Research Journal of

Seed Science

ISSN 1819-3552

Academic Journals Inc.

www.academicjournals.com

Research Journal of Seed Science 3 (2): 70-81, 2010
ISSN 1819-3552

Effects of Gamma Irradiation on Physiological and Phytosanitary Qualities of Brazilian Castor Bean Seeds, *Ricinus communis* (cv. IAC Guarani)

[1]N.N. Kobori, [2]T. Mastrangelo, [1]S.M. Cicero,
[2]P. Cassieri, [3]M.H.D. Moraes and [2]J.M.M. Walder
[1]Seed Analyses Laboratory, Department of Crop Science,
Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP),
Av. Pádua Dias 11, 13418-900 Piracicaba, SP, Brazil
[2]Center for Nuclear Energy in Agriculture (CENA/USP),
Av. Centenário 303, 13400-970 Piracicaba, SP, Brazil
[3]Seed Pathology Laboratory, Department of Phytopathology and Nematology,
Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP),
Av. Pádua Dias 11, 13418-900 Piracicaba, SP, Brazil

Abstract: The castor bean seeds (*Ricinus communis* L.) are raw material for many industrial products and its importance has rise at developing countries in the past few years, since, castor seed oil can be used as substitute of petroleum for biodiesel, plastics and lubricants manufacture. The aim of this study was to observe the effects of a dose range of gamma radiation (0.5 to 10 kGy) over the physiological quality and seed-borne micloflora from two lots of Brazilian castor bean seeds. Seed vigour was assessed by a novel computer imaging analysis system and linear regression analyses were performed for the tests. Castor seed moisture content was not significantly affected by gamma radiation. First counting germination, germination and seedling emergence in sand were inhibited at doses = 2 kGy. The vigour index and seedling length were also severely reduced at higher doses. Both germination and vigour tests identified the lowest vigour of one of the seed lots. The incidence of some fungi as *Alternaria alternata*, *A. ricini*, *Penicillium* sp. and *Aspergillus* sp. was inhibited at doses = 5 kGy, but *Fusarium* sp. and *Cladosporium* sp. survived at exposures up to 8 kGy. Sterilization doses higher than 2-5 kGy would not be suitable as castor bean seeds revealed to be quite radiosensitive up to that dose range.

Key words: Radiation, germination, vigour, castor plant, *Ricinus communis*

INTRODUCTION

The castor bean plant, *Ricinus communis* L., belongs to the Euphorbiaceae family and has naturalized in most tropical and subtropical regions throughout the world (Azevedo and Lima, 2001). *Ricinus communis* seed contains between 40 and 60% oil, which is rich in triglycerides. Its oil has a growing international market assured by more than 700 uses,

Corresponding Author: T. Mastrangelo, Center for Nuclear Energy in Agriculture (CENA/USP), Av. Centenário 303, 13400-970 Piracicaba, SP, Brazil
Tel: +55-19-9152-9662

ranging from medicines and cosmetics to substituting petroleum in the manufacturing of biodiesel, plastics and lubricants (Azevedo and Lima, 2001; Devendra and Ragharan, 1978; Gaydou *et al*., 1982; Weiss, 1971).

The rising fossil fuel prices and security of energy supplies have been the key driver towards the use of renewable and carbon neutral energy sources. Consequently, there is growing interest in the transport industry in the exploitation of fuels produced from the biodegradable fraction of products and residues from agriculture (Demirbas, 2005; Roskilly *et al*., 2008). The use of castor oil as biodiesel stands now as an opportunity for agricultural development especially in arid and impoverished areas throughout the tropics globally. There are several ongoing breeding programs and leading producing areas are India, China, Brazil and Ethiopia (Nylund *et al*., 2008).

According to the Brazilian Institute of Geography and Statistics (IBGE, 2009), the Brazilian area cultivated with castor bean increased from 155, 033 ha in 2008 to 174, 770 ha in 2009 and the estimated seed production in 2008 was about 150, 000 tones.

