Journal of Food Protection, Vol. 76, No. 10, 2013, Pages 1761–1766
doi:10.4315/0362-028X.JFP-12-511

Inactivation of *Penicillium digitatum* and *Penicillium italicum* under In Vitro and In Vivo Conditions by Using UV-C Light

GÜLTEN TİRYAKİ GÜNDÜZ* AND FIKRET PAZIR

Ege University, Engineering Faculty, Food Engineering Department, 35100 Bornova, Izmir, Turkey

MS 12-511: Received 12 November 2012/Accepted 14 June 2013

ABSTRACT

In this study, the effects of UV-C on two of the main wound pathogens of citrus fruits, *Penicillium digitatum* and *Penicillium italicum,* were investigated with different inoculation methods in vitro and on oranges. *P. digitatum* and *P. italicum* spores were inoculated onto the surface of potato dextrose agar or oranges using spread, spot, wound, and piercing inoculation methods. UV-C treatment for 1 min from a working distance of 8 cm reduced the numbers of *P. italicum* and *P. digitatum* by about 3.9 and 5.3 log units, respectively, following spread inoculation under in vitro conditions. Significant reductions were obtained after 1-min UV-C treatments of the tested fungi following inoculation using the spread and spot methods. With inoculation by the wound and piercing methods, the tested spores were not inactivated completely even after 10- and 20-min treatment times, respectively. The application of UV-C (7.92 kJ m^{-2}) on oranges reduced the percentage of oranges infected at least threefold compared with the rate of infection in the untreated control samples. UV-C irradiation could effectively inactivate spores of *P. italicum* and *P. digitatum* inoculated by the spread plate and spot inoculation methods under in vitro and in vivo conditions. On the other hand, because of the low penetration ability of UV-C light, the tested fungi were not completely inactivated following inoculation with the wound and piercing methods. UV-C treatment has potential for use in surface decontamination of citrus fruits.

Penicillium digitatum Sacc. (green mold) and *Penicillium italicum* Wehmer (blue mold) are the main wound pathogens of citrus fruits, causing the most-common and most-devastating postharvest diseases. They occur in all citrus growing countries worldwide and may attack the fruits in packing houses, in transit, in storage, and in the market *(1)*.

There are existing agrochemicals that successfully aid in controlling postharvest diseases *(10)*. On the other hand, a significant public debate concerning pesticide residues in foods emerged in the late 20th century and continues today because of the risks posed by pesticides to the environment, to nontarget organisms, and to humans *(33)*. In addition, the use of fungicides has led to the development of resistant populations. Kinay et al. *(17)* isolated 166 isolates of fungicide-resistant *P. digitatum* from citrus fruit packaging houses in California. For these reasons, there is a pressing need to find alternative treatments for the preservation of citrus fruits.

UV technology has been known for over 60 years, but commercial equipment is primarily manufactured for the pharmaceutical and aquaculture industries, which cannot tolerate chemical disinfecting *(20)*. UV-C radiation in the range of 250 to 260 nm, which shows the maximum effect at 254 nm, is lethal to most microorganisms, including bacteria, viruses, protozoa, mycelial fungi, yeasts, and algae. In practice, UV-C treatments have been used for inhibition of microorganisms on surfaces such as packaging materials, in the disinfection of airborne bacteria in hospitals, and in water sterilization *(4)*. The U.S. Food and Drug Administration approved the use of UV radiation for the processing and treatment of food and food products with an intensity of 1 W (253.7 nm) per 5 to 10 ft^2 (1.076 to 2.152 W m^{-2}) for the control of surface microorganisms *(5)*. The relatively quick exposure time and the lack of any residual compound on the surface of fresh fruits are the advantages of UV-C treatments. The use of UV-C may prove to be beneficial in protecting the safety of fruits and vegetables, in conjunction with good agricultural practices and good manufacturing practices *(34)*.

Many studies have confirmed that the use of UV-C could increase the shelf life of fruits and vegetables, such as strawberry *(22),* broccoli florets *(6),* fresh-cut melon *(21),* zucchini squash *(8),* and grapefruit *(7)*. Researchers have shown antifungal activity of UV-C on *Penicillium* spp., *Aspergillus* spp., *Rhizopus* spp., *Byssochlamys* spp. *(13), Aspergillus flavus, Aspergillus niger, Penicillium corylophilum, Eurotium rubrum (2), A. flavus,* and *A. fumigatus (12)* under in vitro conditions. Gök and Pazır *(11)* determined that UV-C treatment of olives for 10 min from a distance of 10 cm resulted in 1.33-log reductions of yeast and mold counts. Studies on the antifungal activity of UV-C on *P. digitatum* under in vitro conditions are limited *(10),* and to the best of our knowledge, there are no studies about the antifungal activity of UV-C light on *P. italicum* under in vitro and in vivo conditions.

* Author for correspondence. Tel: (+90 232) 311 30 03; Fax: (+90 232) 342 75 92; E-mail: gulten.tiryaki.gunduz@ege.edu.tr.

