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CONTROL OF NATURAL MYCOBIOTA IN MAIZE GRAINS BY ULTRAVIOLET (UVC) IRRADIATION

Carmen Liliana Rodríguez Páez[1], *María Cristina Pérez Reyes*[2], *Claudia Hernández Aguilar*[1], *Flavio Arturo Domínguez Pacheco*[1], *Ernesto Moreno Martínez*[2], *Alfredo Cruz Orea*[3], *José Luis López Bonilla*[1]

[1]Instituto Politécnico Nacional, SEPI-ESIME, Zacatenco, Unidad Profesional "Adolfo López Mateos", Av. Instituto Politécnico Nacional s/n, Unidad Profesional, Colonia Lindavista, Delegación Gustavo A. Madero, México, D.F. C.P. 07738
[2]Unidad de Investigación en Granos y Semillas FESC, UNAM
[3]Departamento de Fisica, Cinvestav-IPN, AP-14 740, Mexico D.F. CP 07360, Mexico
e-mail: carmenlilianapa@hotmail.com

Abstract. The effect of UV-C light as a means of control of natural mycobiota of grains of maize (*Zea mays* L.) hybrids "San Juan" and "H-159" (productive cycle, 2009) was investigated. UV-C lamps of 15 W were used and the exposure times applied were 0, 5, 10, 15, 20, 25 and 30 min. The experiment was established in the randomised complete block design with eight and four replicates. The unit pilot was 50 grains. For the determination of mycobiota the agar plate test was used, after disinfection of the grains with sodium hypochlorite diluted to 3% for 1 min. Differences ($P \leq 0.001$, $P \leq 0.05$) between treatments were found, the best treatments being those of 30 and 10 min, observing reductions of 42.85 and 52.05% in the number of grains infected with Fusarium spp. with respect to control for "San Juan" and "H-159", respectively. For *Fusarium monoliforme* it was found that in 30 min there was a reduction of 53.74% for the hybrid "San Juan", while for H-159 a reduction of 61.7% in 10 minutes was observed. These results show that UV-C radiation may be useful for application as a germicide in future experiments on a wide variety of grains.

Keywords: *Zea mays*, ultraviolet radiation, maize grain, fungi, mycobiota

INTRODUCTION

Climate change has potential effects on yields and quality of food crops, food security being a very important issue worldwide (Magan *et al.* 2011, IPCC 2007), especially from the perspective of fungi control. Various strategies for control of fungi have been used in agriculture, for example crop rotation and avoiding the spread of infested soil and pathogen-carrying plant materials, breeding of fungus-resistant cultivars of crops, and the application of agrochemicals (Cornelissen and Melchers 1993). Initially, agrochemicals provided beneficial results (FIS 1999), but their excessive use caused irreversible damage to soil, environment and – consequently – to human and animal health (Vasilevsky 2003). So, it is important to conduct a larger number of experiments in order to find environmentally friendly ways that can control fungi without harming the environment.

The techniques used for controlling fungi include, for example, fumigation with carbon dioxide (CO_2) (Nilrattanakhun 2003), disinfection with propylene oxide (Almond Board of California 2007), treatment with propionic acid salts (Moreno *et al*. 2000), and application of ozone (Khadre *et al.* 2001). On the other hand, scientific evidence reported by various authors in the world indicates the possibility of usefulness, at a certain range of frequencies belonging to the electromagnetic spectrum, of irradiation sources for the disinfection of pathogenic organisms (Lagunas *et al.* 2006). Radio frequency (RF) heating, operating between 10 and 100MHz, has been successfully used in food processing, and also for insect control in in-shell walnuts (Mitcham *et al.* 2004). The IAEA (1991) reported that gamma irradiation doses in the range of 0.2-1.0 kGy are effective in controlling insect infestation in cereals, and Przybilski (1990) indicated that doses upwards of 5 kGy totally kill the spores of many fungi surviving lower doses. Likewise, gamma radiation treatments at doses of 3.0 kGy completely inhibited the fungus on maize seeds inoculated with Aspergillus flavus (Nagy and Souzan 2004), doses of 1.5 to 2.0 kGy of gamma radiation reduced the fungal load on kernels (Bachir 2004). Food gamma irradiators are costly and there is the concern over public acceptance of irradiated food (Mitcham *et al.* 2004).