Seed technologists have focused on the development or improvement of procedures which allow identification of higher quality seed lots (Demir and Mavi, 2008). The major seed quality consideration used by seed companies to determine the suitability of seed lots is still germination, which has been defined as the ability of a seed lot to produce normal seedlings under favorable conditions (AOSA, 1999). But, a better predictor of stand establishment in the field than germination is a vigour rating. Seed vigour is defined as the ability of a seed lot to have rapid uniform emergence of normal seedlings under a wide range of conditions and there are several procedures of vigour test adopted by seed testing organizations (AOSA, 1999; Delouche, 1986; Malik and Shamet, 2009). Recently, Marcos Filho *et al*. (2006) compared the efficiency of an automated computer imaging system to traditional vigour tests and concluded that the method has a potential use for seed quality control programs.

Despite the importance of those tests, there are still very few reports about the application of such tests for castor bean seeds. Fonseca *et al*. (2004) performed different tests to assess viability and vigour and concluded that the first count germination and the pH of seed exudate test were reliable, unlike the electrical conductivity test.

In order to ascertain the health of commercial seed, seed health tests are required (McGee, 1995). Some plant pathogens can quickly achieve global dissemination by using seeds of their hosts as vehicles for long-distance transport (Elmer, 2001). Hundreds of seedborne pathogens inconspicuously infest the seed and this association spans a continuum of relationships that range from passive hitchhikers on seed coats to infection of embryonic tissue, allowing their introduction into new niches and the occurrence of new outbreaks (Johansen *et al*., 1994; Kimati, 1980; McGee, 1995).

Massola and Bedendo (2005) assessed the sanitary quality of castor bean seeds and reported that *Fusarium oxysporum* f. sp. *ricini* and *Alternaria ricini* are the main pathogenic seed-borne fungi for this crop in São Paulo State, Brazil. Depending on soil and weather conditions, inoculums density and the level of resistance of the cultivar, *Fusarium* wilt can provoke serious damages (Carvalho and Nakagawa, 2000). Zarela *et al*. (2004) detected high frequencies for the genera *Fusarium* in seeds of 6 castor bean varieties, e.g., varieties Guarani and IAC 80. Souza *et al*. (2009) also reported high rates of contamination in castor bean seeds by *Fusarium* sp., *Aspergillus* sp. and *Penicillium* sp. Harvest delay of primary and secondary bunches can result in higher incidences of pathogenic fungi in the field, as *Fusarium* sp. and *Alternaria* sp., but with no withdraw to the physiological quality of seeds (Fanan, 2008; Weiss, 1971).

The World Health Organization encourages the use of irradiation of seeds, which it described as a technique for preserving and improving the safety of food (WHO/FAO, 1988; WHO, 1999). Ionizing radiations have immense applications in agriculture and since decades, they have been employed as an excellent tool for application in food sterilization, preservation and different food engineering processes (Ivanov *et al.*, 2001; Dusan, 2004; Hyun-Pa *et al.*, 2006; Sameh *et al.*, 2006; Maity *et al.*, 2008). Actually, the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard and no special nutritional or microbiological problems (Ingram and Farkas, 1977; WHO, 1981, 1991).

The aim of the present study was to observe the effects of a dose range of gamma radiation on this crop, taking in consideration the physiological quality of the seed and the reduction of the level of some seed-borne pathogenic fungi.

MATERIALS AND METHODS

Samples and Irradiation

The study was conducted at the Seed Analysis Laboratory and the Seed Pathology Laboratory of the University of São Paulo (USP), but irradiation was carried out at the Plant Breeding Laboratory of the Center for Nuclear Energy in Agriculture (CENA/USP, Brazil) in 2009. Castor bean seeds of 2 different lots from cv. IAC Guarani were exposed to a ^{60}Co source (Gamma Cell 220 irradiator®, MDS Nordion International Inc., Canada) with an activity of 26 TBq (714 Ci) and dose rate of 0.51 kGy h^{-1} at 25°C. Radiation doses were 0.5, 1.0, 2.0, 5.0 and 10 kGy. The radiation doses were measured with a Fricke Dosimeter (Fricke and Hart, 1966).

Seed Moisture Content

Determinations were done before and after irradiation by the oven method with air circulation at 105±3°C for 24 h as indicated by the Brazilian Rules for Seed Testing (Brazil, 2009), with two seed samples of approximately 10.0 g for each treatment. Results were expressed as mean percentage moisture content for each treatment (fresh weight basis). Evaluations were performed before germination and seedling emergence tests.

