which permit them to swim and spread in the aquatic medium [6]. However, when swimming zoospores are mechanically disturbed by direct contact with soil surfaces or by vigorous shaking or centrifugation, they lost the flagella [6]. After that, an amorphous cell wall is rapidly

formed and zoospores become a cyst. Ultra structural studies show that the peripheral vesicles on the zoospores membrane discharge their contents within 30 s after encystment and initiate the formation of a cell wall. This cell wall is formed within minutes and consists of a thin wall of microfibrils interwoven and randomly oriented. Cysts begin to germinate within 30 minutes and germ tubes are formed in 2-3 h [6]. This effect could be noticed in the inactivation results of *P. capsici* zoospores showed in figure 1. The zoospore reduction begins within the first hour of treatment, as they were added as cysts in these experiments. After that time, zoospores had initiated their germination and the influx of H_2O_2 will permit their inactivation by internal Haber-Weiss reactions.

Taking into account that all the experiments were carried out simultaneously under the same operating conditions, i.e., temperature, pH and solar UV radiation, and even H_2O_2 concentration demanded was very similar in all cases, the different inactivation time between all the tests may be due to the different osmotic pressure generated inside the spores by the presence of higher amounts of hydrogen peroxide. For the highest concentration of H_2O_2 (10 mg/L), the best inactivation was observed probably due to a higher influx of this oxidant molecule inside the cells causing more detrimental effects.

The low H_2O_2 concentrations used to achieve complete inactivation determines that this disinfection process is a good alternative because it is low cost, and in addition, H_2O_2 concentrations below 50 mg/L have been shown to be non-toxic for crops [24]. Moreover, this technique doesn't require a post-treatment procedure for either reagents removal or pH solution change in contrast with other AOPs, like titanium dioxide photocatalysis or photo-Fenton process.

3.2 Zoospores inactivation by Fe^{2+}/Solar radiation

Inactivation results with 1, 2.5 and 5 mg/L of Fe^{2+} under natural solar radiation are shown in figure 2a. Detection limit was achieved in all cases, no significant differences were observed between the different iron concentrations tested (11 kJ/L of Q_{UV}). Complete spore inactivation was achieved in solar photolysis test with a Q_{UV} of 14 kJ/L

The effect of Fe^{2+} concentration on the viability of *P. capsici* zoospores was evaluated in the dark (Figure 2a). It can be observed that initial zoospores concentration was constant during 4 hours of treatment. The initial iron concentration added to the bottles reactors remained constant and were almost completely dissolved in the water. The results obtained in the dark showed that the iron concentration used had not a negative effect on the zoospores viability.

Maximum temperature measured during solar exposure was 30º C. Thermal inactivation of zoospores was evaluated previously in the dark and results showed high sensitivity for temperatures above 45º C, reducing the spore concentration 3 log until reach the detection limit in one hour. At 40º C the spore concentration decreased 0.6 log in 5 hours; meanwhile at 35º C the viability of zoospores remained constant for 5 h. In this experimental work, water temperature was always below 35 ºC, therefore thermal inactivation of zoospores was discarded in all the experiments. pH dropped from 6.17 to 5.46 with 5 mg/L of Fe^{2+}, and a similar decrease was observed with 2.5 and 1 mg/L of iron; while for solar disinfection pH was remained constant.

Taking into account the importance of acid pH for the correct dissolution of iron in water, the effect of acidic conditions on the zoospores viability was evaluated also in

the dark. At pH 2, zoospores were reduced significantly from 10^3 CFU/mL to DL in few minutes. In the case of pH 3, the viability decreased promptly 1.5-log and after the spores' survival remained constant for 5 hours. At pH 4, the viability was reduced from 10^3 CFU/mL to 10^2 CFU/mL initially and then remained constant during the 5 h of the experiment. The low pH induces the lysis of certain percentage of zoospores while those which presented initial resistance to the low pH, they maintained their survival capacity for at least 5 hours. Kong [33] demonstrated that under unfavourable conditions, motile zoospores prompted cysts or lyse, and even some cysts lysed. The cysts germinated rapidly, although the resultant gemlings grew abnormally. In our experimental work the pH never was lower than 3.5, therefore negative effects on zoospores viability provoked by this factor may not be considered. In addition, zoospores were maintained in the dark for 20 minutes under the experimental conditions before the solar exposure to permit the zoospores adaptation.