Other sustainable methods that are currently applied to reduce microbial load on fruit and vegetables are the ultraviolet light treatments (Yaun *et al.* 2004, Allende *et al.* 2003, Tran and Farid 2004, Guerrero and Barbosa 2004, Allende *et al.* 2006). Besides, the technology of UV-C is widely used in the food industry for the disinfection of air, pollution control of plant surfaces and packaging materials, post harvest storage of fruits and vegetables (Rivera *et al.* 2007, Begum *et al.* 2009).

Gritz *et al.* (1990) reported that UV-C irradiation at 250 mW/cm² was an effective germicide treatment, controlling *Pseudomonas, Streptococcus, Acanthamoeba, Candida and Aspergiullus niger* in 20 minutes. Marquenie *et al*. (2002) demonstrated the inactivation of *Botrytis cinerea* and *Monilinia fructigena* in strawberries and cherries with UV-C treatment at 0.50 and 0.10 J cm^{-2}, respectively.

Stevens *et al.* (1997) reported the inactivation of *Rhizopus stolonifer* in tomato; Kinay *et al*. (2005) used the UV-C treatment to inactivate *P. digitatum* in mandarins. Reports found in scientific literature indicate the application of the UV-C germicidal treatment in strawberry, peach, tangerine and tomatoes (Stevens *et al*. 1997, Baka *et al.* 1999). In grains it has been used to inactivate *Aspergillus* and *Penicilium*, indicating an effect on the quality of wheat grains (Hidaka and Kubota 2006).

According to our knowledge there is no relevant reference in the literature that indicates the usefulness of UV-C for controlling fungi in grain of maize.

Thus, the aim to this study was to evaluate the mycobiota associated with two different varieties of maize when applying a constant amount of hypochlorite to grain after treatment with ultraviolet light at different times of exposure (5, 10, 15, 20, 25 and 30 minutes), under the hypothesis that a constant amount of hypochlorite applied to grain maize after treatment with UV-C at different times of exposure modifies the percentage of grain with fungus.

A total amount of 393.5 million tons of maize (*Zea mays* L.) has been produced in the world (FAO 2009). In 2009, there were 7.72 million hectares planted with maize for grain, with a production of 20.14 million tons, with an average yield of 3.14 tons ha^{-1}, from which 11,824.3 billion tons were used for human consumption (SIAP 2010a), mainly in the form of tortilla (Zepeda *et al.* 2009). Nevertheless, in Mexico there were only 1.32 million tons of maize grain, produced on a cultivated area of 566.43 ha (SIAP 2010b). It demonstrates a deficit of grain, and a necessity to improve the quality of maize grain with a view to various quality attributes, such as the health implications towards improving the quality of food for human and animal consumption and for application in the industry, for mass production of tortilla. Thus, it is important to generate methods to improve food security, impacted by climate change (Chakraborty and Newton 2011).

MATERIALS AND METHODS

Biological material

Maize grains hybrids from San Juan and H-159, provided by researchers from the Autonomous University of Chapingo (UACh) and the National Institute for Forestry, Agriculture and Livestock (INIFAP), respectively, and cultivated in San Miguel Bocanegra, municipality Zumpango, State of Mexico in the spring-summer cycle of 2009, were used in this research.

The grains were homogenised as to their length, thickness and width using a vernier caliper. The selected grains were 1.13, 0.57 and 0.92 cm for San Juan and 1.16, 0.47 and 0.89 cm for H-159. The moisture content of maize grain was calculated according to the International Seed Testing (ISTA 1993) and was 10.18 and 10.75% for the hybrids San Juan and H-159, respectively.

