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Full Length Research Paper

Laser light on the mycoflora content in maize seeds

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Laser light has many applications in agriculture, but there is still much work to provide scientific evidence of its potential use as an alternative for the control of diseases originating in the seed, especially for fungi that are internal. In this study, we investigated the effects of low intensity laser irradiation on the mycoflora content in maize seeds. Five irradiation times (30, 60, 180, 300 and 600 s) and two intensity levels (I_1 = 16.3 e and I_2 = 4.6 mW/cm^2) were applied by using a diode laser (λ = 655 nm and power of 27.4 mW). Consequently, the laser irradiation significantly diminished the quantity of seeds infected with *Fusarium* spp. fungi. The combination of I_1 and I_2, at 5 min of irradiation time, diminished ($p \leq 0.05$) the quantity of infected seeds with *Fusarium* spp. up to 61.11% when compared with the control seed (no irradiation). From these results, we concluded that low intensity laser irradiation could be an alternative method to control seed transmitted diseases in maize seed.

Key words: *Zea mays* L., diode laser, low intensity laser, fungi, *Fusarium.*

INTRODUCTION

The global warming phenomenon has produced changes in the abiotic and biotic environmental factors; as such, these changes have been seen in different agricultural production regions in the world causing changes in plant and at the same time altering the yield and crop production (Parry et al., 2007). Plants undergo stress such as drought, rain, thermal stresses, wind, mechanical contact, pricking by insects, wounds inflicted by phytophages and infection by pathogens (Tafforeau et al., 2004). Among the pathogens that cause losses in crops are fungi (Oerke, 2006). Fungal diseases are controlled by 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). In the case of agrochemicals, they are less suitable to be used as it degrades land, environment, and therefore the human and animal food (Vasilevski, 2003). Thus, it is important to investigate the use of sustainable methods, such as physical methods in this century.

Various physical methods have been used in agriculture for seed treatment such as: Electric field (Moon and Chung, 2000; Nechitailo and Gordeev, 2004), electromagnetic field (Galland and Pazur, 2005; Hernandez et al., 2009a; Dominguez et al., 2010; Zepeda et al., 2010), static magnetic field (Vashisth and Nagarajan, 2008; Carkmak et al., 2010; Aladjadjiyan, 2010) and laser (Aly and Hossam, 2010; Soliman and Harith, 2010; Perveen et al., 2010; Chen et al., 2010; Hernandez et al., 2009b, 2010). In the case of laser, light is applied in agriculture for biostimulation processes depending on the irradiation parameters (positive, negative and zero) in some variables assessed in different phenological stages. The scientific evidence presented by various authors in the world provide the possibility of accelerating the maturity of plants that makes them precocious; increase their resistance to disease; influence alpha-amylase activity and the concentration of free radicals in the seeds of several plants that could deactivate seed dormancy;

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improve their germination rate and percentage; increase seed vigor; and create an impact on the respiration process, photosynthetic activity, chlorophyll content and carotenoid content of seedlings from seeds that are irradiated (Durkova, 1993; Gladyszewska, 2006; Starzycki et al., 2005; Junlin et al., 2007; Hernandez et al., 2005, 2006, 2007, 2008a, 2009b, 2010; Aladjadjiyan and Kakanakova, 2008; Tobgy et al., 2009), so that at the level of crop production, the quality and quantity of plants could be enhanced (Vasilevski and Bosev, 1997a, b; Gładyszewska et al., 1998; Truchliński et al., 2002; Podleśny, 2007; Dziwulska et al., 2006; Wesołowski and Cierpiała, 2006; Szajsner et al., 2007; Szajsner and Drozd, 2007, 2003).

On the other hand, other researches that applied laser irradiation in seeds showed that it could be potentially applied as a fungicide (Bel'skii and Mazulenko, 1984; Dobrowolski et al., 1997; Ouf and Abdel-Hady, 1999; Wilczek et al., 2005a, b; Nenadic et al., 2008), but it is necessary to run more experiments to determine its potential in controlling plant diseases of seed, especially fungi. Therefore, this study aimed at determining the effect of diode laser irradiation from 655 nm semiconductor laser at different intensities and irradiation times in the naturally associated mycoflora of maize seed.

