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RESEARCH ARTICLE

GAMMA RADIATION IN THE MANAGEMENT OF SOFT BELL PEPPER ROT

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ABSTRACT

The objective of this work was to evaluate the effect of gamma radiation at low doses on the post - harvest control of soft rot by *Pectobacterium carotovorum* subsp. *carotovorum* - Pcc in bell pepper, as well as to evaluate the effect of this method on the physical-chemical characteristics of the fruit. Fruit apparently disease-free was inoculated with 10 µl Pcc suspension at 1x109 UFC.mL-1. After inoculation, the fruits were irradiated with the doses of 0.25; 0,5; 1.0 and 1.25 kGy and stored at 28 ± 2 ° C, being evaluated until the controls became unviable for evaluation, 48 h after inoculation. Petri dishes were also irradiated with 36 h growth colonies at the same doses described previously, and afterwards the colonies were inoculated in healthy bell peppers, the fruits being evaluated for pH, Total Soluble Solids (TSS), percentage of Mass Loss (Weight) and firmness. All radiation doses were efficient in handling Pcc and the dose of 1.25 kGy completely inhibited its action. Only the plates irradiated with 0.5 KGy allowed the growth of the colonies after the irradiation of the plates. The physical-chemical characteristics did not present statistical difference.

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INTRODUCTION

The pepper, Capsicum annuum L. (Solanaceae), is a vegetable of great socioeconomic importance for Brazil. It is an excellent production alternative for the irrigated and rainfed areas of the northeastern semi-arid region, as it is easily adapted to the different edaphic and climatic conditions (Lorentz *et al.,* 2002). According to Lima Neto *et al.,* (2013) the pepper is among the ten most important vegetables in the national market, as it is a culture of rapid return to investments, a short period for the beginning of production, for this it is widely explored by small and medium horticulturalists. Fruits and vegetables are susceptible to attack by various pathogens after harvest. Even when transport and storage are carried out properly, losses of 5 to 10% of the production are verified. However, the damage can be total, when due to very severe infections or damages caused during inadequate storage. The factors host, pathogen and environment, greatly influence the development of post-harvest diseases (Eckert, 1991; Mello *et al.,* 2011). *Pectobacterium carotovorum* (Pcc) was originally classified as *Erwinia carotovora* subsp *carotovora*. This species (or subspecies) was a member of the soft rot group of the genus *Erwinia*, and is taxonomically related to *Erwinia chrysanthemi* recently reclassified as several species of *Dickeya*.

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The soft rot, caused by the bacterium *Pectobacterium carotovorum* subsp. *carotovorum* - Pcc (Jones) Hauben *et al.,* is geographically well distributed and its occurrence in Brazil is quite common, both in the field and in the post-harvest phase, becoming a limiting factor for the cultivation of vegetables as well as of the bell pepper. This bacterium is among the 10 most important for causing economic losses in various crops both in the field and in the post harvesting of fruits and vegetables (Jabuonski; Takatsu; Reifschneider, 1986; Mansfield *et al.,* 2012). The management of soft rot is very difficult and requires integration of control measures, since Pcc has the capacity to adapt to a wide temperature range, surviving in water, soil, infected cultural remains and in the rhizosphere/phylosphere of plants cultivated or alternative hosts (De Boer; Kelman, 2001; Pérombelon; Van Der Wolf, 2002). Studies have been carried out with resistant varieties (Ren *et al.,* 2001), biological control (Barra *et al.,* 2009; Carrer Filho *et al.,* 2009), inducers of resistance (Benelli *et al.,* 2004) and use of antibiotics (Zambolim *et al.,* 1997). However, most of these studies were carried out in the laboratory and involving other hosts (Mello *et al.,* 2011). The use of antibiotics for the management of bacterioses should consider the cost, registration for the crop, grace period and, mainly, the interference in the ecosystem involved. The reduced number of agrochemicals registered for bacterial diseases in vegetables favors the use of undue and ineffective products (Mello *et al.,* 2011). In this sense, the use of gamma radiation has contributed significantly to the management of phytopathogenic microorganisms (Moy; Wong, 2002; Gomes; Silveira; Mariano, 2005). Considering the lack of research using gamma radiation in the control of phytopathogenic bacteria, the objective of this work was to evaluate the effect of this radiation, in different doses, on the management of bell pepper rot caused by *P. carotovorum* subsp. *carotovorum* and the direct effect of radiation on the pathogen in vitro.