Determination of the effective dose for UV-C treatment is very important. It is necessary to determine the exposure levels needed to control these organisms under conditions optimum for their survival *(10)*. The aim of this study was to investigate the effect of UV-C in controlling postharvest pathogens of citrus fruits under in vitro and in vivo conditions, using different inoculation methods to simulate the ways in which pathogens contaminate citrus fruits.

MATERIALS AND METHODS

Fungal inoculum. *P. digitatum* and *P. italicum* isolates were obtained from decayed orange (*Citrus sinensis* ''Washington navel'') and mandarin (*Citrus reticulata*), respectively, from İzmir, Turkey. The isolates were identified by considering their cultural and morphological properties *(15, 26, 30, 31)*. The isolates were also evaluated for their pathogenicity on oranges and mandarins *(18)*. According to the pathogenicity tests, the isolates were shown to be pathogenic for oranges and mandarins and caused the typical symptoms of blue and green mold growth. Prior to each experiment, the isolates were grown on potato dextrose agar (PDA, pH 5.6; Oxoid, Basingstoke, England) in slant tubes at 25°C for 7 days. Spores were subsequently harvested by flooding the surface of the medium with 2 ml of sterile distilled water containing Tween 80 (Merck KGaA, Darmstadt, Germany) (0.05% wt/vol) and vortexing the slant tube with a vortex mixer for 30 s to dislodge the spores. The inoculum concentrations were adjusted to 10^6 spores per ml by using a Thoma counting grid with sterile distilled water containing Tween 80 (0.05%, wt/vol) *(25)*.

Plating to petri dishes. Four different inoculation methods were applied, as follows: (i) spread plate, (ii) spot, (iii) wound, and (iv) piercing. In the spread plate inoculation method, 100 ml of the spore suspension described above was spread on PDA previously poured into petri dishes *(12)*. The spot inoculation method consisted of spotting 10 μl of the spore suspension onto the middle of previously poured PDA plates (about 10^4 spores per plate) *(34)*. Wound inoculation was performed by first streaking PDA medium in petri dishes with a sterile needle to about 5 mm in length and <1 mm in depth and then applying 10 μl of inoculum onto the wounded medium *(7, 27)*. The piercing inoculation method consisted of piercing the medium with a stainless steel rod with a diameter of 2 mm to a depth of 2 mm *(3, 16, 25)*. The stainless steel rod was immersed in the spore suspensions prior to each piercing operation. Inoculated plates were left for about 1 h before the UV-C treatments for the absorption of inoculated fungal spores.

UV-C treatments under in vitro conditions. Inoculated petri dishes without the lids were treated with different UV-C doses (0.84 to 30 kJ m^{-2}) under TUV 15 W/G15 T8 lamps (Philips, Roosendaal, Holland). The lamps had a tube diameter of 2.5 cm and a length of 41.3 cm. The UV-C light intensity was kept constant, and the doses applied were varied (0.84 to 30 kJ m^{-2}) by altering the exposure time (from 1 min to 20 min) and the distance (8, 16, and 24 cm) between the plate and the UV-C light in a closed UV sterilizer with four lamps (UV-Entkeimungsschrank, Ernst Schütt Jun. Laborgerätebau, Göttingen, Germany). Four plates were used for each treatment, and the treatments were repeated in three replicates. The UV intensity applied to the plates was determined by using a UVX digital radiometer (UVP, Inc., Upland, CA). The uniformity of the UV intensity in the UV sterilizer was also determined by using the UVX digital radiometer. After the UV-C treatments, the plates were covered immediately and incubated at 25°C for 5 days in the dark to prevent photoreactivation. After the incubation period, the colonies were counted using the spread plate inoculation method, and the reductions in log units were calculated. For the control treatment of the spread plate technique, decimal dilutions of the spore suspension were prepared and duplicate 0.1-ml samples of appropriate dilutions were plated on PDA by using the spread plate technique. In the case of the spot, wound, or piercing inoculation method, the diameter of growth was measured in millimeters after 5 days of incubation and the inhibition of growth was calculated as the percentage of reduction. Untreated plates were used as controls for each of the spot, wound, and piercing inoculation methods.

Inoculation of oranges. Mature oranges (*C. sinensis* var. Washington navel) were obtained from a local market in Bornova, İzmir, Turkey, and stored at 15°C for a maximum of 2 days until use. The oranges were washed with tap water to remove dirt, wiped with a paper towel, and dried. *P. digitatum* and *P. italicum* spore suspensions (10^6 spores per ml) were inoculated by using the spot, wound, and piercing inoculation methods as in the in vitro studies described above. Each orange was inoculated at three sites around the stem end *(7)*. The inoculated oranges were incubated at 25°C for 24 h in plastic boxes to let the inoculums dry.

