RESEARCH ARTICLE

Biocidal efficiency of ultraviolet radiation regarding the excitant of the potato blackened stem *Dickeya solani*

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Abstract

Aim and Scope: There presented results of microbiological studies of the sensitivity of the phytopathogenic bacterium excitant of the blackened stem of potato *Dickeya solani* to ultraviolet (UV) radiation. **Materials and Methods:** As sources of the biocidal radiation, a pulsed xenon lamp and a mercury lamp of low pressure were used. **Result and Discussion:** It is shown that the pulsed xenon lamp has a more pronounced antimicrobial effect with respect to the *D. solani* bacterium than the mercury lamp. **Conclusion:** The obtained results testify the prospects of application of UV disinfection technologies in a complex of phytosanitary measures against bacteriosis during the preparation of seed stock and long-term storage.

Key words: Disinfection, potato blackened stem, pulsed irradiation, ultraviolet radiation

INTRODUCTION

The actual task of the modern biotechnology is the search for effective ways to protect crops. In potato growing, a particular danger is a blackened stem - one of the most harmful bacterial diseases of potatoes.[1] The disease got its name because of decay of the lower part of the stem of young plants. The disease is common in almost all countries where the potato is cultivated.

The excitant agents of the blackened stem are pectolytic, Gram-negative, non-spore-forming bacteria, capable of producing and secreting a set of exoenzymes depolymerizing the components of the cell wall of plants.[2] This allows them to penetrate into plant tissues, due to which they are fed. Bacteria - pathogens of the blackened stem affect the root part of the stems during vegetation and cause decay of the tubers in the field and during their storage.

Conventionally, phytopathogenic bacteria *Erwinia carotovora* are considered to be the causative agents of the blackened stem of vegetating plants.[3,4] However, analysis of the black-stemed potato in the Netherlands in the 1970s revealed for the first time the pathogenic strains of *Dickeya dianthicola* - a species formerly known as *Erwinia chrysanthemi* (as a result of the analysis of rDNA hybridization and a broad study of the biochemical characteristics of pectolytic bacteria, the scientific community decided to completely separate the species of the group "chrysanthemi" from the group of species *Erwinia* and give them the new generic name *Dickeya* (the very group of these pathovars is called *Dickeya* spp.) in honor of the outstanding microbiologist Dickey R.S.).[5] Later, this object was found in many other countries - England, France, Hungary, Sweden, Belgium, Switzerland, Poland, Finland, Scotland, Spain, Georgia, Israel, and Russia.[6] The principal differences of bacteria *Dickeya* spp. from the usual

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causative agents of the potato blackened stem are extremely high aggressiveness at elevated temperature, the ability to transfer from the plant to plant by insects, spread rapidly along the vascular system of the plant, and remain latent during the storage of seeds at low temperature. The European and Mediterranean Organization for plant protection and quarantine included phytopathogenic bacteria *Dickeya* spp. in the list of A2 quarantine organisms. Since 2004–2005, a new species of this pathogen, *Dickeya solani*, has been spreading, adapted to the moderate climate. Recently, interest in this bacterium has increased significantly, since its rapid spread is observed, which entails an increase in potato yield losses.[7]

A particular danger of *Dickeya* spp. pathogens, including their ability to spread with a latent contaminated planting material, requires research and development of new effective phytosanitary approaches aimed at preventing the spread of blackened stem pathogens and reducing economic losses when growing and storing potatoes.

It is known that ultraviolet (UV) radiation is an effective preventive agent aimed at reducing the contamination of microorganisms in drinking water, air, and on the surface.[8-10] Low- and medium-pressure mercury lamps with a monochromatic or polychromatic spectrum of radiation (*Hg*-lamps) are widely used as sources of UV radiation,[10] and stricter environmental standards and quality requirements for food products have led to the emergence of new UV disinfection technologies, based on the use of mercury-free pulsed xenon lamps (*Xe*-lamps).[11-14] The latter is characterized by a continuous emission spectrum and a pulsed power of UV radiation, several orders of magnitude higher than that of traditional *Hg*-lamps.[15]