Treatment of the grains with ultraviolet radiation

In the laboratory, we applied the physical method of ultraviolet radiation treatment of grains using a prototype irradiator consisting of two lamps (4136 G36T6-15W - 254 nm), separated by a distance of 11 cm. Placed in the central part of the lamps assembly was a quartz tube which was used as a container for the grains in order to receive radiation from both sides, as shown in Figure 1. The operation of the lamps was controlled by a timer which was set to exposure times of 5, 10, 15, 20, 25 and 30 min. The grains were placed in a uniform manner horizontally (with the embryo facing up) in the quartz tube. A power meter model 510, Inc. was used to measure the lamps output power. The irradiation intensity was 11 mW cm^{-2}.

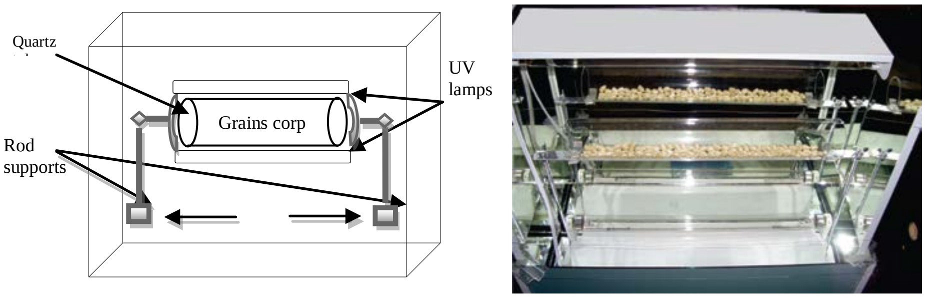

Fig. 1. Experimental setup for UV-C radiation

Determination of the mycobiota

The identification and quantification of the mycobiota and identification of *Fusarium* species: *monoliforme*, *gramineum*, and *dimerium oxysporum* was conducted in the laboratory of the Experimental Unit in Grain Seeds (Unigras). For the determination of mycobiota the agar plate test was used, after disinfection of the grains with sodium hypochlorite (3%) for 1 min. The grains were placed in Petri dishes with culture medium potato dextrose agar (PDA) and incubated at 25°C for 5 days in darkness (Moreno 1988, ISTA 1993). After five days of incubation quantification was performed of the presence of fungi identified to the genus level following the keys of Barnet and Humtah (1998).

For the identification of *Fusarium* to the species level, the isolated *Fusarium* strains were inoculated in Petri dishes with an infusion of PDA and incubated at a photoperiod of 12 hours of light combination of cool white fluorescent light and black fluorescent light, and identified to the species level following the keys of Booth (1971) and Nelson *et al.* (1983) based on the morphological criteria for 12 hours of darkness for seven days at 25°C.

Experimental design and statistical analysis

The experimental design was a randomized complete block with eight and four replicates for the hybrids “San Juan” and “H-159”, respectively, the experimental unit consisting of 50 grains. The analysis of variance and the least significant difference test were conducted with the program SAS (1999).

RESULTS

Table 1 shows the results of analysis of variance (ANOVA) in the incidence of fungi (genus and species) from the grains of maize studied in this research, considering six treatments and the control (grains without radiation) for each one of the varieties, making a total of fourteen different treatments, including control for each variety of maize grain ("San Juan" and "H-159"). The mycobiota found in the grain of maize in the conditions without radiation for the hybrid San Juan was composed of 36.75, 0.5, 0.5 and 0.25% of the genera *Fusarium spp*. *Penicilium spp*., *Alternaria spp*., and *Aspergillus spp*., respectively. The genus *Fusarium spp*. showed a higher incidence in the grain, with four species identified: *monoliforme* (23.5%), *dimerium* (8.25%), *gramineum* (4.0%) and *oxysporum* (1.0%). In the case of hybrid H-159, the genura *Fusarium spp*. (24.5%) and *Penicilium spp*. (3.5%) were found. The identified species of the genus *Fusarium spp*. were *monoliforme* (13.5%), *dimerium* (8.5%) and *gramineum* (6.36%). As can be seen, the genus of higher incidence in both maize hybrids was *Fusarium spp*.