UV-C treatment of oranges. Twenty-four hours after the inoculation of oranges, the oranges were placed stem end up at a distance of 10 cm from the UV-C lamps and treated for 5 min (7.92 kJ m^{-2}). The UV-C dose was selected based on the results of the in vitro studies described above. Each treatment consisted of 12 oranges with three inoculation sites, giving a total of 36 wounds per treatment *(7)*. Twelve untreated oranges were used as a control. After the UV-C treatments, the oranges were stored in the dark in plastic boxes at 25°C for 6 days at 85 to 90% relative humidity. For the spot inoculation, UV-C–treated oranges were streaked about 1 cm in length and 1 mm in depth with a sterile utility knife to give surviving mold spores the opportunity to grow after the UV-C treatment. After the storage period, the percentage of infection was determined as the fraction of infected wound sites and compared with the rate of infection of the untreated control samples *(7)*.

Statistical analysis. All experiments were repeated three times with two parallel treatments, and duplicate plates were analyzed at each treatment. Significant differences between the means were established by analysis of variance and Duncan's multiple range test. Data were analyzed with the SPSS statistical package (SPSS 14.0 for Windows, evaluation version, SPSS, Inc., Chicago, IL).

RESULTS

In vitro studies. The treatment times and distances of the UV-C doses are shown in Table 1. The efficacies of UV-C treatment (0.84 to 30 kJ m^{-2}) on *P. italicum* and *P. digitatum* inoculated onto PDA plates with the spread, spot, wound, and piercing methods are given in Tables 2 and 3, respectively. In the spread plate method, the numbers of *P. italicum* and *P. digitatum* organisms were reduced significantly even after 1 min of treatment with UV-C and were reduced to undetectable levels with the UV-C treatments for 5 min (4.20 to 4.98 kJ m^{-2}) and 3 min (2.99 to 4.50 kJ m^{-2}), respectively.

The growth of spot-inoculated *P. italicum* and *P. digitatum* was reduced by ranges of 82.5 to 100% and 56.2 to 100%, respectively, compared with the growth on the

TABLE 1. *UV-C doses at different times and distances*

Treatment time (min)	Mean (SD) UV-C dose (kJ m^{-2}) at[a]:		
	8 cm	16 cm	24 cm
1	1.50 (0.06)	1.00 (0.05)	0.84 (0.07)
3	4.50 (0.18)	2.99 (0.16)	2.52 (0.20)
5	7.50 (0.30)	4.98 (0.27)	4.20 (0.33)
10	15.00 (0.60)	9.96 (0.54)	8.40 (0.66)
15	22.50 (0.90)	14.94 (0.81)	12.60 (0.99)
20	30.00 (1.20)	19.92 (1.08)	16.80 (1.32)

[a] Values are the means of three replicates.

untreated control plates. The diameters of the *P. italicum* and *P. digitatum* colonies from spot inoculation after 1 min of UV-C treatment (0.84 to 1.50 kJ m^{-2}) were decreased significantly compared with the growth of the nontreated control samples. The growth diameters of *P. italicum* and *P. digitatum* colonies were reduced to undetectable levels with UV-C treatments for 5 min (7.50 kJ m^{-2}) from a distance of 8 cm.

Using the wound inoculation method, reductions of 26.8 to 87.1% and 27.2 to 100% were observed for *P. italicum* and *P. digitatum,* respectively, depending on the treatment times and distances. The spores of *P. italicum* inoculated with the wound method were not completely inactivated even after 10 min of UV-C treatment, regardless of the working distances.

With the piercing inoculation method, maximum reductions of about 10 to 11% were obtained; however, for both fungi, the diameters of the treated colonies did not differ significantly from those of untreated colonies ($P > 0.05$).

In vivo studies. The percentages of oranges infected with *P. digitatum* and *P. italicum* following artificial inoculation with the spot, wound, and piercing methods are given in Table 4. The incidence of *P. digitatum* and *P. italicum* on the oranges treated with UV-C at 7.92 kJ m^{-2} was significantly lower than their incidence on the control oranges regardless of the inoculation method ($P < 0.05$). With the spot inoculation method, no infected oranges were observed after the UV-C treatment, which means that all of the inoculated spores were killed by UV-C treatment.

Significantly higher percentages of infected oranges were obtained in both treated and nontreated oranges inoculated with the piercing method ($P < 0.05$). The percentages of infection of *P. digitatum* and *P. italicum* on the oranges inoculated with the piercing method were reduced about threefold when treated with a UV-C dose of 7.92 kJ m^{-2}. Increasing the UV-C exposure time to 10 min (15.84 kJ m^{-2}) and 20 min (31.68 kJ m^{-2}) resulted in increases in the percentages of *P. italicum*–infected oranges to 50% and 80%, respectively, when inoculated by the piercing method (data not shown).