Germination Test

Four replicates of 50 seeds each per treatment were sowed in moistened paper towel rolls and then germinated at 25±5°C with 8 h of photophase. Seedling evaluations were performed at seven (first counting germination) and 14 days after sowing, considering normal seedlings in accord to the criteria of the Brazilian Rules for Seed Testing (Brazil, 2009). Results were expressed as mean percentage of normal seedlings for each treatment (Nakagawa, 1994).

Seedling Emergence in Sand

Seeds were buried in multicell polystyrene trays containing sand at approximately 3 cm depth. Sand was irrigated constantly and there were four replicates of 50 seeds for each treatment. The evaluation was made only at the 21 days after sowing by counting the number of emerged seedlings. Results were expressed as mean percentage of emerged seedlings.

Assessment of Vigour by the Computer Imaging Analysis

Seeds were germinated in a near vertical orientation (80 to 85°) on moistened rolled paper towels at 20-30°C for 5 days. Four replicates of 25 seeds for each treatment were used. Image acquisition was accomplished by a custom scanning box computer system (HP Scanjet®, 2004) inserted in an Aluminum box (60×50×12 cm) and by a software processing of images (Photosmart®), with 1200 dpi of resolution. Seedlings were transferred to a black paperboard measuring the scanner's area and placed inside the aluminum box. The imaging platform captured multiple images of seedlings, enabling simultaneous measurements of the hypocotyl/primary root axes that were further processed by the Seed Vigour Imaging System software (SVIS®) that quantifies the potential performance of the seed sample. After each seedling was processed and automatically measured, the results of each replicate were combined to produce indices of vigour and uniformity of growth. It was included the penalty term in the uniformity algorithm to avoid the undesirable influence of dead seeds on precision of results. Small adjustments to the software used for soybeans were made to properly recognize and mark the normal seedling hypocotyl/primary root axis. Both indices ranged from a minimum value of zero to a maximum of 1000 and, ideally, high vigour seedlings should be long and exhibit high uniformity within individuals representing the seed sample. Results were expressed as mean values of seedling vigour and seedling length for each treatment (Hoffmaster *et al.*, 2003; Marcos Filho *et al.*, 2006).

Health Seed Test

The blotter test method without freezing (Limonard, 1966) was used as heath seed test. Four replicates with 50 seeds per treatment were incubated in petri dishes (10 seeds $dish^{-1}$) containing three moistened blotter papers. The dishes were incubated for ten days at 20±2°C and photoperiod of 12:12 (L:D). Pathogens that grew were identified following Barnett and Hunter (1972). Results were expressed as pathogen incidence mean percentages.

Statistical Analysis

The treatments and seed lots were distributed in a complete randomized design and analyses were performed separately for each seed test and lot. For statistical analysis of the percentages of seed moisture content, normal seedlings, emerged seedlings, pathogenic fungi incidence and the values of seedling vigour and seedling length, linear regression analysis was performed at the 1% level of significance according to Sokal (1981). The estimated radiation doses that reduces each one of those variables in 90% comparing to the control values (i.e., LD_{90}) with upper and lower confidence limits (α = 0.05) were also calculated. A test for difference of slopes verified the parallelism hypothesis between the regression lines for the two lots ($p<0.01$).

RESULTS AND DISCUSSION

Seed Moisture Content

Castor seeds moisture content was not significantly affected by gamma radiation and no significant difference among the means was found ($p<0.01$) (Table 1). The moisture content remained at about 5 to 6% after irradiation.

Germination and Vigour

Regression analysis indicated significant effects ($p<0.01$) of radiation doses on both seed lots in all performed tests (Table 2, 3). The first counting germination, germination and seedling emergence in sand were inhibited at doses = 2 kGy. The mean percentage for those variables after 2 kGy was considered to be zero since, the seedlings showed abnormalities according to the criteria of the Brazilian Rules for Seed Testing (Brazil, 2009).