MATERIALS AND METHODS

The experiments were conducted at the Post-Harvest Pathology Laboratory of the Rural Federal University of Pernambuco 08° 00' 59" S, 034° 56' 40" W, and at the Laboratory GamaLab, Federal University of Pernambuco 8 ° 3' 25 "S, 34 ° 57'16"W, Recife, PE, Brazil. The isolate of *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc-36), used in the experiment was obtained from the Collection of Cultures of the Laboratory of Phytobacteriology, Rural Federal University of Pernambuco, the same was identified through its biochemical characteristics and molecular biology level. The bell pepper fruits used in this work were obtained from the Center of Supply and Logistics of Pernambuco (CEASA/PE), and selected according to: size, maturation stage and color. To evaluate the effect of gamma radiation and physico-chemical characteristics on soft rot management, the bell pepper fruits were initially washed in running water with neutral soap and then disinfested with 1% sodium hypochlorite, dried at room temperature. Afterwards, each fruit was injured with an entomological pin and 10 μL of the bacterial suspension 1x109 UFC.mL^{-1} was deposited at each wound. The control was also performed by replacing the bacterial suspension with sterile distilled water. After inoculation the fruits were packed in plastic bags and taken to the GamaLab Laboratory of the Regional Nuclear Sciences Center (CRCN/E), where they were submitted to different doses of radiation (0.5, 0.75, 1.0, 1, 25 Kgy) using a Gammacell® 220 Excel radiator (MDS Nordion, Canada), whose source is Cobalt-60 and rate at the time of application 7.303 kGy/h. The fruits were inoculated and immediately irradiated. The positive control was composed of inoculated and non-irradiated fruits and the negative control with fruits without inoculation or irradiation. After irradiation, the fruits were taken to the Post-Harvest Pathology Laboratory (LPPC) and kept in a humid camera for 24 hours at a temperature of 26 ± 2° C and a relative humidity of 70%.

The severity of the disease was assessed over a period of four days after inoculation where the diameter of the lesion was measured on two orthogonal axes as an aid to using a caliper (Mitutoyo, Kaeasaki, Japan). The design was completely randomized with four doses and five replicates, each replicate consisting of three sample units totaling 15 fruits per replicate. The experiment was repeated to prove the results and the data were submitted to statistical analysis using the SISVAR program. For the evaluation of the physical-chemical characteristics, irradiated and non-irradiated fruits were used where it was evaluated: Percentage of Mass Loss (weight); Firmness of the pulp; Hydrogen ionic potential (pH) e; Total Soluble Solids (TSS). Firmness of the pulp was determined using a 327 FT penetrometer (0-13lbs). The pH was verified through the potentiometer Quimis model 400A. Soluble solids content (TSS) was quantified by direct reading in the refractometer, Model Rez (0-32 ° Brix), and results expressed in ° Brix. To verify the direct effect of gamma radiation on Pcc, the isolate was peeled onto Petri dishes containing NYDA medium (Nutrient Agar 23.0g, 10.0g Glucose, Yeast Extract 5.0g, 1000.0 mL Distilled Water, PH to 6.8 with NaOH (2N)), and incubated in BOD at 28 ± 2 °C for 36 h. After this period the Petri dishes containing the bacterium were irradiated in the same doses of gamma radiation from the previous experiment, then they were taken to the LPPC where bacterial suspensions were prepared with posterior inoculation in the bell peppers to observe if the bacterium remained infective. In this experiment, three fruits of bell pepper were used for each dose of gamma radiation. The effect of gamma radiation on Pcc colony growth in vitro was evaluated. To carry out the assay the bacterium was peeled with the aid of a sterile polypropylene loop after being irradiated to Petri dishes containing NYDA medium, with subsequent observation of colony growth.