TABLE 2. *Populations of* Penicillium italicum *recovered after UV-C treatments*

Inoculation method	Treatment time (min)	Mean (SD) population and reduction of *P. italicum* (log CFU/plate[a] or colony diam[b] in mm) at UV-C treatment distance of[c]:					
		8 cm		16 cm		24 cm	
		Population	Reduction	Population	Reduction	Population	Reduction
Spread plate	0	4.3 (0.5)		4.8 (0.1)		4.8 (0.1)	
	1	0.4 (0.1)	3.9 A a	0.1 (0.6)	4.71 B a	0.4 (0.3)	4.4 B a
	3	0.3 (0.0)	4.0 A a	0.2 (0.0)	4.66 B a	0.1 (0.2)	4.8 B b
	5	0.4 (0.7)	3.8 A a	<0 AB b	>4.84 B a	<0	>4.8 B b
	10	0.2 (0.5)	4.0 A a	<0 AB b	>4.84 B a	<0	>4.8 B b
	15	0.9 (0. 6)	3.3 A a	<0 AB b	>4.84 B a	<0	>4.8 B b
	20	0.7 (0.6)	3.6 A a	<0 AB b	>4.84 B a	<0	>4.8 B b
Spot	0	47.8 (7.8)		47.7 (9.3)		47.5 (9.0)	
	1	1.7 (2.9)	96.5 A a	4.8 (8.4)	89.87 A a	8.3 (8.8)	82.5 A a
	5	0.0 (0.0)	100 A a	3.5 (6.1)	92.66 A a	0.0 (0.0)	100 A a
	10	0.0 (0.0)	100 A a	0.0 (0.0)	100 A a	0.0 (0.0)	100 A a
Wound	0	43.7 (3.0)		48.5 (8.8)		48.5 (8.8)	
	1	24.4 (16.5)	44.1 A a	23.2 (5.2)	52.06 A a	35.5 (9.0)	26.8 A a
	5	24.2 (21.0)	44.6 A a	6.2 (5.7)	87.11 A b	14.6 (11.8)	70.0 A b
	10	24.7 (21.6)	43.5 A a	8.5 (7.5)	82.47 A b	11.3 (3.6)	76.6 A b
Piercing	0	39.8 (4.3)		45.3 (6.5)		45.3 (6.5)	
	5	40.0 (1.3)	—[d]	41.8 (3.7)	7.72 A a	44.6 (6.6)	1.6 A a
	10	37.1 (1.7)	6.9 A a	41.1 (3.4)	9.37 A a	45.6 (5.4)	—[d]
	20	36.4 (6.0)	8.6 A a	40.3 (3.8)	11.03 A a	42.0 (9.3)	7.4 A a

[a] With the spread plate method, the numbers of colonies were converted to log CFU per petri dish.
[b] With spot, wound, and piercing methods, diameters of colonies were measured.
[c] Values are the means of three replicates and two parallel treatments. Values within the same row not followed by the same capital letter are significantly different ($P < 0.05$). Within a column with results using the same inoculation method, values not followed by the same lowercase letter are significantly different ($P < 0.05$).
[d] No reduction was observed.

TABLE 3. *Populations of* Penicillium digitatum *recovered after UV-C treatments*

Inoculation method	Treatment time (min)	Mean (SD) population and reduction of *P. digitatum* (log CFU/plate[a] or colony diam[b] in mm) at UV-C treatment distance of[c]: 8 cm Population	8 cm Reduction	16 cm Population	16 cm Reduction	24 cm Population	24 cm Reduction
Spread plate	0	5.3 (0.52)		4.7 (0.1)		5.3 (0.1)	
	1	0.0 (0.00)	5.3 A a	0.1 (0.4)	4.6 B a	0.4 (0.3)	4.9 A a
	3	<0	>5.3 A a	<0	4.7 B a	0.1 (0.2)	5.2 A a
	5	0.6 (0.0)	4.6 A a	<0	>4.7 B a	0.1 (0.2)	5.2 A a
	10	0.1 (0.0)	5.2 A a	<0	>4.7 B a	<0	>5.3 A a
	15	<0	>5.3 A a	<0	>4.7 B a	<0	>5.3 A a
	20	<0	>5.3 A a	<0	>4.7 B a	<0	>5.3 A a
Spot	0	37.0 (8.0)		46.0 (1.7)		40.0 (2.6)	
	1	6.2 (5.6)	83.3 A a	19.4 (19.5)	57.8 A a	17.5 (7.8)	56.2 A a
	5	0.0 (0.0)	100 A b	2.1 (3.6)	95.5 A b	4.2 (3.6)	89.6 A b
	10	0.0 (0.0)	100 A b	0.0 (0.0)	100 A b	0.0 (0.0)	100 A b
Wound	0	40.2 (2.5)		43.3 (5.8)		39.5 (1.8)	
	1	29.2 (9.7)	27.2 A a	30.2 (11.3)	30.2 A a	24.9 (15.8)	36.9 A a
	5	12.8 (14.0)	68.1 A b	19.0 (18.5)	56.2 A b	6.4 (6.9)	83.8 A b
	10	9.9 (15.7)	75.3 A b	1.8 (3.0)	96.0 A b	0.0 (0.0)	100 A b
Piercing	0	39.0 (2.2)		45.3 (7.2)		41.8 (3.5)	
	5	34.9 (4.9)	10.5 A a	46.1 (6.3)	—[d]	40.2 (4.1)	3.8 A a
	10	38.2 (0.9)	1.9 A a	45.8 (6.4)	—[d]	39.2 (4.8)	6.4 A a
	20	37.5 (3.3)	3.8 A a	44.8 (5.0)	1.1 A a	37.8 (6.0)	9.6 A a