The maximum biocidal efficiency is UV-C radiation area (200–280 nm), which causes lethal photochemical modifications of the vital biological structures of the cell, primarily DNA molecules, as well as protein molecules and cell membranes.[16] The biocidal effect depends on the dose of UV radiation, which is determined by the product of the radiation intensity (in W/cm^2) for the exposure time (in sec) - D=I·t (J/cm^2). In the first approximation, the kinetics of inactivation of microorganisms under the action of UV radiation is described by an exponential function of the species:

$$\frac{N}{N_0} = 10 - \frac{D}{D_{10}}$$

Where N_0 and N - the initial number of microorganisms and the number of survivors after UV irradiation with a dose of D microorganisms, respectively; D_{10} - a dose that reduces the initial contamination of microbes by 10 times. The value D_{10} depends on the type of the microorganism (its sensitivity to UV radiation), and also, probably, on the quality of the UV radiation (its spectrum, intensity, etc.). For many bacteria irradiated with the *Hg* lamp, the value D_{10} is determined experimentally; the characteristic values D_{10} are 3-30 mJ/cm^2 surface.[8-10] Literature data on the sensitivity of phytopathogens-causative agents of the potato blackened stem to UV radiation are absent today. Potential possibilities of using UV technologies to protect potatoes from blackened stem causative agents are not investigated.

Objective of the Work

The objectives were microbiological studies *in vitro* of the sensitivity of *D. solani* to UV radiation, a comparative evaluation of the biocidal efficiency of *Xe*- and *Hg*-lamp radiation, as well as the study of the effect of UV radiation on the growth of the macerated tissue of inoculated potatoes.

Sources of Radiation

Experiments included an experimental installation based on the *Xe*-lamp type INP-5/80, developed at the MGTU by N.E. Bauman. The lamp has a cylindrical glowing body with a diameter d_l = 5 mm and a length l_l = 80 mm. The electrical energy of the flash is W_0 = 60 J.

The specific electrical power input per unit volume of the lamp was ~ 600 kW/cm^3. At this level of energy deposition, the radiation spectrum is predominantly continuous,[15] continuously overlapping the UV area of the spectrum.

The radiative characteristics of the lamp were measured using calibrated photoelectric radiation receivers.[17] The brightness temperature in the UV-C area of the spectrum (Δλ = 270+20nm) was 9100 K, the energy yield of the radiation was EUVS = 4.1 J, and the total radiation energy in the spectral area 200 ...1100 nm - 28 J (47% of stored electric energy).

Xe lamp worked in a pulse-periodic mode with a repetition rate of impulses f = 3.3 Hz. The average electric power of the lamp is P_l = 200 W, and the average radiation power in the UV-S range was $P_{UVS\,(Xe)} = E_{UVS} f \approx 13.5$ W.

As an analog in the experiments, we used the *Hg* lamp of the TUV-15 W type with a cylindrical glowing body with a diameter d_{l_rt} = 24 mm and the length l_{l_rt} = 450 mm. The radiation spectrum of such a lamp is monochromatic (linear); 85% of the radiation is concentrated in a narrow line at a wavelength λ_{max} = 253.7 nm. The lamp operates in the continuous burning mode. The average electric power of the lamp is $P_{l\,(Hg)}$ = 15 W.

MATERIALS AND METHODS

The investigated bio-objects (contaminated Petri dishes or potato slices) were placed under the lamps at a distance L = 20 cm.

Comparison of the biocidal effect of lamps was carried out when the energy doses of bioactive objects were equal in the spectral area corresponding to the UV-S range. Equal doses of radiation were provided by appropriate selection of exposure time.

The calculation of the irradiance of the investigated objects was carried out under the assumption of the Lambert source:[18]

$$P_{sUF} = \frac{P_{UF}}{\pi^2 \cdot L^2}\left[\frac{2L^2}{4L^2 + l_l^2} + \frac{L}{l_l}\text{arct}_g\left(\frac{l_l}{2L}\right)\right]; \frac{W}{cm^2}$$

Where L_l - length of the lamp, cm; P_{UV} - average power of UV-S radiation, W.

According to the calculations, the *Xe* lamp provided an average radiation power density in the UV-C range at the object $Ps_{UVS\ (Xe)} \approx 3.3$ mW/cm^2 (the pulse power density was ~ 15 W/cm^2); *Hg* lamp - $Ps_{UVS\ (Hg)} = 0.63$ mW/cm^2. Thus, under conditions of the conducted experiments for collection of the same energy dose of irradiation of the bio-object in the UV-S range, the duration of irradiation with the *Hg* lamp should be ~ 5 times greater than the exposure time of the *Xe* lamp.

The microbiological object of the study was *D. solani* strain *D. fil.* Samples with different initial concentrations of bacteria (from 10^1 to 3.2×10^7 colony forming unit (CFU)/ml (a CFU), CFU (latitude colonia - settlement) - standard indicator, indicating the number of bacteria-forming colonies in 1 ml of the medium) were prepared from the initial sample of the phytopathogen suspension in phosphate-buffered saline by serial (tenfold) dilutions.