The initial dissolved iron measured in the case of 1, 2.5 and 5 mg/L of Fe^{2+} were 0.9, 1.7 and 4.4 mg/L; while at the end of the experiment they decreased slightly to 0.7, 1.3 and 3.9 mg/L of dissolved iron, respectively. Complete inactivation was achieved with solar photolysis, while an enhancement of the inactivation kinetics was observed when Fe^{2+} was added. These results demonstrated a synergistic effect between Fe^{2+} and solar radiation (Fig. 2a). Inactivation mechanisms could be explained by the diffusion of Fe^{2+} inside the cells during the first hour of solar exposure [21]. Once internal iron concentration increased the possibility of $HO^{\bullet}$ generation via intracellular Fenton reactions also increased according to Eq. 2-3. As occurs for H_2O_2/Solar process, the high probability of iron diffusion inside the cells, enhance the reaction possibility between iron and H_2O_2, which is always produced during cellular metabolic activity. Equation 2 can occur also in the dark and the little decrease on zoospores's viability

may be due to this mechanism. Another less important way of damage can be produce by the directly or indirectly oxidation of lipids, proteins, sugars, DNA and site-specific oxidation by the iron [34]. However this ways seems not to be relevant because of similar results were observed between the different Fe^{2+} concentrations tested.

In addition, under solar radiation, the catalase inactivation by UV-A radiation occurs [31], which can favour the inactivation of zoospores by increasing the metabolic H_2O_2, as observed in figure 2a.

All tested Fe^{2+} concentration showed the same efficiency (Fig 2a) as well as 2.5 and 5 mg/L of Fe^{3+} (Fig 2b). This suggests a mechanistic limitation in the process. It is well known that the limiting factor of the photo-Fenton is the availability of H_2O_2. Therefore, the similar inactivation efficacies observed for the three concentrations of Fe^{2+} evaluated (Figure 2) could be due to a limitation of H_2O_2 inside the cells, nevertheless we cannot prove it in these experiments. This hypothesis could explain that with the lowest iron concentration (1 mg/L) the complete inactivation of the zoospores is reached.

3.3 Zoospores inactivation by Fe^{3+}/Solar radiation

Figure 2b shows the inactivation of *P. capsici* zoospores with 1, 2.5 and 5 mg/L of Fe^{3+} under natural solar radiation and in the dark. Results obtained in darkness show similar zoospore behaviour to that observed with Fe^{2+}/Dark. In addition, 1-log spore reduction was attained with 5 mg/L of Fe^{3+}, which was a higher reduction than the observed for 5 mg/L of Fe^{2+}. The pH of the samples with added Fe^{3+} were lower than the pH of samples with Fe^{2+}, for the same initial concentrations; i.e. pH 4.27, 4.14 and 3.71 for 1, 2.5 and 5 mg/L of Fe^{3+} respectively, since ferric nitrate (Fe^{3+} source)

produces a stronger acidification effect in distilled water than ferrous sulphate (Fe^{2+} source). The initial iron concentration measured for the case of Fe^{3+} was 1 mg/L lower than for the case of Fe^{2+}. Nevertheless, a similar effect on the spore viability is observed in both systems.

The very little reduction of zoospores concentration in the dark found has been previously observed by Byrt et al [35]. They studied the effect of different inorganic ions on the encystement and lyses of *P. cinnamomi* zoospores. They demonstrated that 15 µM of Fe^{3+} induced encystment with a slight reduction (20 %) on the zoospores viability in 30 minutes [35].

Under natural solar radiation the DL was achieved in all cases. The best inactivation results were obtained with 5 mg/L of Fe^{3+}, which attained the DL from 496 (±46) CFU/mL with 5 kJ/L of Q_{UV}. 2.5 mg/L of Fe^{3+} led to a complete inactivation from 468 (±52) CFU/mL with 7 kJ/L of Q_{UV}. With 1 mg/L of Fe^{3+}, very little enhancement was obtained compared with mere photolysts, as DL required a Q_{UV} of 12.5 kJ/L. The maximum temperature achieved was 32 ºC. As expected, and according to Eq. 3, Fe^{3+} was completely transformed to Fe^{2+} at the end of the experiments via hydroxyl radicals generation. The initial dissolved iron measured in the case of 1, 2.5 and 5 mg/L of Fe^{3+} were 0.3, 1.8 and 3.7 mg/L; while at the end of the experiment were 0.2, 1.2 and 3.5 mg/L, respectively.