[a] With the spread plate method, the numbers of colonies were converted to log CFU per petri dish.
[b] With spot, wound, and piercing methods, diameters of colonies were measured.
[c] Values are the means of three replicates and two parallel treatments. Values within the same row not followed by the same capital letter are significantly different ($P < 0.05$). Within a column with results using the same inoculation methods, values not followed by the same lowercase letter are significantly different ($P < 0.05$).
[d] No reduction was observed.

DISCUSSION

In this study, the efficacy of UV-C treatment on wound pathogens of citrus fruits was investigated in vitro and on oranges (*C. sinensis* var. Washington navel) with different inoculation methods. The spread plate inoculation technique was used to investigate the inactivation of spores on the surface of food by using agar to model the food surface. The other three inoculation methods were used to represent the natural ways in which citrus fruits are contaminated, as used for the inoculation of molds onto fruits and vegetables in most studies.

TABLE 4. *Percentages of oranges infected following artificial inoculation with* P. digitatum *and* P. italicum *and UV-C treatment*

Mold	Inoculation method	% of oranges infected[a] Untreated	% of oranges infected[a] UV-C treated
P. digitatum	Spot	22.2 a A	0 a B
	Wound	45.0 b A	15.3 b B
	Piercing	63.8 c A	21.2 c B
P. italicum	Spot	13.9 d A	0 a B
	Wound	18.0 e A	5.6 d B
	Piercing	77.5 f A	23.8 e B

[a] Values are the means of two replicates and two parallel treatments. Values within the same column not followed by the same lowercase letter are significantly different ($P < 0.05$). Values within the same row not followed by the same capital letter are significantly different ($P < 0.05$).

Higher reductions of the tested fungi were obtained when the spread plate inoculation method was used. Similar to our results, Fernandez and Hall *(10)* reported that a UV-C dose of 3.96 kJ m^{-2} completely killed *P. digitatum* inoculated onto PDA plates. Green et al. *(12)* reported that 0.35 and 0.54 kJ m^{-2} germicidal irradiation can cause 90% inactivation of *A. flavus* and *A. fumigatus,* respectively, on an agar surface. UV-C exposure of 60 s reduced the survival of tested fungi (*Alternaria alternata, Aspergillus carbonarius, A. niger, Cladosporium herbarum,* and *Penicillium janthinellum*) isolated from grapes and raisins by more than 90% in plates inoculated by spreading the inoculum onto the surface of agar *(32)*. Hamanaka et al. *(13)* reported that *Penicillium* spp. isolated from citrus fruits were reduced about 3 log by a UV-C treatment of 6 kJ m^{-2} in vitro. Hamanaka et al. *(14)* observed that the number of *Rhodotorula mucilaginosa* cells in PDA plates were reduced by 1.50 log units after treatment with 1 kJ m^{-2} of UV irradiation. With the wound and piercing inoculation methods, the inoculated spores penetrate into the agar and spores are protected from UV-C irradiation compared with the exposure of spores on the surface of agar. The efficacy of UV-C light treatment could be reduced by shadowing effects or internalization of microorganisms in food tissues *(21)*. Begum et al. *(2)* also indicated that tested spores were

most susceptible to UV-C inactivation when spread in a monolayer on an agar surface. *A. niger* on the surface of agar showed a 2-log reduction after 30 to 60 s, whereas UV-C exposure for 120 to 180 s was required to achieve the same level of inactivation in aqueous Tween 80. UV-C radiation is not very penetrating, and microorganisms are not affected if they are protected by solids, such as particles, dust, or covers *(20)*. The presence of irregularities which would protect microorganisms from incident UV is a limiting factor of this technology *(4)*.

Significant differences in the levels of reduction of *P. italicum* or *P. digitatum* were not obtained for the treatment distances of 8, 16, and 24 cm for each treatment time in the spread plate (only *P. digitatum*), spot, wound, and piercing inoculation methods ($P > 0.05$). On the other hand, in the spread plate inoculation method, significant differences in the reduction of *P. italicum* were obtained between the working distances of 8 cm and 16 or 24 cm for each treatment time ($P < 0.05$). Differences among the reductions between the treatment distances or times are related to the inoculation methods, as well as the tested pathogens. The UV-C doses applied to plates for 1 min at the treatment distances of 8, 16, and 24 cm were 1.5, 1.0, and 0.84 kJ m^{-2}, respectively. As similar doses were applied for each distance, no significant effect of distance was observed for a given treatment time.