Table 1 shows that the hybrid "San Juan" presented highly significant differences ($p \leq 0.001$) among the treatments for the variables PFT, PFM and PFT; in the case of variable PFD statistically significant differences were obtained ($p \leq 0.05$). For the maize hybrid "H-159" statistically significant differences ($p \leq 0.05$) were found for the variables PFT and PTF.

The effects of ultraviolet irradiation applied prior to sodium hypochlorite for both maize hybrids ("San Juan" and "H-159"), observed in the variables PTF, PFT and PFM, are presented in Figures 2, 3 and 4 respectively. In these graphs there is a tendency of similar behaviour of the resulting curves of incidence of the percentage of grains that contain naturally associated fungi.

In Figure 2 we see the percentage of grain of maize hybrids "San Juan" and "H-159" infected with identified fungi total. The performance of irradiated grains mycobiota is evidenced in differences compared with the control (not irradiated), the percentage of infested grain being lower for the irradiation times of 30 and 10 min in the hybrids "San Juan" and "H-159" respectively.

Figure 3 shows the percentage of grains of maize hybrids "San Juan" and "H-159" infected with *Fusarium spp.* (PFT). It is noted that the treatments that had the smallest percentage in the amount of grains infested were with the exposure times of 30 and 10 minutes, with 52.05 and 42.86% reduction in the incidence of PFT in "San Juan" and "H-159", respectively, compared to control.

Table 1. Analysis of variance in the incidence of fungi in grains of maize hybrids San Juan and H-159 cultivated in Zumpango, Edo de Mexico

S.V.	DF	PFT	PFM	PFO	PFG	PFD	PP	PAF	PM	PC	PA	PRZ	PTF
"San Juan" Hybrid													
Treatments	6	141.64**	88.51**	32.13ns	14.73ns	88.19*	25.65ns	1.18ns	1.18ns	1.18ns	2.073ns	–	149.67**
Repetitions	7	81.95	195.47	149.19	149.48	73.99	38	1.18	1.18	1.18	36.14	–	75.06
Error	42	7.29	6.26	16.94	18.97	22.46	16.38	1.18	1.18	1.18	7.96	–	8.56
MEDIA													
R^2		0.81	0.87	0.63	0.58	0.52	0.37	0.23	0.23	0.23	0.44	–	0.79
CV		9.52	11.30	98.76	56.33	41.72	152.71	748.33	748.3	748.33	186.53	–	9.73
"H-159" Hybrid													
Treatments	6	41.34*	17.2ns	–	31.24ns	49.32ns	13.34ns	–	–	–	3.93ns	2.36ns	34.26*
Repetitions	3	37.82	9.47	–	10.15	60.45	8.26	–	–	–	3.14	2.36	13.26
Error	18	14.59	20.66	–	33.41	25.88	16.63	–	–	–	4.98	2.36	12.39
MEDIA													
R^2		0.57	0.26	–	0.26	0.5	0.25	–	–	–	0.26	0.33	0.52
CV		15	24.86	–	126.3	35.15	35.25	–	–	–	384.41	529.15	12.20

SV = source of variation; DF = degrees of freedom; CV = coefficient of variation; R^2 = coefficient of determination, calculated for each one of the variables evaluated in this research (PFT, PFM, PFO, PFG, PFD, PP, PM, PAF, PC, PA, PRZ and PTF); PFT = percentage *Fusarium spp*.; PFM = Percentage *Fusarium moniliforme*; PFO = percentage *Fusarium oxysporum;* PFG = percentage *Fusarium graminearun*; PFD = percentage *Fusarium dimerium;* PP = percentage *Penicilium*; PM = percentage *Mucor*, PAF= percentage *Aspergillus flavus*; PC = percentage *Cladosporium*, PA = percentage *Alternaria*; PRZ = percentage *Rizhopus*, PTF = percent total fungi, **: highly significant ($p \leq 0.001$); *: significant ($p \leq 0.05$), ns = not significant.