Using a dispenser, 0.1 ml of the solution from the prepared samples was transferred to a Petri dish (Ø90 mm) with YDC medium (medium composition: Yeast extract 10 g, $CaCo_3$ - 20 g, glucose - 10 g, agar - 18 g, and distilled water - 950 ml) and the spatula was evenly triturated on the surface of the nutrient medium. The maximum density of contamination of the surface by microorganisms in the experiments was given as follows:

$n_s = No/S = 3.2 \times 10^7\ 0.1/64 = 5 \times 10^4$ CFU/cm^2,

Where S = 64 cm^2 - area of the Petri dish.

Irradiation of the dishes was performed at different energy doses of UV-S radiation. The dose (mJ/cm^2) was calculated by the following formula:

$D_i = Ps_{UVS} t_i$,

Where Ps_{UVS} - average irradiation of the dish in the UV-S spectral range, mW/cm^2; t_i - exposure time, s.

The control dishes were not irradiated.

After the experiment, the dishes were placed in a thermostat at 27°C. Evaluation of the results was carried out 24 h after irradiation by counting the number of grown colonies and averaging the data by three replicates in each variant of the experiment.

To evaluate the effect of UV radiation on the growth of the macerated potato tissue after its inoculation, the bacteria were selected as maximally homogeneous potato tubers. The tubers were washed, cleaned, and treated with a 96% solution of ethyl alcohol. Then, square potato slices with a side of 4 cm and a thickness of 0.5 cm were cut and placed on a pre-wetted phosphate-buffered saline buffer filter paper in a Petri dish.

The slices were inoculated using a dissection needle with the *D. solani* suspension (the concentration of bacteria in the suspension was 10^9 CFU/ml) and irradiated.

Control samples prepared in a similar manner were not subjected to UV irradiation. Furthermore, the samples that were inoculated with distilled water to control extraneous microflora were not irradiated.

Evaluation of the results of the experiments was carried out at 48 h after irradiation by measuring the diameter of the macerated tissue of the potato and averaging over three replicates in each variant of the experiment.

Statistical processing of the received information was carried out by the method of the dispersing analysis. The final statistical indicator in the methodology was the smallest significant difference (SSD). With the help of the SSD, the significance of the differences between the variants of the experiment was established. If, when comparing the two variants, it was established that the difference between them is greater than or equal to the SSD, then this difference was recognized as a significant one and conditioned influence of the variant.

RESULTS AND DISCUSSION

Table 1 shows the results of a series of experiments carried out at high levels of contamination of the surface by microorganisms and at relatively high energy doses of UV radiation.

As it can be seen from the presented data, effective UV inactivation of *D. solani* bacteria occurs under the influence of UV radiation - both *Hg*- and *Xe*-lamps [Figure 1]. At a sufficiently high contamination of the surface ~ 5.10^4 cells/cm^2, the density of contamination of the surface with microorganisms decreases by more than 5 orders of magnitude at UV-C doses of 30 mJ/cm^2, the degree of disinfection was:

$\eta = (1 - Nk/N_0)\ 100\% \geq 99.999\%$

Table 1: Results of microbiological studies (HCP_{05}=1)

Concentration of *D. solani* in the suspension, CFU/ml		3.2×10^7	3.2×10^6	3.2×10^5	3.2×10^4	3.2×10^3
Initial contamination on the test object (N_0), CFU		3.2×10^6	3.2×10^5	3.2×10^4	3.2×10^3	3.2×10^2
Radiant variant		**Residual contamination on the test object (N_k), CFU**				
Control without radiation		loan	loan	loan	loan	320
Dosage UV-S 30 mJ/cm²	*Xe*-lamp	2	0	3	3	1
	Hg-lamp	7	2	0	0	2
Dosage UV-S 300 mJ/cm²	*Xe*-lamp	0	5	0	0	0
	Hg-lamp	0	0	0	0	2

Residual contamination at the level of the count of individual bacteria indicates the probabilistic nature of the UV disinfection process or is associated with errors in the microbiological experiment.

In a series of experiments with small doses of radiation (2–24 mJ/cm²) and low levels of contamination of the surface (from single bacteria to 2.8×10^3 CFU), after treatment with UV radiation, single colonies (from 2 to 5) were observed only in experiments with a minimum radiation dose (2 mJ/cm²) and a maximum initial contamination at the test object $N_0 = 2.8 \times 10^3$ CFU; in all other implementations, both with the use of *Hg*- and *Xe*-lamps, colonies growth was not observed.