The basis of this mechanism could be the existence of phytochrome, not only in plants and seeds but also some viruses, bacteria and fungi (Lamparter, 2004; Levskaya et al., 2009, Mathews et al., 2003; Li et al., 2009). The absorption spectrum of these photoreceptors is in the red and infrared light (Smith, 2000; Quail, 2002; Shimizu et al., 2002; Casal and Yanosvsky, 2005; Kneissl et al., 2008). In this way, fungi associated naturally in the maize seed could be affected with the red laser light treatment.

Maize is the third most important crop in the world after wheat and rice. In Mexico, the area cultivated with maize is around 8 million hectares, in which 18.3 million tons are produced every year (SIAP, 2009). Different species of *Fusarium*, such as *F. moniliforme* and *F. graminearum,* are considered among the most destructive diseases of the crop (Morán, 1993), which are transmitted by seed and which affect the germination, vigour and longevity of seeds in addition to causing losses in mass and physiological and biochemical changes. Several species of *Fusarium* associated with these diseases are producers of potent toxins, such as fumonisin and zearalenone. These species are toxic and they pose a serious problem for human and animal health which accentuated the problem in developing countries.

MATERIALS AND METHODS

Biological material

A single cross hybrid ($CL_1 \times CL_4$) of *Zea mays* L. of the agricultural cycle (1998 to 1999), associated naturally with mycoflora and provided by the Mexican Genetic Quality Control Assurance Institute (IREGEP) Seed Program, Post-graduated College (Mexico), was used in this study. The seed lot was graded for uniformity using 8 mm screen and the 1000-seed weight was 273 g.

Optical characterization of the seed

The instrumentation used to obtain the coefficient of irradiation was from photoacoustic spectroscopy. The photoacoustic (PA) experimental set-up consisted of 1000 W Xe lamp (Oriel), a mechanical chopper operating at 17 Hz, a monochromator (Oriel), and a homemade brass PA cell provided with an electret microphone. The PA signal from the microphone was fed into the input channel of a Standford Research (SR)-850 lock-in amplifier interfaced to a personal computer that displayed simultaneously both the amplitude and the phase of the wavelength-dependent PA signal (Figure 1). Fifteen seeds were selected at random to obtain their optical absorption coefficients (β) in the wavelength range from 620 to 700 nm. The optical absorption coefficient β was determined from the PA signal amplitude by using the Rosencwaig and Gersho model (Fesquet et al., 1984). The total optical absorption coefficient for thermally thick ($a_s\ l_s >> 1$) samples was obtained from the normalized PA signal by using Equation (1):

$$\beta = \frac{(a_s)\ \{q^2 + q(2-q^2)^{1/2}\}}{(1-q^2)} \quad (1)$$

$$a_s = (\pi f / \alpha)^{1/2} \quad (2)$$

Where, q is the normalized PA intensity, $a_s = \pi\phi/\alpha)^{1/2}$ is the thermal diffusion coefficient at the modulation frequency f (Hernandez et al., 2009), $\alpha = 4.44\ 10^{-3}\ cm^2\ s^{-1}$ is the thermal diffusivity of starch (Fernández et al., 2001) and l_s refers to the sample's thickness, in which the mean β value from 15 seeds was evaluated. Once this value was obtained, the optical length penetration was calculated (l_β).

Laser treatment application

As a source of radiation, we used a diode laser with an output power of 27.4 mW and 655 nm wavelength and spectral half bandwidth of 0.3 nm, which was obtained with the laser spectrum analyzer mod E210LSA034 Brand IST. Two levels of intensity and five irradiation times in design randomized complete block (Table 1) were applied. The seeds were photosensitized before the irradiation by soaking in red methyl at a dilution of $1:10^4$ for 25 min. The maize seeds were placed randomly for the pre-sowing irradiation in a fixed position besides embryos (Figure 2). A laser power meter model 45-545 of the company, "Metrologic Instruments, Inc., USA", was used to measure the laser output power.