Inactivation enhancement observed in the case of Fe^{3+}/solar light system could be attributed to several factors acting together: (i) $HO^{\bullet}$ generated by Eq. 3, would lead to the first attack over the cell wall. The efficiency of Fe^{3+}/solar light has been widely investigated for chemical contaminants degradation, i.e., Mailhot [36] remarked the importance of Fe^{3+} aquacomplexes in the $HO^{\bullet}$ photogeneration for the abatement of

diethyl phthalate under solar radiation. (ii) Fe^{3+} is likely to be adsorbed over cells generating exciplexes on bacterial membrane. This is provoked by the diffusion of Fe^{3+} into the cells leading to its binding to specific proteins [21]. It is well known that the arquitecture of proteins related with the transport of iron and molybdenum across membranes in Oomycetes is maximized compared to prokariotas. Moreover, there are evidences of ATP binding cassette (ABC) superfamily in *Phytophthora ramorum* and *P. sojae* genomes, which are comparable in size to the *Arabidopsis thaliana* and rice genomes, and significantly larger than two fungal pathogens, *Fusarium graminearum* and *Magnaporthe grisea* [37]. Thus, the high presence of iron interchange specific-sites and the influence of osmotic forces, provoke a high Fe^{3+} adsorption rate on the zoospores cell wall. This may enhance the inactivation under solar radiation, which also explains why higher Fe^{3+} concentrations achieve complete inactivation faster than lower concentrations. (iii) Finally, Fe^{2+} generated from Eq. 3 can also diffuse into cells and cause internal injury by Haber-Weiss reaction inside spore with the metabolic H_2O_2, as it occurs in Fe^{2+}/Solar system.

The effect of Fe^{2+} and Fe^{3+} (0.6 mg/L) with UV/Vis radiation has been found in literature to inactivate *E. coli* [21]. Opposite to our results, this contribution showed better inactivation results with Fe^{2+} than with Fe^{3+} in demineralised water. Moreover, in this work different iron concentrations were not evaluated. García-Fernández *et al* also showed good inactivation results in *E. coli* and *Fusarium solani* spores using Fe^{3+}/Solar system [38].

3.4 Zoospores inactivation by Fe^{2+}/H_2O_2/Solar radiation and darkness.

Figure 3a shows the inactivation of *P. capsici* zoospores in dark Fenton. Several Fe^{2+}/H_2O_2 concentrations ratio: 1/ 2.5, 2.5/ 5 and 5/ 10 mg/L, were tested. For the two lowest reagent concentrations, the pH were 5.5 and 4.3 respectively. The maximum

temperature measured was 25 ºC. Hydrogen peroxide added to the system was not completely consumed; 0.8 mg/L, 1.33 mg/L and 3.16 mg/L respectively in 5. At the end of the experiments, high loses of dissolved iron were measured in the case of 2.5 mg/L and 5 mg/L of Fe^{2+} (2.31 and 4.76 mg/L, respectively).

The two lowest concentrations produced very little decrease on the zoospores concentration probably due to the very low reagents concentrations used. The DL was achieved with 5/10 mg/L of Fe^{2+}/H_2O_2 within 2 hours. In this case the complete inactivation was due to the effect of $HO^{\bullet}$ generated by Eq. 2 and the increased stress provoked by the acidic conditions of the water for these reagents concentrations (initial pH 3.9).

Figure 3a shows also the zoospores inactivation under natural solar radiation and photo-Fenton treatment at similar reagents concentrations. In all photo-Fenton experiments the inactivation efficiency enhanced the Fenton results. Nevertheless, DL was only achieved with 5 mg/L of Fe^{2+} and 10 mg/L of H_2O_2 in an hour of treatment (Q_{UV} = 6 kJ/L).

A comparative view of photo-Fenton and photolysis results shows that there a linear tendency in photo-Fenton curves while the solar photolysis shows the typical shoulder followed by a linear region without reaching the detection limit. For the photo-Fenton experiments the solar photolysis mechanisms disappear or becomes irrelevant probably because the oxidative process is acting strongly from the beginning of the process.

The inactivation mechanisms in the case of 5 mg/L of Fe^{2+}/ 10 mg/L H_2O_2 could be explained by several factors acting together: (i) The generation of extracellular $HO^{\bullet}$ by photo-Fenton reactions (Eq. 2-3) which attack the cell wall, initiating lipid

peroxidation chains. (ii) Proteins and sugars from cell wall, like β-glucanes, are also susceptible of being attacked. (iii) The stress generated by the acidic pH, initially 3.9.