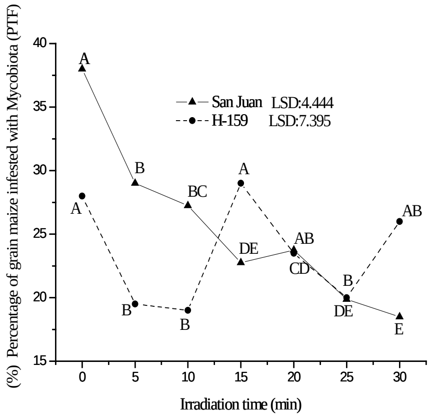

Fig. 2. Percentage of grain maize infested with Mycobiota (PTF) in the varieties San Juan and H-159. Means with different letters are statistically different (LSD, $p \leq 0.05$); LSD = least significant difference; 0 = Control (without radiation)

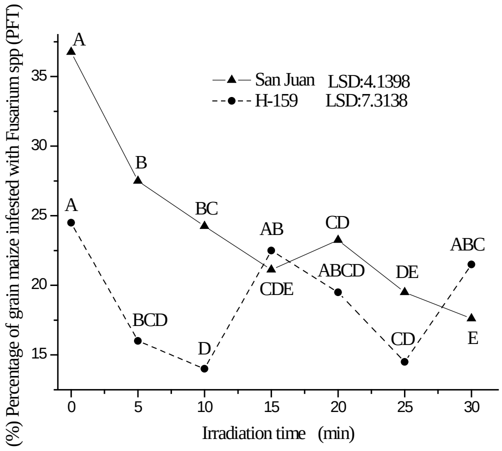

Fig. 3. Percentage of grain maize infested with *Fusarium spp* (PFT) in the varieties San Juan and H-159. Means with different letters are statistically different (LSD, $p \leq 0.05$); LSD = least significant difference; 0 = Control (without radiation)

Figure 4 illustrates the behaviour of *Fusarium monoliforme* (PFM), when the hybrid “San Juan” and “H-159” in combination with radiation times are compared to the control. For hybrid “San Juan”, the greatest reduction is observed in 30 minutes (53.74%), while that for “H-159” was the largest decrease at 10 minutes (61.7%).

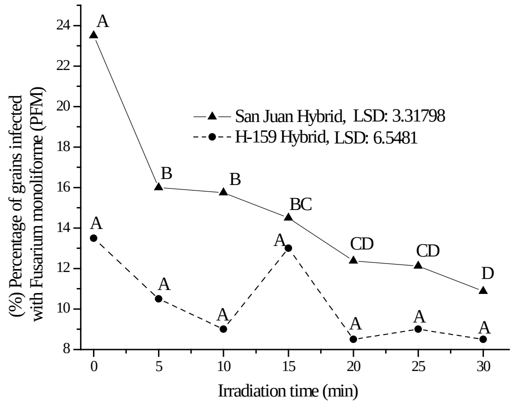

Fig. 4. Percentage of grain maize infested with *Fusarium monoliforme* (PFM) in the varieties San Juan and H-159. Means with different letters are statistically different (LSD, $p \leq 0.05$); LSD = least significant difference; 0 = Control without radiation

DISCUSSION

These results show that irradiation of the grain of hybrids of maize "San Juan" and "H-159" with ultraviolet light and UV-C at different times of exposure reduced the percentage of grain infected with *Fusarium spp*. and *Fusarium moniliforme*, suggesting that ultraviolet radiation with UV-C in combination with sodium hypochlorite could be used as a germicide treatment for the control of incidence of the genus *Fusarium spp*. Other authors demonstrated the germicidal effect of UV-C radiation at 254 nm wavelength, and have applied it in the disinfection of different foods (Allende *et al*. 2006).

Also it has been used as an alternative for chemical sterilisation of microorganisms and their inactivation on fruit surface (Stevens *et al.* 1998). In this research, the amount of grain infested with fungus was decreased, so we can say that it is an alternative for surface sterilisation of grains of maize. We observed that the effects depend on the maize hybrid grain studied and on the times of exposure to radiation, which is consistent with reports by other authors on the control of other types of microorganisms with UV light (Guerrero and Barbosa 2004, Rivera *et al*. 2007). In our research, the treatments had greater reduction in variables (percentage of grain infected by *Fusarium spp*. and *Fusarium monoliforme*) being radiation time from 10 and 30 minutes, using UV-C lamps with 15W power for hybrid "H-159" and "San Juan", respectively.