Table 1: Castor seed moisture content (fresh basin) before and after gamma irradiation

Moisture content (%) (fresh basin)	Dose (kGy) 0	0.5	1	2	5	10	Significance of the linear regression ($p<0.01$)
Before irradiation							
Lot 1	5.6	5.7	6.3	5.7	6.1	6.2	$MSE^{\alpha} = 0.08$
Statistical analysis							$F_{1,23} = 2.05^{ns\,b}$
MSE^{α}							$p = 0.22$
Lot 2	5.5	6.8	6.3	6.7	6.3	6.5	MSE = 0.15
Statistical analysis							$F_{1,23} = 1.8^{ns}$
MSE^{α}							$p = 0.3$
After irradiation							
Lot 1	5.5	5.5	5.9	5.9	6.7	6.1	MSE = 0.25
Statistical analysis							$F_{1,23} = 0.21^{ns}$
MSE^{α}							$p = 0.67$
Lot 2	5.7	6.6	6.3	6.3	6	6.7	MSE = 0.14
Statistical analysis							$F_{1,23} = 2.26^{ns}$
MSE^{α}							$p = 0.21$

[a]MSE: Residual mean-square error, [b]Analysis of variance with F-test indicates if a significant linear regression can be fitted to the data or not (ns = not significant, $p>0.01$)

Table 2: Physiological tests for two lots of irradiated castor bean seeds cv.IAC Guarani (mean percentage±standard error)

Test	Dose (kGy) 0	0.5	1	2-10	Significance of the linear regression ($p<0.01$)	Slope±SE	Test for difference of slopes	LD_{90} (95% CI)
First count germination								
Lot 1	73±3.4	54±6.9	11±2.2	0	$MSE^{\alpha} = 596.1$ $F_{1,23} = 13.8^{b}$ $p = 0.001$	-5±1.4 ($p = 0.001$)	$F_{1,44} = 3.1$ ns $p = 0.08$	5.5 (3.1; 7.9)[c]
Lot 2	29±6.5	28±7.4	9±1.6	0	MSE = 152.5 $F_{1,23} = 11.9$ $p = 0.002$	-3±0.7 ($p = 0.002$)		3.5 (1.4; 5.6)
Germination (14° day)								
Lot 1	86±2.5	78±.2	18±1.7	0	MSE = 874.4 $F_{1,23} = 15.8$ p<10-3	-7±1.7 ($p<10^{-3}$)	$F_{1,44} = 3.0$ ns $p = 0.09$	6.01 (3.6; 8.4)
Lot 2	56±6.2	22±1.1	9±1.6	0	MSE = 309.1 $F_{1,23} = 10.9$ $p = 0.003$	-3±1.03 ($p = 0.003$)		4.4 (2.01; 6.7)
Seedling emergence in sand								
Lot 1	72±.7	70±3.6	12±2.9	0	MSE = 700.9 $F_{1,23} = 14.1$ $p = 0.001$	-6±1.5 ($p = 0.001$)	$F_{1,44} = 0.23$ ns $p = 0.63$	5.8 (3.3; 8.2)
Lot 2	61±5.3	50±2.9	23±1.6	0	MSE = 383.03 $F_{1,23} = 18.3$ $p<10^{-3}$	-5±1.1 ($p<10^{-3}$)		5.6 (3.5; 7.7)

[a]MSE: Residual mean-square error, [b]Analysis of variance with F-test indicates if a significant linear regression can be fitted to the data or not (ns = not significant, $p>0.01$).[c]Confidence Interval stated at 95% confidence level

The estimated mean LD_{90}s for these tests (Table 2) ranged from 3.5 to 6 kGy and the 95% confidence intervals overlapped. The slopes of the linear regressions did not differ significantly (Table 2).

The percentages of seedling emergence in sand from the control were slighter lower comparing to the mean percentages from germination test control (Table 2). This difference is attributed to the less favorable conditions of the greenhouse.

Vigour index and seedling length were also severely reduced at higher doses (Table 3), but the estimated mean LD_{90}s values were higher (e.g., 7.5-8 kGy) comparing to those estimated for first counting germination, germination and seedling emergence in sand. This dose overestimation was caused by the fact that both the vigour index and the seedling length took into account the entire length of the hypocotyls/primary root axis at 2 and 5 kGy.