In the case of low reagent concentrations (1, 2.5 mg/L of Fe^{2+}/ 2.5, 5 mg/L of H_2O_2) the DL was not achieved, and they have less inactivation efficiency compared with the photolysis and the Fe^{2+}/Solar process. These unexpected results could be explained by the very low dissolved iron concentrations, which were 0.30 mg/L (final 0.45 mg/L), 1.66 mg/L (final 0.47 mg/L) for 1 and 2.5 mg/L of added iron respectively. However, in the case of Fe^{2+}/Solar higher concentrations of dissolved iron were measured. The presence of H_2O_2 generates the quick precipitation of iron in forms of aqua complexes at far-pH of Fenton optimal [ref.]. These aqua complexes produce aggregates and colour the water which absorbs the sunlight, but also act as protective screen for microorganisms.

On the other hand, the individual effect of H_2O_2 and Fe^{2+} with sunlight had been explained with mechanisms based on diffusion of the reagent into cells. Nevertheless, in photo-Fenton the mechanism has not to be necessarily the addition of both mechanisms, and the diffusive mechanisms maybe don't occur because of the fast kinetics of the photo-Fenton reaction. For this reason we found very different curves tendency and final inactivation results.

These results demonstrated that only at higher Fenton reagents concentration (5 mg/L of Fe^{2+} with 10 mg/L of H_2O_2), the generation of external $HO^{\bullet}$ produces an effective damage in the cell. The low iron concentrations don't generate enough amount of $HO^{\bullet}$ for the inactivation of zoospores and the diffusion of Fenton reagents inside the cells is limited.

3.5 Zoospores inactivation by Fe^{3+}/H_2O_2/Solar radiation and darkness.

Zoospores were exposed to dark Fenton with 1/ 2.5, 2.5/ 5 and 5/ 10 mg/L of Fe^{3+}/H_2O_2. Figure 3b shows that the spore viability remained constant during the treatment time for 1 mg/L and 2.5 mg/L of Fe^{3+}. However, with 5 mg/L of Fe^{3+} the spore viability was reduced until DL in 2 hours. In this case, the initial pH was lower for all iron concentrations tested compared to the system with Fe^{2+}/H_2O_2/Dark, especially at 5 mg/L of iron (pH 3.7) which could be the main factor that produces the strong loss of zoospores viability. In this case, the H_2O_2 consumption was around the 50% compared with the consumption observed in the case of Fe^{2+} system for all iron concentrations tested.

Solar photo-Fenton inactivation of *P. capsici* zoospores under natural sunlight with 1/ 2.5, 2.5/ 5 and 5/ 10 mg/L of Fe^{3+}/H_2O_2 are also shown in figure 3b. Complete zoospores inactivation was achieved in the case of 2.5 and 5 mg/L of Fe^{3+} (Q_{UV} of 4 and 2 kJ/L, respectively), while in the case of 1 mg/L the initial spore concentration of 387 (±12) CFU/mL was reduced to 33 (±12) CFU/mL with a Q_{UV} of 5 kJ/L, the DL was not achieved in 2 hours of solar exposure. In this case, the total added hydrogen peroxide was consumed within the first two hours of solar treatment. The initial dissolved iron measured was 0.5 (final 0.1 mg/L), 0.95 mg/L (final 0.35 mg/L) and 2.5 mg/L (final 2 mg/L) for 1, 2.5 and 5 mg/L of added Fe^{3+} to the water.

A high zoospores inactivation rate was obtained with 5 mg/L of Fe^{3+} and 10 mg/L of H_2O_2. The inactivation pathways are similar to those described for photo-Fenton results with Fe^{2+}, i.e, the main injure generated over zoospores may be due to the $HO^{\bullet}$ generated by photo-Fenton reactions (Eq. 2-3). Nevertheless, inactivation kinetics were enhanced in the case of 5 mg/L of Fe^{3+} if they are compared with similar conditions for Fe^{2+}/H_2O_2/solar. This could be explained by the presence of site-specific to adsorb Fe^{3+} on the cell wall of encysted zoospores, as explained in previous section,

by the low acidic water conditions produced by ferric nitrate (4.4, 4 and 3.7 for 1, 2.5 and 5 mg/L of Fe^{3+}), and by the higher H_2O_2 consumption during the solar exposure.

Comparing the results obtained with photo-Fenton and Fe^{3+}/Solar radiation, it must be highlighted that for 2.5 and 5 mg/L of Fe^{3+}, zoospores inactivation rates were enhanced when photo-Fenton was applied (Fig. 3a and 3b), meanwhile a concentration of 1 mg/L of added Fe^{3+} did not affect the spores- viability. These results demonstrate that concentrations lower than 1 mg/L of Fe^{2+} or Fe^{3+} in photo-Fenton system are not adequate to inactivate zoospores of *P. capsici* at least under our experimental conditions, while 5 mg/L of both kind of iron produces successfully inactivation rates.