RESULTS AND DISCUSSION

The bell pepper fruits inoculated and treated with gamma radiation had a positive effect on Pcc management (Table 1). It was found that all inoculated and immediately irradiated fruits decreased the diameter of the lesions at all doses (0.5, 0.75, 1.0 and 1.25 KGy) or had no symptoms compared to the control (Figure 1). The doses 0.5, 0.75 and 1.0 KGy reduced the diameter of the lesion by 4.304; 0.996 and 1.182mm respectively on the first day of evaluation. Inhibition of 100% Pcc at the dose of 1.25 KGy was observed. On the second day of evaluation, the diameter of the lesions decreased by 9.932, 1.318 and 2.220 mm respectively and by 100% at the dose of 1.25 KGy (Table 1).

Table 1. Effect of treatment of different doses of gamma radiation (0.5, 0.75, 1.0, 1.25 KGy) on Pcc severity in bell pepper fruits *in vivo*

Radiation Doses Range	Diameter of the lesion (mm)	
	1° day	2° day
0,00 (1)	9,266 c *	35,770 c *
0,50	4,304 b	9,932 b
0,75	0,996 a	1,318 a
1,00	1,182 a	2,320 a
1,25	0,000 a	0,000 a
CV %	38,05	36,68

(1) Witness = inoculated pathogen and not treated with gamma radiation.
* Average of five replicates. Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability.

Figure 1. Overview of bell pepper fruits (*Capsicum Anuum*) inoculated with *Pectobacterium carotovorum* sbsp. *carotovorum*, 2 days after gamma radiation at doses (0.5, 0.75, 1.0, 1.25 kGy), compared to the non-irradiated (0.0 kGy) control (Test.); and fruit inoculated with previously irradiated culture (PI). Recife, UFRPE and UFPE, Recife, Pernambuco, Brazil, 2015

It is possible to observe the decrease in the diameter of the lesion as the dose of gamma radiation increases (Figure 2). In the control treatment, non-irradiated fruits, the diameter of the lesion was 35mm, in the irradiated fruit, it is possible to observe in the dose of 1.25 KGy absence of symptoms of Pcc.

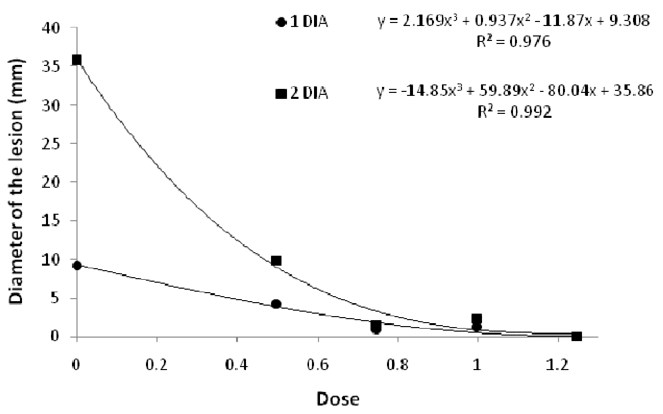

Figure 2. Severity of soft rot in bell pepper fruits at different doses of gamma radiation on the two days of evaluation

The irradiation process was first described by O'Beirne. (1989), and consists of the use of gamma rays in food, without risk of radioactive contamination. The efficiency of the use of gamma radiation in food conservation is linked to three factors: the type of food to be irradiated, the dose to be applied and the time of exposure of the food to the source (Vieites, 1998). According to Lacroix; Quattara (2000), relatively low doses, are between 1.0 and 3.0 kGy, promote reduction of microbial population. However, there were no studies on gamma radiation in the management of phytobacteria, being this result similar to those found by other researchers in different patosystems. Nahed (1999), observed that radiation leads to the elaboration of certain chemical substances that stimulate or retard the growth process. Alexandre *et al.* (2012) working with gamma radiation at a dose of 1.0 kGy verified the inhibition of mycelial growth of *Colletotrichum gloeosporioides in vitro*. According to Lima *et al.* (2001), the use of food irradiation is a promising technique, with good results in post-harvest losses. According to Nagaraja (2007), the irradiation of fruits and vegetables in the post-harvest has as main interest the reduction of the damages caused by phytopathogens. However, it is also used in conservation, prolonging the storage and budding of some plant products, due to physiological changes. In the case of the direct effect of the gamma radiation on Pcc, where Petri dishes containing Pcc were irradiated, followed by the preparation of the colonies suspensions with posterior inoculation in the chili fruits, no lesions were observed, since the bacterium was not more infective (Figure 1). It was also observed that after the plates were irradiated and pegged to NYDA medium plates, they did not grow when subjected to the 0.75 gamma radiation doses; 1.0 and 1.25 KGy (Figure 3).

