# Effectiveness of the CleanLight UVC irradiation method against pectolytic *Erwinia* spp.

## Zon Fruit & Vegetables

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## 1 Introduction

The Ultraviolet Germicidal Irradiation (UVGI) method is a disinfection technique employing the ultraviolet light at sufficiently short wavelengths – UVC, known as the germicidal UV. Such an approach has been implemented in the CleanLight UVC technology to protect crops against potential phytopathogenic organisms.
The effectiveness of the CleanLight UVC irradiation method against pectolytic *Erwinia* spp. in paprika has been verified by ALcontrol Food & Water laboratory by means of an *in vitro* experiment performed on red paprika samples exposed to the UVC light generated by the CleanLight Hobby Unit.

## 2 Method principle

The UVC light is being produced by a mercury-vapor lamp which emits the UV at the germicidal wavelength deactivating nucleic acids and thereby effecting the growth of microorganisms. This type of treatment alters DNA molecular structure of living organisms disabling basic cell functions as reproduction and multiplication what in consequence cause cell death (Figure 1).

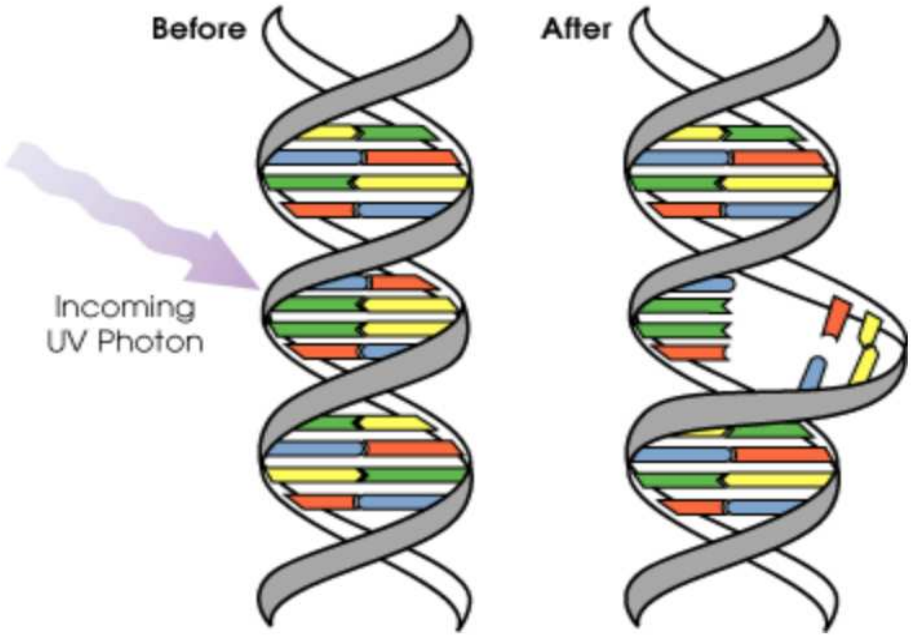



*Fig 1 Exposure of DNA molecule to the UVC irradiation*

The CleanLight technology employs 11 Watt UVC lamps capable of inducing the UVC light which damages microorganisms, viruses and moulds (Appendix 1). On top of that, the application of specific exposure times in the range of up to 10 seconds does not harm plants and crops themselves.
The UVGI is found to be an effective surface sterilisation method however its output depends on a number of factors as:

- Exposure time of microorganisms to the UVC light,
- Microbial susceptibility to the UVC light,
- Power fluctuations of the UVC source,
- The presence of particles which might protect microorganisms from the UVC light,
- The distance between disinfected surface and exposed microorganisms.