3.6 Conclusions

The resistance of *P. capsici* in distilled water to different solar photo-chemical treatments: H_2O_2, Fe^{2+}, Fe^{3+} and Fe^{2+}/H_2O_2 or Fe^{3+}/H_2O_2 with and without natural sunlight has been evaluated under several reagents combination. High spore inactivation results have been observed (2- to 3-log within 1-4 hours) for the different treatments under investigation.

The following order of the treatment efficiency was observed: 5/ 10 mg/L of Fe^{3+}/H_2O_2/solar radiation > 2.5/ 5 mg/L of Fe^{3+}/H_2O_2/solar radiation > 10 mg/L of H_2O_2/solar radiation > 5/ 10 mg/L of Fe^{2+}/H_2O_2/solar radiation > Solar photolysis.

The experimental results have demonstrated the effectiveness of this technology to obtain a high water quality for using in irrigation crops, reducing completely the risk of plant diseases due to *P. capsici* zoospores.

Acknowledgements

The authors wish to thank the European Commission for financial support for the RITECA II project (0401_RITECA_II_4_E) under FEDER fund, in the "Competitiveness and Employment Promotion" subject.

References

1. FAO Statistical Yearbook. FAO Electronic Publishing. ISBN 92-5-005506-4, p. 55 (2006).
2. W.T. Runia, S. Boonstra, Mededelingen. 66 (2001) 73-82.
3. L.L. Granke, S.T. Windstam, H.C. Hoch, C.D. Smart, M.K. Hausbeck, Phytophatology. 99 (2009) 1258-1264.
4. A.J. Gevens, R.S. Donahoo, K.H. Lamour, M. K. Hausbeck, Phytopathology. 97 (2007) 421-428.
5. M.K. Hausbeck, K.H. Lamour, Plant Disease. 88 (2004) 1292-1303.
6. D.C. Erwin and O.K. Ribeiro (eds), *Phytophthora* Disease Worldwide. American Phytopathological Society Press, St Paul, MN, 1996.
7. E.A Bernhardt, R.G. Grogan, Phytopathology. 72 (1982) 507-511.
8. K.H. Lamour, M.K. Hausbeck, Genertics and Resistance. 90 (2000) 396-400.
9. E.A. Van Os, J.J. Amsing, A.J. van Kuik, H. Willers, Acta Horticulture. 481 (1999) 519-525.
10. H. Yamamoto, T. Terada, T. Naganawa, K. Tatsuyama, Ann Rev Phytopathol Soc Japan. 56 (1990) 250-251.
11. D. Downey, D.K. Giles, M.J. Delwiche, J.D. MacDonald, Trans ASAE. 41 (1998) 849-857.
12. S.L. Von Broembsen, J.W. Deacon, Phytopathology. 87 (1997) 522-528.
13. P. Diby, Y.R. Sarma, Archives of phytophathology and Plant Protection. 39 (2006) 113-118.
14. S. Malato, P. Fernández-Ibáñez, M.I. Maldonado, J. Blanco, W. Gernjak, Catalysis Today. 147 (2009) 1-59.
15. I. Paspaltsis, C. Berberidou, I. Poulios, T. Sklaviadis, Journal of Hospital Infection. 71 (2009) 149-156.