Deep freezing blotter test

Deep freezing blotter test was used to determine the effect of irradiation with laser light in the incidence of fungi naturally associated to the maize seed (Neergaard, 1979). Prior to the establishment of the test, the seeds of each treatment were divided into two equal lots. One of the lots were disinfected (D) with sodium hypochlorite (10%) for 3 min and rinsed with distilled water, while the other was left without sterilizing and was not disinfected prior to laser light treatment (ND).

The experimental design was a randomized complete block with 5 replications; each replication consisted of 20 seeds. The seeds

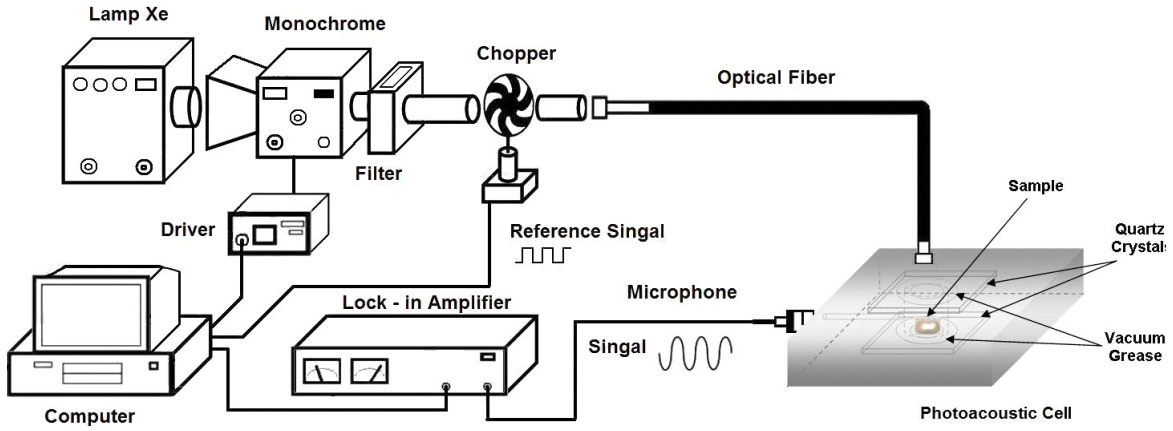

Figure 1. Experimental setup of the photoacoustic spectrometer used to obtain the optical absorption spectrum of maize seeds used in this research project.

Table 1. Intensities and irradiation times of the laser diode used to test the naturally infected maize seed infested with fungi.

Intensity (mW/cm^2)	Irradiation time (s)					
	0	30	60	180	300	600
0	Control					
4.6		139.5	279	558	1,395	2,790
16.3		489.3	978.6	1,957	4,893	9,786