# 3 Study performance

## 3.1 Materials

### *3.1.1 UVC instrument*

The effectiveness of the UVC irradiation technique against *Erwinia* spp. in paprika samples has been investigated employing the CleanLight Hobby Unit (Figure 2).
The technical specifications of this instrument are as listed below:

- UV lamp 11 Watt
- UVC output 3.6 Watt
- UVC after 45000 hrs 85%
- µW/cm$^2$ (1m) 33
- Ballast 230V AC 50/60Hz

*Fig. 2 CleanLight Hobby Unit*

### *3.1.2 Matrix under study*

Since the soft rot caused by *Erwinia* spp. in paprika originates in the peduncle tissues and therefrom spreads into the paprika fruit this part attached to harvested fruit has been sampled for the performance of the study (Figure 3).
Paprika samples for this investigation were provided by Zon Fruit & Vegetables and these were represented by fruits without any visible symptoms of bacterial soft rot. Therefore in the context of the study the peduncle parts have been artificially contaminated using the reference strain available at the laboratory.

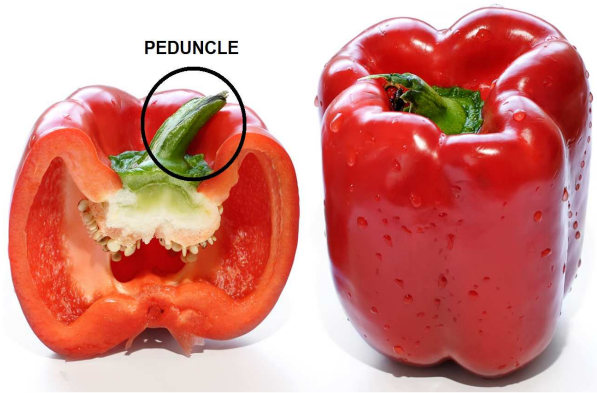


*Fig. 3 Red paprika samples*

### *3.1.3 Reference strain*

The positive strain used in the experiment, *Erwinia carotovora* subsp. *carotovora* (LMG2401/ATCC495) has been purchased from the BCCM culture collection.
Characteristics properties of this microorganism have been checked at the laboratory employing selective culture media and tests and these are as respectively:

- Degradation of pectin in DL-CVP agar,
- Lactose fermentation onto MCC agar,
- Oxidation/fermentation in O/F medium,
- Catalase test,
- Oxidase test,
- Soft Rot Test (Figure 4).


*Fig. 4 Soft Rot Test (positive reaction on left)*

## 3.2 Methods

### *3.2.1 Reference preparation*

The reference strain, *Erwinia carotovora* subsp. *carotovora* involved in the performance of spiking on paprika samples have been grown overnight at 37℃ in the Brain Hart Infusion Broth (BHI).
Its fresh culture has been controlled for homogeneity, purity and contamination level on a day before the execution of artificial contamination on paprika peduncles. This has been performed by means of pipetting series decimal dilutions of the bacterial suspension onto the surface of DL-CVP isolation medium (Figure 5) dedicated for growing pectolytic *Erwinia* spp.

*Fig. 5 E. carotovora subsp. carotovora grown onto DL-CVP isolation agar*

### *3.2.2 Sample preparation and irradiation technique*

In order to verify the effectiveness of applied UVGI treatment against pectolytic *Erwinia* spp. in paprika samples, there were in total 6 red paprika samples analyzed – 3 ones not exposed to the impact of the UVC light considered as the experimental controls and 3 samples subjected to the direct influence of the UVC rays.
Top parts of analyzed paprika samples which contained the research object, peduncle tissues have been cut off from the fruit (Figure 6).

*Fig. 6 Cutting off paprika top part with a peduncle*

Afterwards these were placed in 14-cm Petri dishes with their peduncles directed to the top (Figure 7). This approach has been chosen in order to expose the peduncle tissues to the UVC light generated by the CleanLight instrument during the execution of the ultraviolet germicidal irradiation test.