On the other hand, in the control grain sample of the hybrid “San Juan”, the presence of *Fusarium* spp. was 36.75%, while in the case of hybrid H-159, *Fusarium spp*. was fount at 24.5% and *Penicilium spp*. 3.5%, the identified species of *Fusarium spp*. being *monoliform*e (13.5%), dimerium (8.5%) and gramineum (6.36%). As can be seen, the genus of higher incidence in both maize hybrids was *Fusarium spp*. In both cases it was lower than the level reported by Hernandez *et al.*, 2007, in maize cultivated in northern Tamaulipas, Mexico (76%). These differences may be due to the different climates in each of those regions - Zumpango Edo de Mexico has a dry temperate climate with average temperature of 15°C (García 1987), and Rio Bravo, Tamaulipas (Mexico), a semidry climate, with average temperature above 28°C (Hernández *et al.* 2007). Some authors (Shelby *et al*. 1994, Shephard *et al.* 1996) suggest that hot and dry climates are suitable for a higher incidence of *Fusarium spp*.

Among the species of *Fusarium* found in maize grain, the *monoliform*e and *gramineaum* species are producers of fumonisin (Fandohan *et al.* 2003, Munkvold 2003), toxic for human and animal consumption (Placinta *et al.* 1999), being also species that cause ear rot (De León 1984, García and Martínez 2010). Thus it is necessary to develop treatments that control different species of fungi. In this research we found that irradiation of maize grain with UV-C prior to disinfection with sodium hypochlorite can modify the amount of grain with fungus when compared with control grains (control samples – without irradiation UVC).

On the other hand, it was observed that the species *Fusarium oxysporum* was less abundant in the maize hybrid “San Juan” and was not present in “H-159”. According to Garcia and Martinez (2010), it has a great capacity to produce toxins with serious consequences in health and in the economic aspect.

Moreover, the control of fungi by the application of UV treatment has been reported. UV-C at dose of 1.3 to 4.0 KJ m^{-2} was effective in reducing diseases caused by *Alternaria*, *Botrytis cinérea* and *Rhizopus stolonifer* in tomatoes (Liu *et al.* 1993). The effects of UV irradiation may be beneficial or cause damage, depending on the characteristics of the radiation (power, intensity, distance, area and UV exposure times) and on the characters of the biological material to be subjected to the treatment.

In mandarin, application of UV-C for 10 minutes caused inactivation of *Penicilium*, but it also caused lesions such as burns and darkening of the fruit surface (Kinay *et al.* (2005). For his part, Stevens *et al*. (1997) reported that treatment with UV-C was effective in counteracting rot caused by *M. fructicola* in peach and deterioration by attack of *P. digitatum* in mandarin (*Citrus reticulata* Blanco), as well as rotting caused by *R. stolonifer* in tomato. In the peach it reduced 55% of the rot. Erkan *et al.* (2001) report that times of 10 and 20 min of exposure to UV-C significantly reduced the microbial activity and deterioration during storage of slices of zucchini (Cucurbita pepo L., cv. Tigress). Whereas, Hidaka and Kubota (2006) reported an estimate of 90% reduction in wheat grain infected with *Aspergillus* spp. and *Penicilium* spp. at 5.6 hours of UV-C sterilisation at 97 W m^{-2} intensity.

Due to the positive and negative effects of UV-C radiation found in this research, it can be usefully applied in future experiments in a wide variety of grains with the intent of germicide. Besides that, it is a global and increasing problem to combat, and that is within the complex web of impacts produced by climate change (Garret *et al*. 2011, Ghini *et al*. 2011, Eastburn *et al*. 2011)

According to the results of this research it can be said that UV-C light produced by lamps proved to be a valid low-cost method that could improve maize seed health quality. However, future research would have to be carried out on a wider germplasm spectrum, irradiation parameters and grains of various degrees of infection.