[1]non-irradiated seed; [2]Dose = mJ/cm²

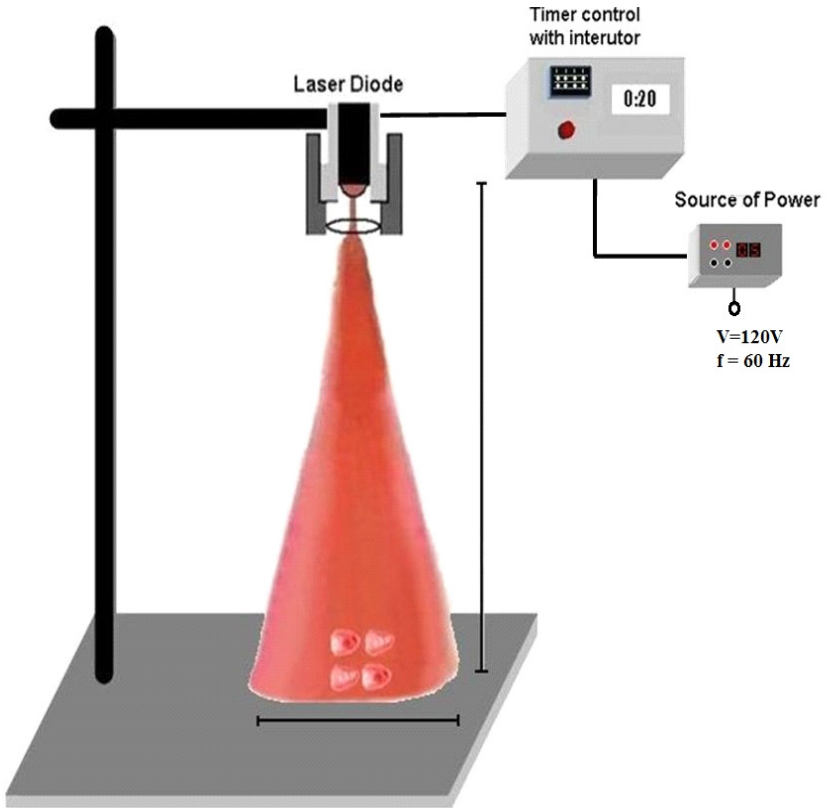

Figure 2. Experimental setup used for pre-sowing seed treatment.

Table 2. Comparison of the mean of the variables percentage obtained from the pathogens under laboratory conditions in Montecillo, Mexico.

Treatment	I (mW/cm^2)	Irradiation time (seconds)	TP	FUSAP	ASPERP	PENIP	ALTERP
			Genotype	CL-4 X CL-1 (Semilla desinfestada)			
1	4.6	30	48^{bc}	36^{bdc}	12^{ba}	4^{a}	0^{a}
2	4.6	60	40^{bc}	32^{dc}	0^{b}	0^{a}	4^{a}
3	4.6	180	48^{bc}	48^{bac}	16^{a}	0^{a}	0^{a}
4	4.6	300	28^{c}	24^{b}	0^{b}	4^{a}	0^{a}
5	4.6	600	40^{bc}	36^{bdc}	4^{ba}	0^{a}	0^{a}
6	16.3	30	60^{ba}	56^{ba}	4^{ba}	0^{a}	4^{a}
7	16.3	60	48^{bc}	48^{bac}	0^{b}	0^{a}	4^{a}
8	16.3	180	52^{ba}	52^{bac}	0^{b}	0^{a}	0^{a}
9	16.3	300	28^{c}	24^{d}	8^{ba}	0^{a}	0^{a}
10	16.3	600	48^{bc}	40^{bdc}	8^{ba}	4^{a}	0^{a}
11	0	0	72^{a}	64^{a}	12^{ba}	0^{a}	0^{a}
			Genotype	CL-1 X CL-4(ND)			
1	4.6	30	100^{a}	100^{a}	12^{ba}	8^{c}	8^{b}
2	4.6	60	100^{a}	100^{a}	0^{b}	16^{bc}	8^{b}
3	4.6	180	100^{a}	100^{a}	16^{a}	36^{ba}	8^{b}
4	4.6	300	100^{a}	100^{a}	0^{b}	36^{ba}	4^{b}
5	4.6	600	100^{a}	100^{a}	4^{ba}	20^{bc}	28^{a}
6	16.3	30	100^{a}	100^{a}	4^{ba}	32^{ba}	0^{b}
7	16.3	60	100^{a}	100^{a}	0^{b}	48^{a}	12^{ba}
8	16.3	180	100^{a}	100^{a}	0^{b}	28^{bac}	12^{ba}
9	16.3	300	84^{b}	84^{a}	8^{ba}	28^{bac}	8^{b}
10	16.3	600	100^{a}	100a	8^{ba}	16^{bc}	4^{b}
11	0	0	100^{a}	100^{a}	12^{ba}	20^{bc}	4^{b}

The mean values with the same letters are statistically equal (LSD, $\alpha = 0.05$). LSD, Least significant difference probability level; Treatment 11 = Control without laser irradiation; *TP* = Fungi total percentage (%); FUSAP = *Fusarium* spp. percentage (%); ASPERP = *Asperguillus* sp. percentage (%); PENIP = *Penicillium* spp. percentage (%); I = intensity level; and ALTERP = *Alternaria* spp. percentage (%).

were placed at regular intervals on two layers of sterile blotting paper moistened with sterile distilled water, contained in a transparent polystyrene box (base = 27 × 16 cm, height = 4.5 cm). After sealing with parafilm, boxes with seeds were kept at 25°C for 48 h, -15ºC for 24 h and 25°C for 72 h, with 12 h alternating cycle of light and darkness placed in the germination camera (Seedburo Equipment Company, USA).