16. L. Zan, W. Fa, T. Peng, Z.Gong, Journal of Photochemistry and Photobiology B: Biology. 86 (2007) 165-169.
17. J.I. Nieto-Juarez, K. Pierzchla, A. Sienkiewicz, T. Kohn, Environmental Science Technology. 44 (2010) 3351-3356.
18. J.Y. Kim, L. Changha, L. David, Sedlak, Y. Jeyong, L.K. Nelson, Water Research. 44 (2010) 2647-2653.
19. C. Sichel, J. Tello, M. de Cara, J. Blanco, P. Fernández-Ibáñez, Catalysis Today. 129 (2007b) 152-160.
20. P.S. Hartman, A. Eisenstark, J. Bacteriology. 133 (1978) 769-774.
21. D. Spuhler, J.A. Rengifo-Herrera, C. Pulgarín, Appl. Catalysis B: Environ. 96 (2010) 126-141.
22. M.I. Polo-López, P. Fernández-Ibáñez, I. García-Fernández, I. Oller, I. Salgado-Tránsito, C. Sichel, J. Chem. Technol. Biotechnol. 85 (2010) 1038-1048.
23. M.I. Polo-López, I. García-Fernández, I. Oller, P. Fernández-Ibáñez, Photochemical & Photobiological Sciences. 10 (2011) 381-388.
24. C. Sichel, P. Fernández-Ibáñez, M. de Cara, J. Tello, Water Research. 43 (2009) 1841-1850.
25. F. Méndez-Hermida, E. Ares-Mazás, K.G. McGuigan, M. Boyle, C. Sichel, P. Fernández-Ibáñez, J. of Photochemistry and Photobiology B: Biology. 88 (2007) 105-111.
26. H.N. Anathaswamy, A.J. Eisenstark, Bacteriology. 130 (1977) 187-191.
27. F. Sciacca, J.A. Rengifo-Herrera, J. Wéthé, C. Pulgarin. Chemosphere, 78 (2010) 1186-1191.
28. M.O. Ahonsi, S. Doan, A. Demuren, T.J. Banko, W.E. Copes, C.X. Hong, Pest Management Science. 66 (2010) 696-704.
29. C.W. Jones. Applications of hydrogen peroxide and derivates. Edited by James H. Clark. ISBN 0-85404-536-8 .Royal Society of chemistry, Cambrigde, UK. (1999).
30. M. Kruszewski, Mutat. Res. Fund. Mol. Mech. Mutagen. 531 (2003) 81-92.
31. J.A. Imlay, Annual Review of Biochemistry. 77 (2008) 755-776.
32. M.I. Polo-López, I. García-Fernández, T. Velegraki, A. Katsoni, I. Oller, D. Mantzavinos, P. Fernández-Ibáñez, Applied Catalysis B: Environmental. 111-112 (2012) 545– 554.

33. P. Kong, G.W. Moorman, J.D. Lea-Cox, D.S. Ross, P.A. Richardson, C.X. Hong, Applied and Environmental Microbiology, 75 (2009) 4307-4314.
34. B. Halliwell, J.M.C. Gutteridge. Biochem. J. 219 (1984) 1-14.
35. P.N. Byrt , H.R. Irving, B.R. Grant, Journal of General Microbiology. 128 (1982) 1189-1198
36. G. Mailhot, M. Sarakha, B. Lavedrine, J. Caceres, S. Malato, Chemosphere. 49 (2002) 525-532.
37. P.F. Morris, V. Phuntumart, J Mol Evol. 68 (2009) 563-575.
38. I. García-Fernández, M.I. Polo-López, I. Oller, P. Fernández-Ibáñez, App Cat B: Environ 121-122 (2012) 20-29.

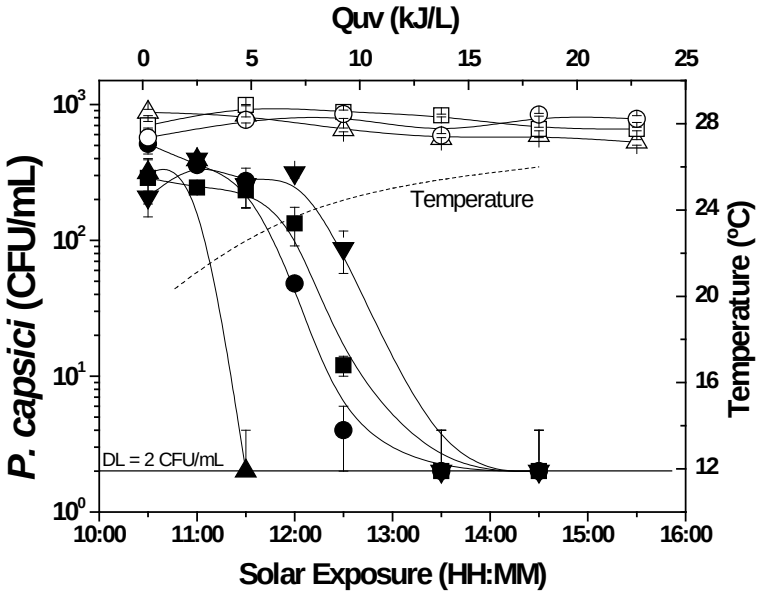
Captions

Figure 1. *P. capsici* zoospores inactivation under H_2O_2/Solar radiation with 2.5 mg/L (-■-), 5 mg/L (-●-) and 10 mg/L (-▲-) of H_2O_2 and solar photolysis (-▼-). Empty symbols correspond to same experiment in the dark.