Most of the treatment conditions applied in the in vitro studies showed that an increased treatment time and a decreased working distance did not result in any additional significant reductions in the numbers of the citrus pathogens tested. For this reason, additional UV-C treatment or increased dose did not increase the reductions of test pathogens significantly. This unexpected result can be explained by the rapid inactivation of the tested fungi after 1-min UV-C treatments. The National Advisory Committee on Microbiological Criteria for Foods *(23)* has also indicated that UV-C treatment does not demonstrate linear inactivation kinetics and that rapid inactivation is often followed by a tailing of survival.

In vivo studies on oranges treated with 7.92 kJ m^{-2} of UV-C (working distance of 10 cm for 5 min) showed about threefold reductions of the percentage of disease incidence with the wound and piercing inoculation methods. No growth of pathogens was observed on spot-inoculated oranges. Other studies have also confirmed that the percentage of peaches naturally infected with brown rot was reduced fourfold compared with the results for the control when they were treated with a UV-C dose of 7.5 kJ m^{-2} *(29)*. Stevens et al. *(28)* determined that the incidence of *P. digitatum* on tangerines treated with a UV-C dose of 1.3 kJ m^{-2} was 55% after the storage period of 53 h, whereas in the control, it was 100%. Manzocco et al. *(21)* also reported that UV-C light treatment represents a novel technology with a high potential to achieve surface decontamination of ready-to-eat fruit products while improving their sensory properties.

In the present study, the lower UV-C dose of 7.92 kJ m^{-2} (working distance of 10 cm for 5 min) showed greater inhibitory effects on *P. italicum* inoculated onto oranges than the higher UV-C doses of 15.84 and 31.68 kJ m^{-2} (working distance of 10 cm for 10 and 20 min, respectively). High levels of UV-C (15.84 kJ m^{-2}) adversely affected oranges by causing darkened color of the oranges. Also, high UV-C doses increased the susceptibility of oranges to *P. italicum*. The increase in the UV-C dose to 15.84 and 31.68 kJ m^{-2} resulted in increases in the percentage of infected oranges that were inoculated with *P. italicum* by the piercing method, to 50 and 80%, respectively. Escalona et al. *(9)* observed similar results for *Salmonella* counts. They reported that low UV-C doses (2.4 to 7.2 kJ m^{-2}) showed greater inhibitory effects than high UV-C doses (12 to 24 kJ m^{-2}) on *Salmonella* spp. Nigro et al. *(24)* also found that *Botrytis cinerea* inoculated onto table grapes was reduced by using low UV-C doses, ranging from 0.125 to 0.5 kJ m^{-2}; however, higher UV-C doses resulted in an increasing number of infected berries and larger lesion diameters. It was indicated that the percentage of decay caused by *A. alternata* on tomatoes treated with a UV-C dose of 7.5 kJ m^{-2} was 29% and that of the control was 85%, while on the other hand, when the UV-C dose was increased to 40 kJ m^{-2}, the rate of decay increased to 77% *(19)*. The authors concluded that higher UV-C doses on tomatoes caused noticeable dull skin blemishes and increased the susceptibility of tomato fruits to storage rots. Stevens et al. *(29)* also indicated that high UV-C doses (20 to 40 kJ m^{-2}) increased the susceptibility of peaches to *Monilinia fructicola*. Escalona et al. *(9)* observed that higher doses of UV are detrimental and lower doses stimulate beneficial reactions in the quality of baby spinach leaves. Studies under both in vitro and in vivo conditions have confirmed that higher doses of UV-C are not efficient to inactivate microorganisms. On the other hand, Ben-Yehoshua et al. *(3)* reported that a lower UV-C dose (5 kJ m^{-2}) was not effective in reducing the percentage of lemon fruit with decay, and an 88% incidence of decay was obtained for both UV-C–treated and untreated control samples.

To the best of our knowledge, this is the first study to use the inoculation methods of spot, wound, and piercing to determine the efficacy of UV-C treatment in vitro. Even though we obtained greater reductions with the spread and spot inoculation methods, the ways in which contamination occurs under natural conditions should be taken into consideration. Since the spread and spot inoculation methods cannot give an exact quantification of the reduction if there are wounds and crevices on the surfaces of fruits and vegetables, it is better to use the wound and piercing inoculation methods to determine the efficacy of UV treatment on wound pathogens of fruits and vegetables.

The results obtained in the present study show that if spores are on the surfaces of citrus fruits, UV-C treatments with 7.92 kJ m^{-2} will be enough to inactivate them without any damage to the sensory properties. If there were wounds and cracks on the surfaces of fruits, the percentage of infected oranges decreased threefold compared with the results for the nontreated control samples. A higher UV-C dose of about 16.8 kJ m^{-2} is needed to reduce the tested fungi to safe levels in vitro. On the other hand, the in vivo studies showed that higher UV-C doses increased the susceptibility of oranges to wound pathogens and affected

sensory properties. The results of this study also indicate that the ways in which fruits are contaminated should be taken into consideration in designing studies about the efficacy of UV-C treatments on postharvest pathogens of fruits. UV-C treatment at lower doses would be a good alternative to chemical fungicides for citrus fruits.