Elapsed incubation observations were performed with stereoscopic microscope to record the development of fungal colonies in the seeds and to determine their gender, according to the type of mycelium and spores, and proceeded to quantify the numbers of seed containing each type of fungus.

Statistical analysis

A randomized complete block experimental design with 5 replications was used. The experimental unit was 20 seeds. Data were subjected to analysis of variance using the SAS GLM procedures (SAS, 1998 version). The least significant difference (LSD) test at the 5% probability level was used for comparing treatments (Steel and Torrie, 1980).

RESULTS

Naturally associated mycoflora to maize seed (CL1 × CL4, of the agricultural cycle of 1998 to 1999) corresponded to the genera: *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp. *Alternaria* spp. *Cladosporium* spp. *Rhizopus* spp., *Trichoderma* spp. and *Helminthosporium* spp. The gender of the fungus that predominated more in the seed sample evaluated was *Fusarium* spp. Laser irradiation significantly ($p \leq 0.05$) affected the incidence of infective mycoflora that were naturally associated to the seed; although the effect was dependent on intensity levels I_1 and I_2 (16.3 and 4.6 mW/cm^2, respectively) and the irradiation time (Table 2 and Figure 3).

There was a reduction of over 60% seed infected with *Fusarium* fungi and total fungi (that is, any gender of fungi) in treatments with radiation as compared to the control treatment. Treatments I_1-300 and I_2-300 recorded the lowest percentage of seed infected with *Fusarium*