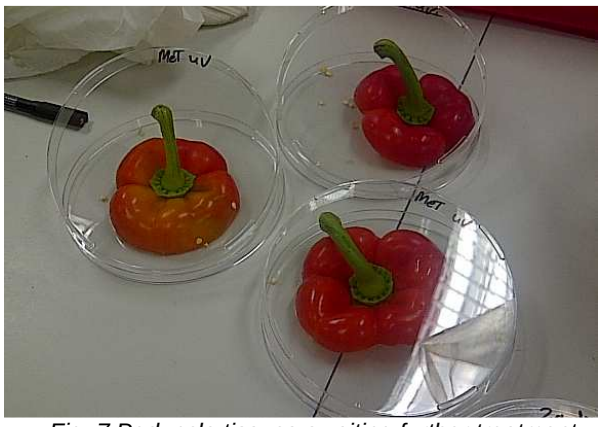


*Fig. 7 Peduncle tissues awaiting further treatment*

Peduncle tissues of all prepared paprika samples have been spiked with the laboratory reference strain, *Erwinia carotovora* subsp. *carotovora* (Figure 8) at equal contamination levels.
This inoculum distributed on the surface of paprika peduncle tissues has been left for 30 minutes at room temperature in order to adapt to the specific matrix conditions.

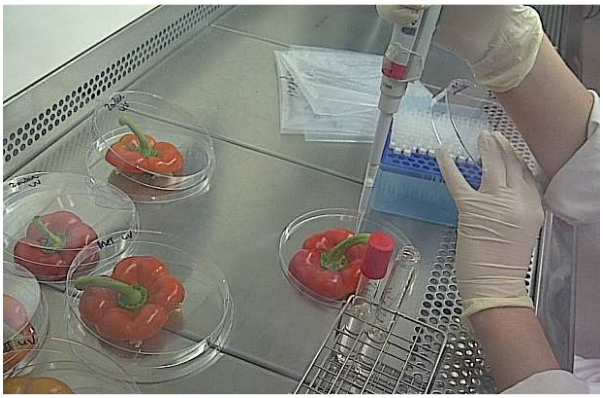


*Fig. 8 Spiking of paprika peduncle tissues*

Subsequently 3 paprika samples have been disinfected with the UVC rays using the CleanLight instrument (Figure 9).
The employed exposure time of spiked paprika samples to the generated UVC light was 3 seconds while the UVC lamp has been located 0.5 m away from the target objects.

*Fig. 9 UVC treatment of paprika samples*

Paprika peduncle tissues of 3 samples exposed to the UVC light as well as 3 control samples not treated with the UVC rays have been cut off using a scalpel from paprika fruit (Figure 10).

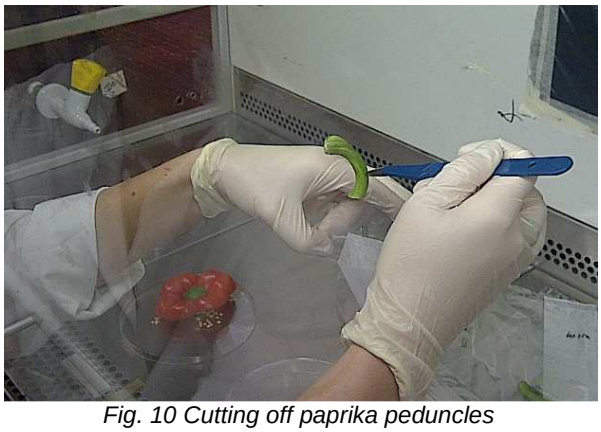


*Fig. 10 Cutting off paprika peduncles*

Accordingly the performance of primary dilutions of removed paprika parts followed by the isolation and confirmation of pectolytic *Erwinia* spp. has been executed (Figure 11).

*Fig. 11 Performance primary dilution*

## 4 Study results

### 4.1 Reference contamination levels

The results of the actual contamination levels of the reference strain, *Erwinia carotovora* subsp. *carotovora* verified beforehand the performance of spiking on paprika samples are displayed in Table 1.

| Dilution | Enumeration result cfu/ml |
|---|---|
| $10^{-3}$ | > |
| $10^{-4}$ | > |
| $10^{-5}$ | 287 |
| **$10^{-6}$** | **75** |
| $10^{-7}$ | 7 |
| $10^{-8}$ | 2 |

*Table 1 Contamination levels of the reference strain*

In order to achieve the target concentration of *Erwinia carotovora* subsp. *carotovora* in artificially contaminated peduncle tissues of paprika samples at the level of approx. 5 $\log_{10}$ cfu/g, the matrix under study have been spiked with 2 ml of bacterial suspension at dilution $10^{-2}$.