Table 3: Vigour index and seedling length of irradiated castor bean seeds cv. IAC Guarani (Mean±SE)

Vigour parameters	Dose (kGy) 0	0.5	1	2	5	10	Significance of the linear regression (p<0.01)	Slope± standard error	Test for difference of slopes	LD_{90} (95% CI)
Vigour index										
Lot 1	587±18.6	522±17.7	457±15.5	347±8.6	305±9.8	0	MSE[a] = 2659.6 $F_{1,23}$ = 312.4[b] $p<10^{-3}$	-53±3.01 ($p<10^{-3}$)	$F_{1,44}$ = 21.5 $p<10^{-3}$	8.2 (7.4; 8.9)[c]
Lot 2	383±16.5	339±37.1	325±16.7	288±15.3	219±6.5	0	MSE = 1469.1 $F_{1,23}$ = 256.2 $p<10^{-3}$	-36±2.2 ($p<10^{-3}$)		7.5 (6.7; 8.3)
Seedling length (mm)										
Lot 1	30±0.9	25±0.8	17±1.0	8±0.1	7±0.6	0	MSE = 32.3 $F_{1,23}$ = 58.7 $p<10^{-3}$	-3±0.3 ($p<10^{-3}$)	$F_{1,44}$ = 5.6 ns p = 0.02	8.0 (6.5; 9.1)
Lot 2	20±0.9	15±1.9	12±0.9	9±0.9	7±0.5	0	MSE = 9.3 $F_{1,23}$ = 86.3 $p<10^{-3}$	-2±0.2 ($p<10^{-3}$)		8.2 (6.6; 9.4)

[a]MSE: residual mean-square error. [b]Analysis of variance with F-test indicates if a significant linear regression can be fitted to the data or not (ns = not significant, p>0.01). [c] Confidence Interval stated at 95% confidence level

Table 4: Effects of gamma radiation on castor seed-borne fungi (mean percentage of incidence±standard error)

Pathogenic fungi	Dose (kGy) 0	0.5	1	2	5-10	Significance of the linear regression (p<0.01)
***Aspergillus* sp.**						
Lot 1	41±27.0	14±1.0	1±0	0	0	MSE a = 367.1 $F_{1,23} = 0.27^{ns\,b}$ p = 0.61
Lot 2	40±1.0	31±3.0	12±2.0	0	0	MSE = 192.9 $F_{1,23} = 4.5^{ns}$ p = 0.06
***Penicillium* sp.**						
Lot 1	5±3.0	2±1.0	0	0	0	MSE = 11.6 $F_{1,23} = 2.4^{ns}$ p = 0.15
Lot 2	5±1.0	2±2.0	0	0	0	MSE = 5.5 $F_{1,23} = 0.0001^{ns}$ p = 0.99
A. alternata						
Lot 1	6±2.0	6±2.0	3±1.0	4±2.0	0	MSE = 5.2 $F_{1,23} = 9.01^{ns}$ p = 0.01
Lot 2	8±4.0	6±2.0	5±1.0	3±3.0	0	MSE = 9.7 $F_{1,23} = 7.1^{ns}$ p = 0.02

[a]MSE: Residual mean-square error. [b]Analysis of variance with F-test indicates if a significant linear regression can be fitted to the data or not (ns = not significant, p>0.01)

The slopes of the regression lines for the two lots vigour indexes differed significantly ($F_{1,44}$ = 21.5; $p<10^{-3}$), what could suggest a higher radiosensitivity of lot 2. However, the mean LD_{90} values did not differ significantly, since, the 95% confidence intervals overlapped (Table 3).

Effects of Gamma Radiation on Seed-borne Fungi

The percentage of incidence of all gamma irradiated fungi decreased in a dose dependent manner (Table 4), but for *Alternaria ricini* (lot 2), *A. alternata*, *Aspergillus* sp. and *Penicillium* sp., the linear regression was not significant, not allowing the estimation of the LD_{90}s. One of the reasons for this was the very low natural incidence (i.e., less than 10%) for some fungi as *A. alternata* and *Penicillium* sp. Besides it, no incidence of *Aspergillus* sp. and *Penicillium* sp. was observed at doses = 2 kGy and = 5 kGy for *A. alternata*.