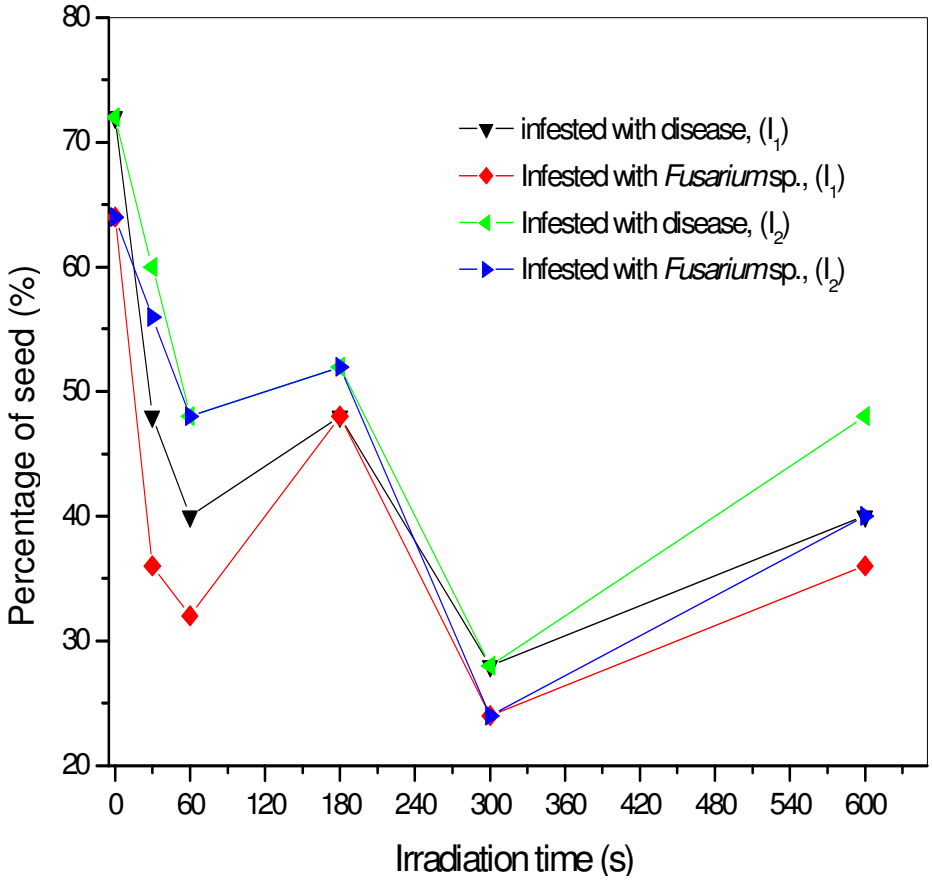

Figure 3. Effect of laser light (655 nm, 27.4 mW) on the incidence of total *Fusarium* spp. naturally associated with the hybrid maize seed (CL_1 x CL_4). The seed that was previously disinfested with sodium hypochlorite (10%) for 3 min and rinsed with distilled water was irradiated with an intensity of I_1 = 16.3 mW/cm^2 or I_2 = 4.6 mW/cm^2. The results of the sample showed that the seed was not irradiated on time (s = 0). Total fungi (LSD = 23.95); *Fusarium spp.* (LSD = 23.73, $p \leq 0.05$).

spp. and total fungi in relation to other treatments with irradiation, with the exception of I_2-300 treatment, where radiation treatments recorded 100% of infested seed, similar to the control, that is, irradiation apparently did not affect the development or incidence of mycoflora that naturally infested the seed (Figures 4 and 5). In contrast, treatment I_2-300 recorded less than 16% infested seeds with respect to *Fusarium* spp.

DISCUSSION

In the present study, for the particular case of the single cross maize $CL_1 \times CL_4$, laser irradiation was seen as an alternative for the control of diseases originating in the seed, especially for pathogens that were seen internally in the seed. Other authors have reported the effects of laser irradiation on growth and development of prokar yotic microorganisms, such as bacteria *Escherichia coli*, although eukaryotic has been reported in the literature on microbiology (Karu, 1987, 1989). Irradiation of the bacteria *E. coli* with a He-Ne laser (632.8 nm) within a range of values produced two maximum values in the growth stimulation before reaching a minimum value, after which the growth was observed (Karu et al., 1994). On the other hand, in fungi, Ouf and Abdel (1999) reported that laser irradiation of soybean seeds for 3 min caused a clear reduction in the number of seed-borne fungi which became more pronounced when the irradiation time was extended using a laser of He-Ne with λ= 632.8 nm and power of 7.3 mW. The pattern of behavior within the time window was used to present a maximum value of the fungus decreased seed. As such, *Rhizoctonia solani*, *Alternaria tenuissima*, *Cercospora kikuchii* and *Colletotrichurn truncatum* were completely eliminated when the seeds were pretreated with a dye and irradiated for 10 min.

In this research, the seeds were stained with methyl red, but they did not fully achieve the required amount of fungi, instead they achieved only a percentage of total fungi and the fungus *Fusarium* spp. Also, we used another wavelength and a different type of laser spectral