Figure 3. Effect of different doses of gamma radiation (0.5, 0.75, 1.0, 1.25 kGy) on the growth of *Pectobacterium carotovorum* sbsp. *carotovorum in vitro*, compared to the non - irradiated control (0.0 kGy) (Test.) UFRPE and UFPE, Recife, Pernambuco, Brazil, 2015

In relation to physicochemical characteristics, no significant differences were observed between the different doses of gamma radiation (Table 2), although there is research confirming that the irradiation of foods is efficient in promoting the maintenance of fruit firmness due to the fact of altering Components and provide them with better appearance, with increased firmness. Low doses of radiation result in the hydrolysis of certain components, which may increase the useful life of the product and the conversion of starches and sugars (Lima *et al.,* 2001). The firmness of the pepper, in experiments performed by Milagres *et al.,* (2012) was strongly influenced by storage at 25 °C, in addition to the use of irradiation, according to work done. The authors also observed a difference in the increase in resistance to penetration of the cylindrical probe of 2mm in diameter, and the loss of turgidity of the cells.

Table 2. Mean values of physicochemical characteristics of chili fruits submitted to different doses of ionizing irradiation

Irradiation Doses in KGy	Characteristics evaluated			
	pH	SST	Mass loss	Firmness
0,0	5,69	3,76	101,17	2,77
0,5	5,46	4,41	101,118	2,33
0,75	5,68	4,50	101,168	2,25
1,0	5,78	4,30	98,829	2,60
1,25	6,06	4,50	103,574	2,26
CV (%)	2,68	15,62	13,95	11,59
General Media	5,69	4,41	101,168	2,33

Means do not differ by Tukey test at 5% probability.

In the tests where the hydrogen ionic potential (pH) was verified, the analysis of variance showed no statistical difference between the different doses of gamma radiation, observing that even small variations in this characteristic can cause marked alterations in the fruit flavor Silva *et al*. (2008). There were no differences between the applied doses for total soluble solids. Silva *et al*. (2008) state that the increase in the amount of soluble solids is closely associated with the degree of maturation of the fruits. However, Santos (2008) and Françoso *et al*. (2008) obtained a reduction in OSH contents in irradiated pequi and strawberry, respectively. According to Kluge *et al.,* (2002) after prolonged storage the sugar content decreases. In relation to mass loss, no significant difference was observed between the irradiated and non-irradiated bell pepper fruits at all doses of gamma radiation to which the fruits were submitted. The loss of fruit mass is a factor that occurs naturally during the storage of fruits and vegetables, mainly due to the transpiration process (CHITARRA; CHITARRA, 2005). No statistical difference was found between the different irradiated doses and the fruits not irradiated. As to the visual appearance of the fruits, no interference was observed in the coloration between the irradiated and non-irradiated fruits, since they presented uniformity in color and brightness, diverging from the results found by Milagres *et al*. (2012), where it was verified a significant increase of the coloration of bell peppers.

Conclusion

It is concluded that irradiation is a useful tool in post-harvest handling of bell pepper rot and the dose of 1.25 kGy of gamma rays is the most recommended because it provides a decrease in the severity of soft rot by *Pectobacterium carotovorum* subsp. c*arotovorum*, without changing the physical-chemical characteristics of the fruit.

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