CONCLUSIONS

1. In the present investigation it was found that the effect of UV-C radiation in combination with sodium hypochlorite on mycobiota, applied to control corn hybrids “San Juan” and “H-159”, depended on the exposure time of UV radiation, the variety of grain and the fungus associated with the maize grain to be controlled.
2. The variables that were statistically significant ($P \leq 0.05$) between treatments with respect to control were PFD, PFT and PTF for hybrids “San Juan” and “H-159” respectively. In the same way the PFT variables, PFM and PTF were significantly different ($p \leq 0.001$) for the San Juan hybrid.
3. The UV-C radiation treatments that produced the lower percentage of grains infested with *Fusarium spp*. were 30 and 10 minutes of exposure (15 W, 254 nm) with 52.05 and 42.85% for hybrids “San Juan” and “H-159” compared to the control, respectively.
4. Regarding the effect of UV-C radiation in the control of *Fusarium moniliforme* it was found that the time of 30 minutes presented a reduction of 53.74% for hybrid “San Juan”, whereas “H-159” showed a reduction in the number of infested grain (61.7%) in 10 minutes.

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ZWALCZANIE NATURALNYCH MIKOBIOT W ZIARNIE KUKURYDZY ZA POMOCĄ PROMIENIOWANIA ULTRAFIOLETOWEGO (UVC)

Carmen Liliana Rodríguez Páez[1], María Cristina Pérez Reyes[2], Claudia Hernández Aguilar[1], Flavio Arturo Domínguez Pacheco[1], Ernesto Moreno Martínez[2], Alfredo Cruz Orea[3], José Luis López Bonilla[1],

[1]Państwowy Instytut Politechniczny, SEPI-ESIME, Zacatenco,
Unidad Profesional "Adolfo López Mateos", Av. Instituto Politécnico Nacional s/n,
Unidad Profesional, Colonia Lindavista,
Oddział im. Gustavo A. Madero, Meksyk, D.F. C.P. 07738
[2]Ośrodek Badania Ziarna i Nasion FESC, UNAM
[3]Wydział Fizyki, Cinvestav-IPN,AP-14 740, Meksyk D.F. CP 07360, Meksyk
e-mail: carmenlilianapa@hotmail.com

S t r e s z c z e n i e. Badano wykorzystanie promieniowania UV-C jako metody zwalczania naturalnych mikobiot ziarna hybryd kukurydzy (*Zea mays* L.) "San Juan" i "H-159" (cykl produkcyjny, 2009). Zastosowano lampy UV-C o mocy 15 W, a czasy ekspozycji wynosiły 0, 5, 10, 15, 20, 25 oraz 30 min. Doświadczenie założono metodą kompletnych bloków randomizowanych w ośmiu i czterech powtórzeniach. Jednostka pilotażowa wynosiła 50 ziaren. Dla określenia mikobiot zastosowano test agarowy po uprzedniej dezynfekcji ziarna 3% roztworem podchlorynu sodu przez 1 min. Stwierdzono różnice ($P \leq 0{,}001$, $P \leq 0{,}05$) pomiędzy obiektami doświadczenia. Najlepsze wyniki uzyskano dla czasów ekspozycji 30 i 10 min, uzyskując odpowiednio 42,85 oraz 52,05% redukcję ilości ziaren zakażonych *Fusarium spp*. dla ziarna hybryd "San Juan" i "H-159" w odniesieniu do kontroli. W odniesieniu do *Fusarium monoliforme* stwierdzono, że po czasie naświetlania 30 min nastąpiła redukcja o 53,74% dla hybrydy "San Juan", a dla H-159 stwierdzono spadek o 61,7% po 10 minutach. Te wyniki wykazują, że promieniowanie UV-C może być użyteczne do stosowania jako zabieg zarodnikobójczy w przyszłych doświadczeniach na rożnych rodzajach ziarna.

S ł o w a k l u c z o w e: *Zea mays*, promieniowanie ultrafioletowe, ziarno kukurydzy, grzyby, mikobioty