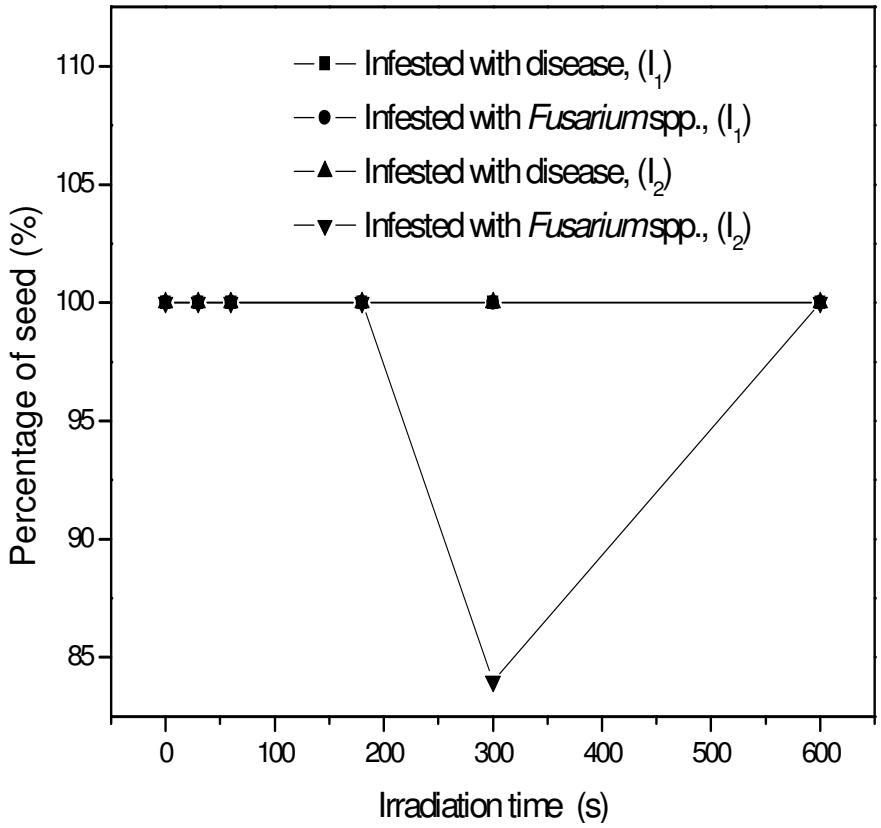

Figure 4. Effect of laser light (655 nm, 27.4 mW) on the incidence of total *Fusarium* spp. naturally associated with the agricultural cycle (1998 to 1999) of the hybrid maize seed (CL_1 x CL_4). I_1 = 16.3 mW/cm^2 or I_2 = 4.6 mW/cm^2. The results of the sample (not irradiated) is in time (s = 0). *Fusarium* spp. (DMS = 8.4438, $p \leq 0.05$).

(half width of 0.3 nm), greater than that of He-Ne, to carry out a research with a larger laser power of 27.4 mW. In addition, a different wavelength of laser He-Ne (655 nm) was applied. Besides, optical characteristics, phenotypic, genetic and thermal properties of the seed were different from that of soybeans.

The influence of laser irradiation has also been reported by some authors who conducted a research in other types of seeds, for example: Bel'skii and Mazulenko (1984) found that pre-sowing laser treatment of seeds barely increased the resistance of the plant against infection amongst the other fungi with *Fusarium* spp. Also, a 7-fold decrease in the infection by *Ustilago hordei* has been found by the authors. On the other hand, Wilcek et al. (2005a), using irradiation with divergent He-Ne laser beam amid a surface power density in the irradiation plane of 0.3 and 6 mW/cm^2 significantly decreased the percentage of those seeds infected with fungi except for the Regime 6 x 1 dose, whereas seed dressing completely eliminated fungal disease on the seeds. Conversely, they indicated that the single irradiation process should be avoided in practice when applied to hybrid alfalfa seed, as it causes a more intense development of fungi, especially of the Penicillium type. Wilcek et al. (2005b) irradiated divergent He-Ne laser light with a surface power density in the irradiation plane of 0, 3 and 6 mW cm^{-2} applied for 1, 3 and 5 times. A single irradiation treatment with a dose of 3 mW cm^{-2} resulted in a significant increase in the number of these strains as compared to the control. A double dose of power with a single irradiation also resulted in a significant growth in fungi numbers of the *Penicillium* type, whereas a laser light beam with a surface power density of R 6 x 3 and R 6 x 5 destroyed them completely. Fungi of the *Alternaria* type were completely destroyed by such dressings as the Funaben T and Sarfun T 65 DS.

The effects found are similar to the behavior reported in seed biostimulation processes, which can be positive, negative or zero. The basis of biostimulation mechanism could be associated with the behavior of the phytochromes, which are affected by red and infrared light. The phytochromes are ubiquitous in plants, but are also identified in many prokaryotic species and fungi (Lamparter, 2004; Levskaya et al., 2005, Li et al., 2009; Mathews, 2006). The pattern according to the results obtained passes through a minimum value, so that the minimum value found may serve to eliminate *Fusarium* fungus associated with the maize seed.