From the data obtained, it follows that *D. solani* is characterized by a very high sensitivity to the action of UV radiation - already at doses of UV-S radiation at a level of 2 mJ/cm², the surface contamination is reduced by more than two orders of magnitude. This allows an upper estimate of the energy dose of D_{10} for *D. solani* bacteria of the order of 1 mJ/cm² (for comparison - D_{10} for *Escherichia coli* is 3 mJ/cm² and for staphylococcus 5 mJ/cm² surface).[8-10]

The high sensitivity of the test microorganisms under investigation did not make it possible to draw a reasoned conclusion about the effect of UV radiation (its spectrum and intensity) on biocidal efficacy within the framework of the conducted experiments. In this context, experiments can be made to evaluate the effect of UV radiation on the growth of macerated potato tissue after its inoculation with *D. solani*. The results of these experiments, averaged over three replicates, are shown in Table 2, as well as in Figure 2.

As it can be seen from the presented data, UV radiation suppresses the growth of macerated tissue on inoculated potato slices in comparison with the control one, i.e., unirradiated, sample, while the pulsed broadband radiation of the *Xe*-lamp has a more pronounced effect than the *Hg*-lamp.

Thus, the relatively recently discovered and extremely harmful phytopathogen *D. solani* is characterized by a high sensitivity to the action of UV radiation as a biocidal agent.

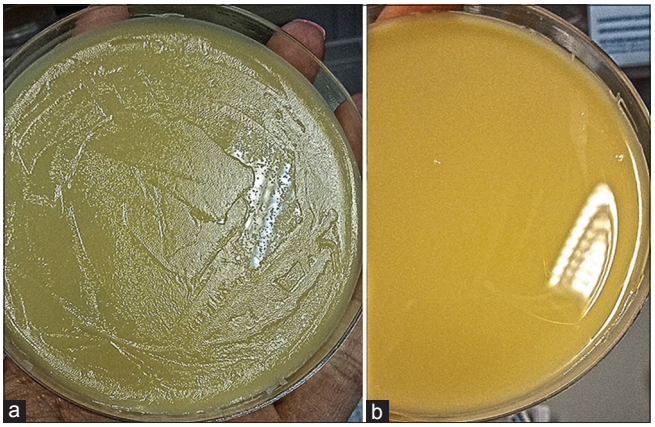

Figure 1: The efficiency of irradiation by the example of *Xe* lamp treatment: (a) Control sample; (b) treatment with the impulse xenone lamp with the dosage UV-S 30 mJ/cm². Initial contamination on the test object - 3.2×10^6 CFU

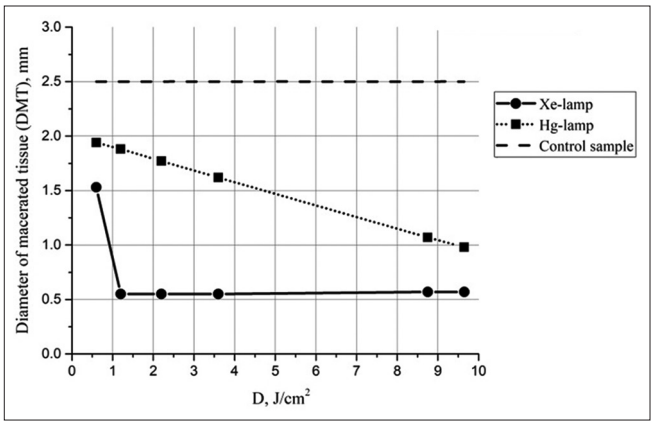

Figure 2: The results of irradiation of inoculated *Dickeya solani* slices of potatoes with *Xe*- and *Hg*-lamps

CONCLUSION

The obtained results show the prospects of using UV disinfection technologies and corresponding technical means in a complex of phytosanitary measures to combat the bacterial bacteriasis in seed stock preparation and long-term storage.

Table 2: The results of irradiation of inoculated *D. solani* slices of potatoes with *Hg*- and *Xe*-lamps, HCP_{05}=0.81

Experiment variant	Diameter of the macerated tissue, mm	
Control - H_2O without radiation	no	
Control - *D. fil* culture without irradiation	2.5	
Dosage UV-S, mJ/cm²	**Impulse xenon lamp**	**Mercury lamp**
600	15.3	19.4
1200	5.5	18.8
2200	5.5	17.7
3600	5.5	16.2
8750	5.7	10.7
9650	5.7	9.8

D. solani: Dickeya solani, UV: Ultraviolet

With respect to *D. solani*, the *Xe* lamp as a source of biocidal radiation has a more pronounced antimicrobial effect than the traditional *Hg* lamp. High flow density of biocide radiation of *Xe* lamps allows to significantly reduce the processing time, which makes it possible to create on their basis high-performance disinfection plants, including the ones of the conveyor type.

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