Figure 2.a) *P. capsici* zoospores inactivation under Fe^{2+}/Solar radiation with 1 mg/L (-■-), 2.5 mg/L (-●-), 5 mg/L (-▼-) of Fe^{2+} and solar photolysis (-▲-). Empty symbols correspond to same experiment in the dark. **b)** *P. capsici* zoospores inactivation under Fe^{3+}/Solar radiation with 1 mg/L (-■-), 2.5 mg/L (-●-), 5 mg/L (-▼-) of Fe^{3+} and solar photolysis (-▲-). Empty symbols correspond to same experiment in the dark.

Figure 3. a) *P. capsici* zoospores inactivation under photo-Fenton treatment with 1/ 2.5 mg/L (-■-), 2.5/ 5 mg/L (-●-), 5/10 mg/L (-▼-) of Fe^{2+}/H_2O_2 and solar photolysis (-▲-). Empty symbols correspond to same experiment in the dark. **b**) *P. capsici* zoospores inactivation under photo-Fenton treatment with 1/ 2.5 mg/L (-■-), 2.5/ 5 mg/L (-●-), 5/ 10 mg/L (-▼-) of Fe^{3+}/H_2O_2. Empty symbols correspond to same experiment in the dark

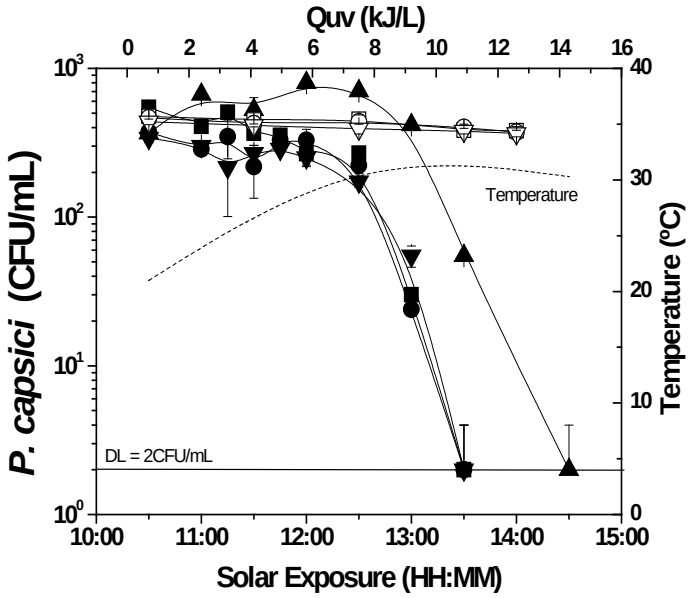
1 **Figure 1**


Quv (kJ/L)
0
5
10
15
20
25
10^3
10^2
10^1
10^0
P. capsici (CFU/mL)
28
24
20
16
12
Temperature (ºC)
Temperature
DL = 2 CFU/mL
10:00
11:00
12:00
13:00
14:00
15:00
16:00
Solar Exposure (HH:MM)