Table 5: Effects of gamma irradiation on castor seed-borne fungi (mean percentage of incidence±standard error)

Vigour parameters	Dose (kGy) 0	0.5	1	2	5	10	Significance of the linear regression ($p<0.01$)	Slope± standard error	Test for difference of slopes	LD_{90} (95% CI)
***Fusarium* sp.**										
Lot 1	97±1.0	91±9.0	92±2.0	54±14.0	15±3.0	2±2.0	MSE[a] = 309.01 $F_{1,23} = 48.6$[b] $p<10^{-3}$	-10±1.5 ($p<10^{-3}$)	$F_{1,20} = 3.4^{ns}$ p = 0.07	7.9 (6.02; 9.7) c
Lot 2	75±1.0	71±5.0	59±3.0	44±2.0	15±3.0	7±1.0	MSE = 133.5 $F_{1,23} = 52.4$ $p<10^{-3}$	-7±0.9 ($p<10^{-3}$)		8.2 (6.3; 10.1
***Cladosporium* sp.**										
Lot 1	64±2.0	58±6.0	52±22.0	46±4.0	22±10.0	2±0.1	MSE = 173.3 $F_{1,23} = 31.03$ $p<10^{-3}$	-6±1.1 ($p<10^{-3}$)	$F_{1,20} = 4.7^{ns}$ p = 0.04	8.1 (5.7; 10.5)
Lot 2	87±9.0	81±3.0	82±6.0	58±4.0	21±1.0	1±1.0	MSE = 113.6 $F_{1,23} = 107.2$ $p<10^{-3}$	-9±0.9 ($p<10^{-3}$)		8.03 (6.7; 9.3)
A. ricini										
Lot 1	32±8.0	18±10.0	23±1.0	19±3.0	3±3.0	0	MSE = 79.6 $F_{1,23} = 16.6$ p = 0.002	-3±0.7 (p = 0.02)	---	4.7 (2.6; 6.8)
Lot 2	8±4.0	6±5.0	3±1.0	1±1.0	0	0	MSE = 10.6 $F_{1,23} = 5.3$ ns p = 0.04	---		---

[a]MSE: residual mean-square error. [b]Analysis of variance with F-test indicates if a significant linear regression can be fitted to the data or not (ns = not significant, p>0.01). [c]Confidence Interval stated at 95% confidence level

For *Fusarium* sp., 90% reduction of the incidence was achieved at mean doses about 7-8 kGy in either lots (Table 5). The slopes of the regression lines did not differ significantly ($F_{1,20} = 3.4$; p = 0.07), revealing that the decrease of incidence occurred proportionally in the same way.

Cladosporium sp. also survived the higher radiation exposures and the mean estimated LD_{90}s were about 8 kGy. The slopes of the regression lines were not significantly different ($F_{1,20} = 4.7$; p = 0.04).

For most seeds, the equilibrium moisture content depends mainly on the relative humidity and temperature conditions of the environment (Yang *et al*., 2003; Reddy and Chakraverty, 2004). Although, the castor seeds moisture content has not been significantly affected by gamma radiation, further investigations on possible changes of the inner structure of castor seed caused by gamma irradiation should be carried out, since radiation can alter the drying characteristics of many foods over time (WHO, 1999). For example, Yu and Wang (2005) showed that, when gamma irradiation is used as a pretreatment method before drying, the drying characteristics of rough rice are affected. Ashaye (2006) found that the amount of water absorbed by irradiated cowpeas (*Vigna unguiculata*) is generally higher than non-irradiated cowpeas. Yu and Wang (2007) found that the values of the equilibrium moisture content of wheat decreased with an increasing dose of gamma irradiation due to the changes of wheat starch granules and their water bound ability caused by different radiation doses.

Castor seeds are usually slow to germinate and seedling emergence may take 7 to 14 days under optimal conditions. But depending on the physiological quality of seed and the environment, it can take 7 to 20 days (Carvalho, 2005). No delay in germination provoked by radiation was noticed on this work.

Regression analysis indicated significant effects of radiation doses on both seed lots in all performed tests of germination and vigour. Biologically, parallelism can be interpreted to mean that changes in activity per unit change in rate are the same (i.e., potency as defined by Finney (1971). Thus, as the slopes of the linear regressions did not differ significantly (Table 2), the damages were induced by gamma radiation proportionally in the same manner in the three germination tests.