### 4.2 Applied UVC dose

The achieved UVC dose emitted by the CleanLight instrument and employed on spiked peduncle tissues removed from analyzed paprika samples is calculated using the following formula:

$$D_{UVC} = I_{1m} \times IF \times T$$

Where:

$D_{UVC}$ - Dose UVC light [µWs/cm$^2$]
$I_{1m}$ - Intensity as the UVC lamp output at the distance of 1 meter [µW/cm$^2$]
IF - Intensity Factor at distances different than 1 meter
T - Time of the exposure to the GVC light [s]

Based on the technical specifications of the CleanLight unit – $I_{1m}$= 33 µW/cm$^2$, conditions applicable during the performance of this experiment – T= 3s as well as the estimated Intensity Factor (IF) of 3.6 for the lamp placed at the distance of 0.5 m, the actual dose of the UVC light ($D_{UVC}$) achieved in this study has been assessed at the level of 356.4 µWs/cm$^2$.
Intensity Factors (IF) corresponding to particular distances of the UVC light source are given in Appendix 2.

## 4.3 Effectiveness UVC treatment

The population of *Erwinia carotovora* subsp. *carotovora* quantified on peduncle tissues of paprika samples following the artificial inoculation was found to be at the intended contamination level of approx. 5 $log_{10}$ giving in average 4.86 $log_{10}$ cfu/g (Table 2).

| Sample | No UV treatment cfu/g | No UV treatment $log_{10}$ cfu/g | UV treatment cfu/g | UV treatment $log_{10}$ cfu/g |
|---|---|---|---|---|
| *Replicate 1* | 70000 | 4,85 | 550 | 2,74 |
| *Replicate 2* | 35000 | 4,54 | 1800 | 3,26 |
| *Replicate 3* | 160000 | 5,20 | 3400 | 3,53 |
| **Average** | **88333** | **4,86** | **1917** | **3,18** |

*Table 2 Effect of the UVC irradiation on E. carotovora subsp. carotovora in paprika samples*

After the application of the disinfection treatment with the CleanLight instrument the numbers of this phytopathogen in paprika samples exposed to the influence of the UVC rays have been decreased into 3.18 $log_{10}$ cfu/g. This can be seen in Figure 12.