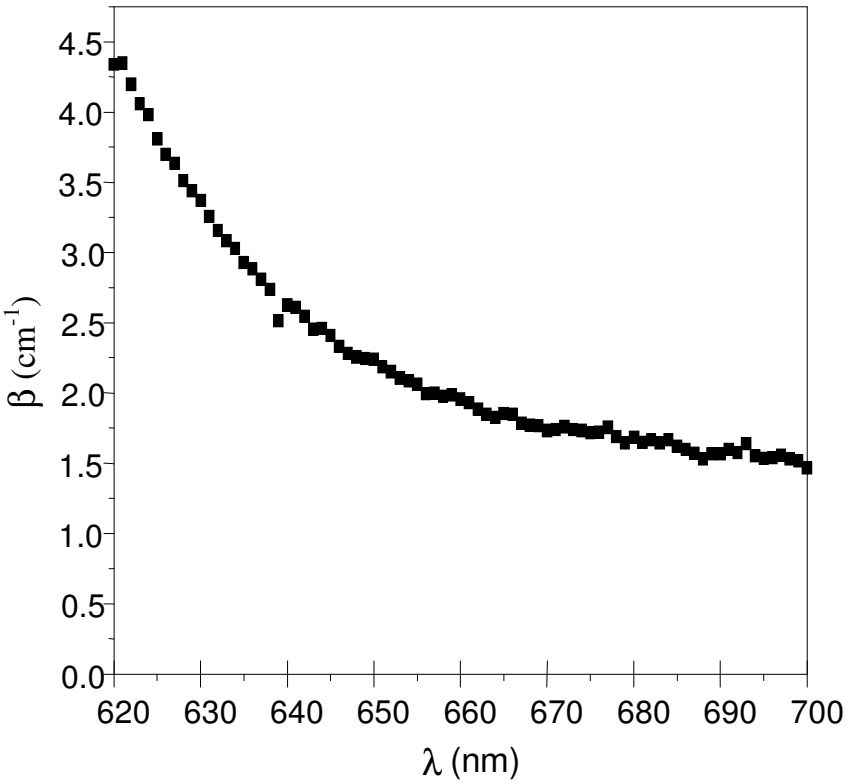

Figure 5. Optical absorption spectra for maize seed, as a function of wavelength obtained by PAS (At λ = 655 nm: β = 2.06 cm^{-1} and $l_{\square}$ = 0.48 cm).

The improvement in the sanitary quality of seed in this research, by irradiation laser, resulted in a favorable decrease in the percentage of seed infested by fungus, which can be associated with the increment in seed quality because the total seed infested by any type of fungi and gender *Fusarium* spp. decreased. Consequently, this could reduce crop losses by reducing the amount of seed that could transmit fungi in the establishment of field crops. This indicates that laser irradiation affects not only the physiology and biochemistry as we have reported in other scientific reports of pre-sowing laser treatment, but also the fungi associated with seed.

A practical limitation to the use of lasers in the seed industry was due to the possible size of the laser footprint and cost. Now, with the development of technology, laser diode arrays could be used to radiate greater amounts of seeds; so it becomes a viable option as well. These diodes have been lowering their costs and thus would be a sustainable technology for treatment of pre-sowing seed to improve the quality of care and thus avoid use of fungicides that harm the environment.

Conclusions

In the present research project, the following conclusions were drawn:

1. The diode laser 655 nm wavelength and 27.4 mW power significantly decreased the incidence of the fungi that were internally associated with the hybrid maize seed $CL_1 \times CL_4$.
2. The combination of irradiation parameters (λ = 655 nm, intensity = 16.3 mW/cm^2 or I = 4.65 mW/cm^2) and irradiation time of 5 min significantly reduced ($p \leq 0.05$) the amount of infected seed *Fusarium* spp. and total fungi by 62.5 and 61.11%, respectively.
3. In ND seeds, the combination of I = 16.35 mW/cm^2, the irradiation time of 5 min and λ = 655 nm was the treatment that reduced the number of infested seed and total fungi *Fusarium* spp.; although the decrease in incidence was 16%.
4. Laser diodes can become an alternative for the control of diseases associated with the seeds in different species by applying the appropriate laser irradiation parameters.

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