The estimated mean LD_{90}s values for the vigour index and seedling length were higher (Table 3) than those estimated for first counting germination, germination and seedling emergence in sand. However, as the LD_{90} values are based on the assumptions of the regression model, they are estimates, not measurements. Therefore, sampling error and natural variation may always contribute to the appearance of different LD values from the same samples in repeated bioassays (Robertson *et al.*, 2007).

Gamma radiation induced physiological changes were plenty effective in avoiding germination in exposures after 5 kGy (Table 2 and 3). Free radicals generated in irradiated seeds and the induced genetic damages might be related to full growth inhibition (WHO, 1999; Kumagai *et al.*, 2000). Basyony *et al.* (1989) irradiated soybean seeds and verified that 3 kGy reduced the percentage of seed germination and the seed oil content was affected. Younis and Hammouda (1960) detected no stimulating effect on growth and development when dry cotton seeds were subjected to 11.4 to 262 Gy of gamma radiation, but at higher doses they also noticed reduction of emergence, retardation of flowering and lower production of bolls.

In general, the seedling vigour index and seedling length that comprised the computer imaging system of seed performance evaluation (Table 3) were consistent with results for the first counting germination, germination and seedling emergence in sand and also identified the lowest vigour of lot 2. This lower vigour can be explained by the fact that lot 2 was comprised by freshly harvested seeds for which some level of dormancy was expected. The intensity and persistence of the dormancy depends on the cultivar and the seed maturation stage at the moment of the harvest. In addition, the type of castor raceme has influence on seed degree of dormancy and the dormancy also decreases with the increase of the storage time of seeds (Weiss, 1971).

The automated computer imaging system here applied can assess seed vigour of many species and was successfully used for the evaluation of lettuce (Sako *et al.*, 2001), soybean (Hoffmaster *et al.*, 2003) and melon seed vigour (Marcos Filho *et al.*, 2006). Such system brings some advantages as image storability and similarity to the information provided by traditional vigour tests (McCormac *et al.*, 1990; Marcos Filho *et al.*, 2006).

Differences in radiation induced effects have been reported for many seed-borne fungi species and gamma radiation at doses up to 10 kGy can be very effective in eliminating microflora (Aziz *et al.*, 2007; Bari *et al.*, 2003; Maity *et al.*, 2008; Rajkowski and Thayer, 2001; Saleh *et al.*, 1988).

The radiosensitivity of *Alternaria* sp. and *Aspergillus* sp. is well documented in literature. The presented data (Table 4) for these two fungi contrast to the findings of some authors. Saleh *et al.* (1988) estimated an inactivation dose range for *A. alternata* at higher doses (e.g., 11.5-13.9 kGy). Cuero *et al.* (1986) also reported microflora inhibition on maize seeds at a high dose (12 kGy). Complete growth inhibition of *Aspergillus* sp. on the powder black pepper at a higher dose range (5 and 10 kGy) was reported by Emam *et al.* (1995). Decontamination of *Nigella sativa* seed from *Aspergillus* sp. can be achieved at 6 kGy (El-Bazza *et al.*, 2001). However, Maity *et al.* (2008) observed the efficiency of gamma exposure in controlling fungi at lower doses, reporting that the total growth inhibition of *Alternaria* sp. was achieved at 2.5 kGy and *Aspergillus* sp. was completed inhibited at 3 kGy. Similarly, Saleh *et al.* (1988) achieved total inactivation of *Aspergillus flavus*, *Aspergillus* *niger*, *Aspergillus fumigatus* and *Aspergillus parasiticus* at a lower dose range (1.7-3 kGy). Maity *et al.* (2009) demonstrated that *Alternaria* sp. and *Aspergillus* sp. can survive up to 3-4 kGy immediately after irradiation, but the total inhibition is exhibited after 1.5 month storage seed.