ACKNOWLEDGMENT

The financial support of Ege University Research Foundation (project no. 2010-MUH-056) is gratefully acknowledged.

REFERENCES

1. Barkai-Golan, R. 2001. Postharvest diseases of fruits and vegetables—development and control. Elsevier Science, Amsterdam.
2. Begum, M., A. D. Hocking, and D. Miskelly. 2009. Inactivation of food spoilage fungi by ultra violet (UVC) irradiation. *Int. J. Food Microbiol.* 129:74–77.
3. Ben-Yehoshua, S., V. Rodov, J. J. Kim, and S. Carmeli. 1992. Preformed and induced antifungal materials of citrus fruits in relation to the enhancement of decay resistance by heat and ultraviolet treatments. *J. Agric. Food Chem.* 40:1217–1221.
4. Bintsis, T., E. Litopoulou-Tzanetaki, and R. K. Robinson. 2000. Existing and potential applications of ultraviolet light in the food industry—a critical review. *J. Sci. Food Agric.* 80:637–645.
5. Code of Federal Regulations. 2011. 21 CFR 179.39. Title 21—Food and drugs; chap. I—Food and Drug Administration, Department of Health and Human Services; subchap. B—Food for human consumption (continued); part 179—Irradiation in the production, processing and handling of food; subpart B—Radiation and radiation sources; sec. 179.39—Ultraviolet radiation for the processing and treatment of food. Code Fed. Regul. 3:456–457.
6. Costa, L., A. R. Vicente, P. M. Civello, A. R. Chaves, and G. A. Martínez. 2006. UV-C treatment delays postharvest senescence in broccoli florets. *Postharvest Biol. Technol.* 39:204–210.
7. Droby, S., E. Chalutz, B. Horev, L. Cohen, V. Gaba, C. L. Wilson, and M. Wisniewski. 1993. Factors affecting UV-induced resistance in grapefruit against the green mould decay caused by *Penicillium digitatum*. *Plant Pathol.* 42:418–424.
8. Erkan, M., C. Y. Wang, and D. T. Krizek. 2001. UV-C irradiation reduces microbial populations and deterioration in *Cucurbita pepo* fruit tissue. *Environ. Exp. Bot.* 45:1–9.
9. Escalona, V. H., E. Aguayo, G. B. Martínez-Hernández, and F. Artés. 2010. UV-C doses to reduce pathogen and spoilage bacterial growth *in vitro* and in baby spinach. *Postharvest Biol. Technol.* 56:223–231.
10. Fernandez, Y. J., and D. J. Hall. 2004. *In vitro* response of *Penicillium digitatum* and *Geotrichum candidum* to ultraviolet (UV-C) exposure. *Proc. Fla. State Hortic. Soc.* 117:380–381.
11. Gök, S. B., and F. Pazır. 2011. Efficacy of some non thermal operations on shelf life of packaged black olive in the absence of brine. Presented at the 11th International Congress on Engineering and Food, Athens, Greece, 22 to 26 May 2011. Available at: www.icef11.org/content/papers/nfp/NFP102.pdf. Accessed 20 March 2013.
12. Green, C. F., P. V. Scarpino, P. J. Nancy, J. Jensen, and S. G. Gibbs. 2004. Disinfection of selected *Aspergillus* spp. using ultraviolet germicidal irradiation. *Can. J. Microbiol.* 50:221–224.
13. Hamanaka, D., G. G. Atungulu, F. Tanaka, and T. Uchino. 2010. Effect of combining infrared heating with ultraviolet irradiation on inactivation of mold spores. *Food Sci. Technol. Res.* 16:279–284.
14. Hamanaka, D., N. Norimura, N. Baba, K. Mano, M. Kakiuchi, F. Tanaka, and T. Uchino. 2011. Surface decontamination of fig fruit by combination of infrared radiation heating with ultraviolet irradiation. *Food Control* 22:375–380.
15. International Mycological Association. 2010. MycoBank online taxonomic novelties. Fungal databases: nomenclature and species banks, *Penicillium italicum* and *Penicillium digitatum*. Available at: http://www.mycobank.org/MycoTaxo.aspx?Link=TRec=162660 and http://www.mycobank.org/MycoTaxo.aspx?Link=T&Rec=169502. Accessed 26 April 2010.
16. Kim, J. J., S. Ben-Yehoshua, B. Shapiro, Y. Henis, and S. Carmeli. 1991. Accumulation of scoparone in heat-treated lemon fruit inoculated with *Penicillium digitatum* Sacc. *Plant Physiol.* 97:880–885.