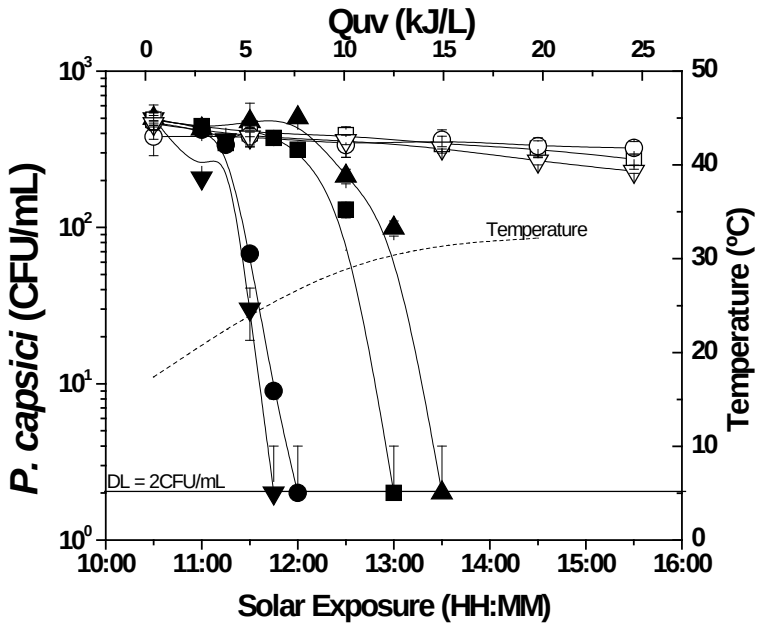
2

3

Figure 2

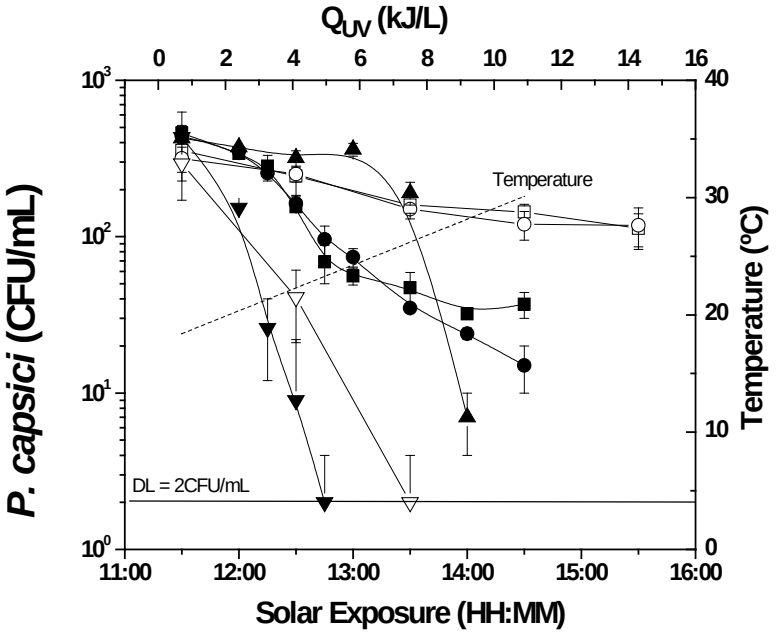

Quv (kJ/L)
0 2 4 6 8 10 12 14 16
P. capsici (CFU/mL)
Temperature (ºC)
Temperature
DL = 2CFU/mL
10:00 11:00 12:00 13:00 14:00 15:00
Solar Exposure (HH:MM)

a)

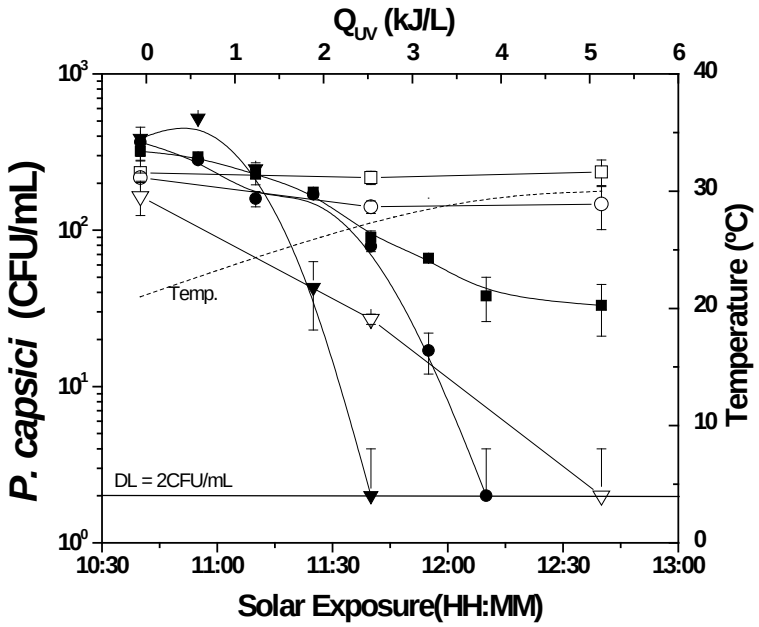

Quv (kJ/L)
0 5 10 15 20 25
P. capsici (CFU/mL)
Temperature (ºC)
Temperature
DL = 2CFU/mL
10:00 11:00 12:00 13:00 14:00 15:00 16:00
Solar Exposure (HH:MM)

b)

Figure 3


Q_{UV} (kJ/L)
0 2 4 6 8 10 12 14 16
10^3 10^2 10^1 10^0
40 30 20 10 0
Temperature
DL = 2CFU/mL
P. capsici (CFU/mL)
Temperature (ºC)
11:00 12:00 13:00 14:00 15:00 16:00
Solar Exposure (HH:MM)

a)


Q_{UV} (kJ/L)
0 1 2 3 4 5 6
10^3 10^2 10^1 10^0
40 30 20 10 0
Temp.
DL = 2CFU/mL
P. capsici (CFU/mL)
Temperature (ºC)
10:30 11:00 11:30 12:00 12:30 13:00
Solar Exposure(HH:MM)

b)