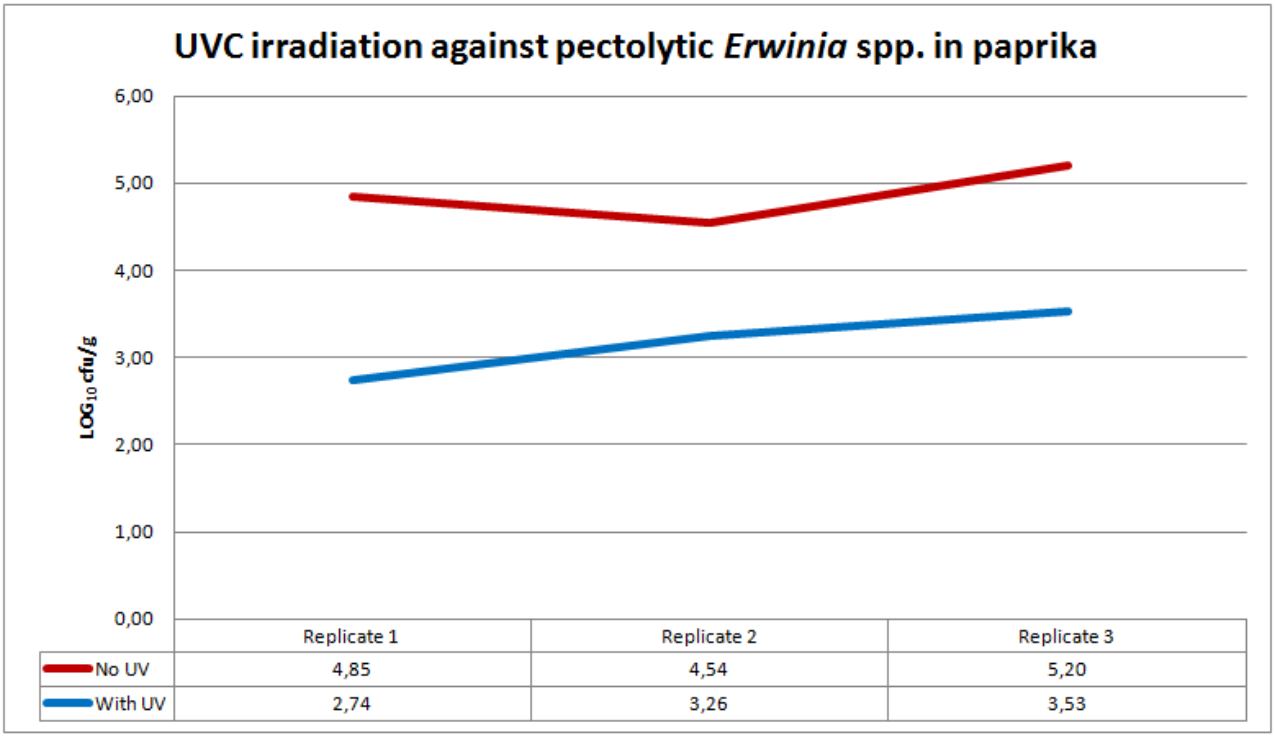



*Fig. 12 UVC irradiation effectiveness against E. carotovora subsp. carotovora in paprika samples*

## 5 Results discussion and recommendation

As demonstrated in the current *in vitro* trial performed on the effectiveness of the Ultraviolet Germicidal Irradiation (UVGI) technique employing the CleanLight unit against *Erwinia carotovora* subsp. *carotovora* reference strain which was surface inoculated onto peduncle tissues of paprika samples this method reduced significantly the level of the phytopathogenic bacterium in analyzed material in comparison to the untreated control samples.
The application of the UVC treatment at the UVC dose of 356.4 µWs/cm$^2$ resulted in 1.68 $\log_{10}$ cfu/g reduction of *Erwinia carotovora* subsp. *carotovora* growth in tested paprika samples under laboratory conditions.
Since the principal limitation of the UVC germicidal irradiation method is related to the requirement for direct exposure of target surfaces to this disinfection tool the operability of the UVGI technique shall be confirmed *in situ* as well.
In addition, adequate exposure cycles to the UVC irradiation in combination with the optimal UVC doses specified for paprika shall be established. This in order to avoid possible side effects of verified disinfection technique on exposed paprika plants and/or fruit which might lead to the degradation of their tissues. Hereby care shall be also taken to assess the photoreactivation potential of *Erwinia* spp. after the employed UVC treatment as well as the effect of flora developed on paprika plants and fruit under such modified conditions since yeast and fungi with their spores are known to be less susceptible to the UVC light than microorganisms.