17. Kinay, P., M. F. Mansour, F. M. Gabler, D. A. Margosan, and J. L. Smilanick. 2007. Characterization of fungicide-resistant isolates of *Penicillium digitatum* collected in California. *Crop Prot.* 26:647–656.
18. Köhl, J., G. B. Haas, H. G. Geijn, A. Speksnijder, P. Kastelein, S. Hoog, and B. G. Ende. 2009. Pathogenicity of *Stemphylium vesicarium* from different hosts causing brown spot in pear. *Eur. J. Plant Pathol.* 124:151–162.
19. Liu, J., C. Stevens, V. A. Khan, J. Y. Lu, C. L. Wilson, O. Adeyeye, M. K. Kabwe, P. L. Pusey, E. Chalutz, T. Sultana, and S. Droby. 1993. Application of ultraviolet-C light on storage rots and ripening of tomatoes. *J. Food Prot.* 56:868–872.
20. López-Malo, A., and E. Palou. 2005. Ultraviolet light and food preservation, p. 405–421. *In* G. V. Barbosa-Cánovas, M. S. Tapia, and M. P. Cano (ed.), Novel food processing technologies. CRC Press, Boca Raton, FL.
21. Manzocco, L., S. Da Pieve, and M. Maifreni. 2011. Impact of UV-C light on safety and quality of fresh-cut melon. *Innov. Food Sci. Emerg. Technol.* 12:13–17.
22. Marquenie, D., C. W. Michiels, A. H. Geeraerd, A. Schenk, C. Soontjens, J. F. Van Impe, and B. M. Nicolaï. 2002. Using survival analysis to investigate the effect of UV-C and heat treatment on storage rot of strawberry and sweet cherry. *Int. J. Food Microbiol.* 73:187–196.
23. National Advisory Committee on Microbiological Criteria for Foods. 2006. Requisite scientific parameters for establishing the equivalence of alternative methods of pasteurization. *J. Food Prot.* 69:1190–1216.
24. Nigro, F., A. Ippolito, and G. Lima. 1998. Use of UV-C light to reduce *Botrytis* storage rot of table grapes. *Postharvest Biol. Technol.* 13:171–181.
25. Obagwu, J., and L. Korsten. 2003. Integrated control of citrus green and blue molds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water. *Postharvest Biol. Technol.* 28:187–194.
26. Pitt, J. I., and R. H. Cruickshank. 1990. Speciation and synonymy in *Penicillium* subgenus *Penicillium*—towards a definitive taxonomy, p. 113–117. *In* R. A. Samson and J. I. Pitt (ed.), Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, New York.
27. Porat, R., A. Lers, S. Dori, L. Cohen, B. Weiss, A. Daus, C. L. Wilson, and S. Droby. 1999. Induction of chitinase and β-1,3-endoglucanase proteins by UV irradiation and wounding in grapefruit peel tissue. *Phytoparasitica* 27:233–238.
28. Stevens, C., V. A. Khan, J. Y. Lu, C. L. Wilson, P. L. Pusey, E. C. K. Igwegbe, K. Kabwe, Y. Mafolo, J. Liu, E. Chalutz, and S. Droby. 1997. Integration of ultraviolet (UV-C) light with yeast treatment for control of postharvest storage rots of fruits and vegetables. *Biol. Control* 10:98–103.
29. Stevens, C., V. A. Khan, J. Y. Lu, C. L. Wilson, P. L. Pusey, M. K. Kabwe, E. C. K. Igwegbe, E. Chalutz, and S. Droby. 1998. The germicidal and hormetic effects of UV-C light on reducing brown rot disease and yeast microflora of peaches. *Crop Prot.* 17:75–84.
30. Stolk, A. C., R. A. Samson, J. C. Frisvad, and O. Filtenborg. 1990. The systematics of the terverticillate penicillia, p. 121–137. *In* R. A. Samson and J. I. Pitt (ed.), Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, New York.
31. Timmer, L. W., S. M. Garnsey, and P. Broadbent. 2003. Diseases of citrus, p. 163–195. *In* R. C. Ploetz (ed.), Diseases of tropical fruit crops. CABI Publishing, Wallingford, UK.
32. Valero, A., M. Begum, S. L. Leong, A. D. Hocking, A. J. Ramos, V. Sanchis, and S. Marín. 2007. Effect of germicidal UVC light on fungi isolated from grapes and raisins. *Lett. Appl. Microbiol.* 45:238–243.
33. Winter, C. K. 2005. Pesticide residues in fruit and vegetables, p. 135–155. *In* W. Jongen (ed.), Improving the safety of fresh fruit and vegetables. Woodhead Publishing Limited, Cambridge, UK.
34. Yaun, B. R., S. S. Sumner, J. D. Eifert, and J. E. Marcy. 2004. Inhibition of pathogens on fresh produce by ultraviolet energy. *Int. J. Food Microbiol.* 90:1–8.