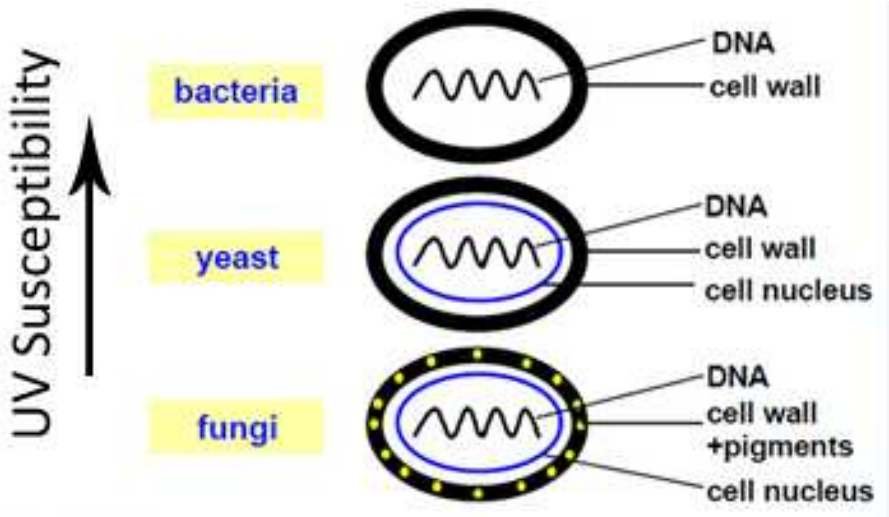
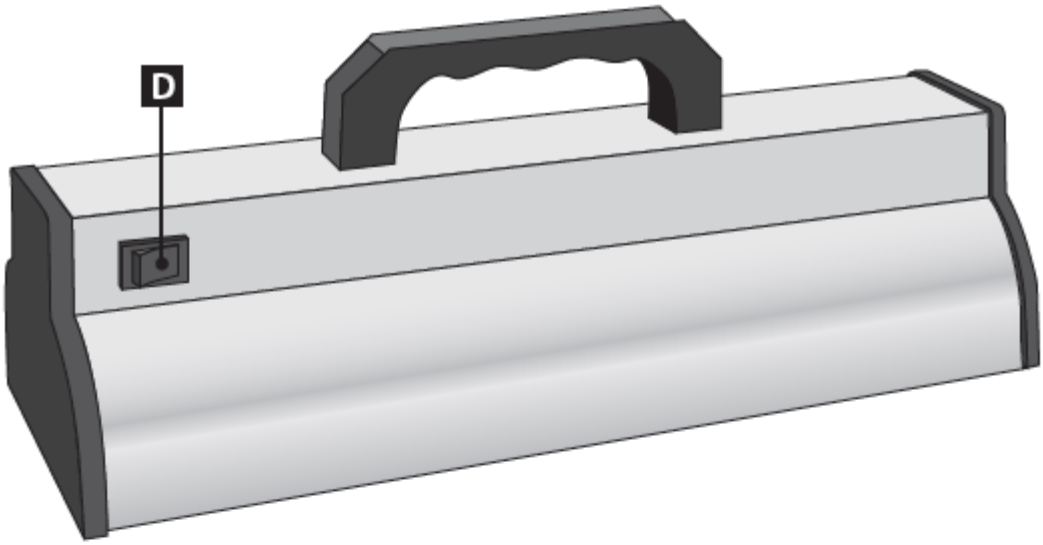


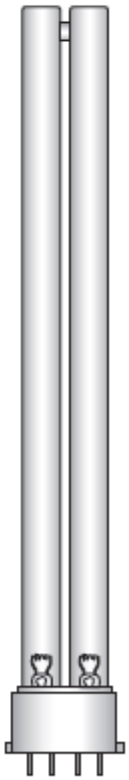

*Fig. 13 Susceptibility of different organisms groups to the UVC exposure*

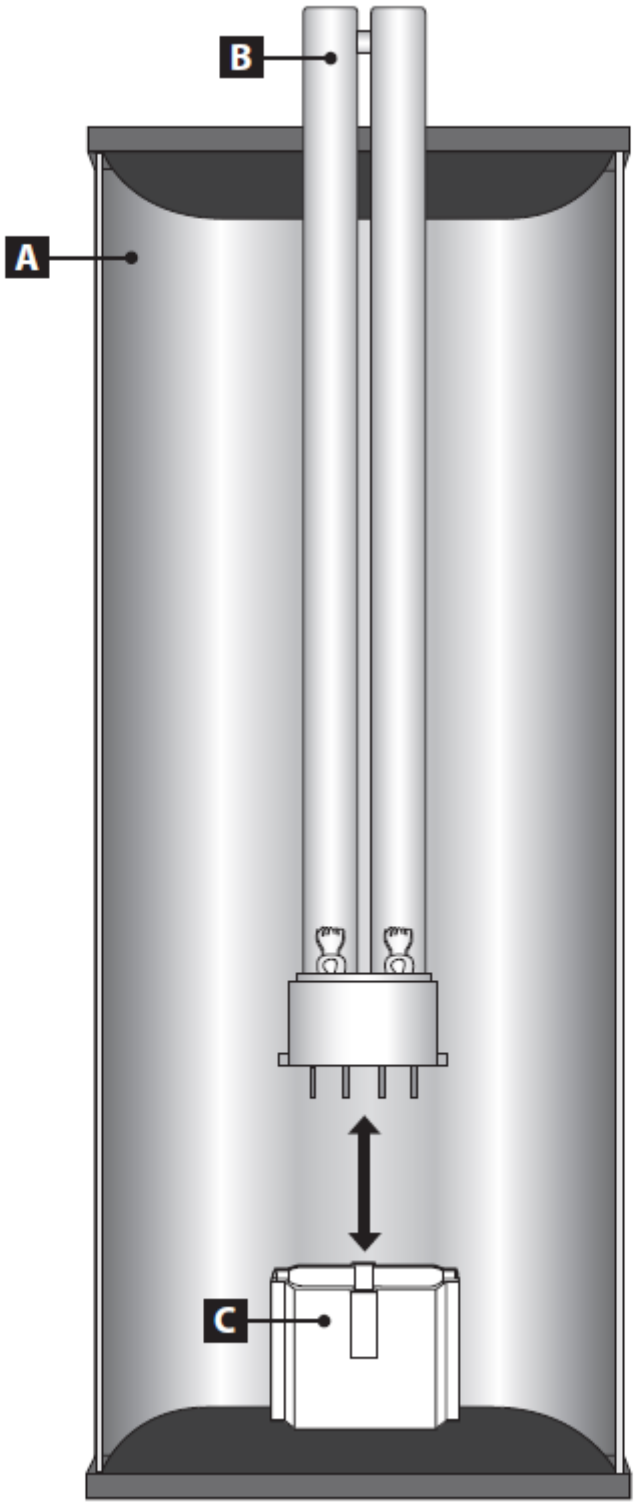
Therefore it can be assumed that the Ultraviolet Germicidal Irradiation (UVGI) method applied at appropriate doses and frequency can serve as a potential effective tool for the limitation of the prevalence of pectolytic *Erwinia* spp. in paprika protecting it against this phytopathogenic bacterium.

# 6 Appendices

## Appendix 1: CleanLight Hobby Unit device






Descriptions of the CleanLight Hobby Unit device:

A - CleanLight Luminaries
B - CleanLight Lamp
C - CleanLight Luminaries'' lamp holder
D - On/Off button

## Appendix 2: Intensity Factor (IF) calculation

| Distance [in] | Distance [m] | Intensity Factor |
|---|---|---|
| 2 | 0,05 | 32,3 |
| 3 | 0,08 | 22,8 |
| 4 | 0,10 | 18,6 |
| 6 | 0,15 | 12,9 |
| 8 | 0,20 | 9,85 |
| 10 | 0,25 | 7,94 |
| 12 | 0,30 | 6,48 |
| 14 | 0,36 | 5,35 |
| 18 | 0,46 | 3,6 |
| 24 | 0,61 | 2,33 |
| 36 | 0,91 | 1,22 |
| 39,37 | 1,00 | 1 |
| 48 | 1,22 | 0,681 |
| 60 | 1,52 | 0,452 |
| 80 | 2,03 | 0,256 |
| 100 | 2,54 | 0,169 |
| 120 | 3,05 | 0,115 |