Penicillium sp. was inhibited at 1 KGy. According to Saleh *et al.* (1988), *Penicillium* sp. was complete inactivated at likely doses (1.7-2.5 kGy). At 10 kGy (Table 5), the observed *Fusarium* sp. incidence was 2±2.0 and 7±1.0% for lots 1 and 2, respectively, what may suggest a lower radiosensitivity of infecting local species. In contrast, *Fusarium solani* is inhibited at 1.7-2.5 kGy (Saleh *et al.*, 1988). Aziz *et al.* (2007) also reported that *Fusarium* sp. was inhibited at 4.0 kGy for barley and 6.0 kGy for wheat and maize seeds. At 5 kGy, the observed *Cladosporium* sp. incidence was about 20%, what is close from literature. *Cladosporium cladosporioides*, for example, can be inactivated at 6-6.5 kGy (Saleh *et al.*, 1988).

It is necessary to state that comparisons between different studies in radiosensitivity of seed-borne fungi must be done carefully, since different methodologies to assess the sensitivity are applied. Natural radioresistance already vary among fungi species and also depends on age of inoculums and the medium. Conidia suspended in water are inactivated at lower doses than in agar media for instance. In agar cultures, in their turn, the nutrient conditions favor the function of fungi's repair system and the organic compounds in the medium compete with the fungus for the hydrolytic products of irradiation (Saleh *et al.*, 1988; Silverman, 1983; Urbain, 1986).

Other several factors can contribute to an increased radioresistance of some fungi species, as a higher number of chromosomes. In addition, macroconidia thick walled, multicelled and containing melanin pigment, as occurs for *Cladosporium* sp. and *Curvularia* sp. can show increased radioresistance (Saleh *et al.*, 1988).

CONCLUSIONS

The performed tests indicated that the decrease in germinating potential and in fungal incidence of the treated castor bean seeds was noticed in a dose-dependent manner. Seed vigour was successfully assessed by the automated computer imaging analysis system, revealing the potential of this recent method as a vigour test. The reduction in percentage of incidence of seed-borne fungi by gamma radiation was significantly different in different species. As castor bean seeds revealed to be quite radiosensitive to gamma radiation up to 2-5 kGy as other oil seeds, sterilization doses higher than that dose range would not be suitable when considering the maintenance of physiological quality.

ACKNOWLEDGMENTS

Authors thank the Instituto Agronômico de Campinas (IAC) for providing castor bean seeds and the Conselho Nacional de Desenvolvimento Tecnológico e Científico (CNPq) for scholarships.

REFERENCES

AOSA, 1999. Rules for Testing Seeds. AOSA, Lincoln NE.

Ashaye, O.A., 2006. Effect of gamma irradiation on moisture sorption isotherms of cowpeas. Afr. J. Biotech., 5: 1505-1507.

Azevedo, D.M.P. and E.F. Lima, 2001. O Agronegócio da Mamona No Brasil [The Castor Plant Agribusiness in Brazil]. 1st Edn., Embrapa Informação Tecnológica, Brasília, ISBN: 8573831162.

Aziz, N.H., F.M. El-Far, A.A.M. Shahin and S.M. Roushy, 2007. Control of *Fusarium* moulds and fumonisin B1 in seeds by gamma-irradiation. Food Control, 18: 1337-1342.

Bari, M.L., E. Nazuka, Y. Sabina, S. Todoriki and K. Isshiki, 2003. Chemical and irradiation treatment for killing *Escherichia coli* 0157: H7 on alfalfa, radish and mung bean seeds. J. Food Prot., 66: 767-774.

Barnett, H.L. and B.B. Hunter, 1972. Illustrated Genera of Imperfect Fungi. 3rd Edn., Burgess Publishing Company, Minneapolis, ISBN-10: 0808702661, pp: 331.

Basyony, A.E., M.I. El-Refaei, M.S. Galal and M.I.E. Barakat, 1989. Effect of gamma irradiation on seed-bome fungi and soybean seed components during storage. Agric. Res. Rev., 67: 619-628.

Brazil, 2009. Ministério da Agricultura e Reforma Agrária. 3rd Edn., SNDV/CLAV, Brasília, ISBN: 978-85-99851-70-8.

Carvalho, B.C.L., 2005. Manual do Cultivo da Mamona [Castor Bean Production Manual]. 1st Edn., EBDA, Salvador, ISBN: 978-85-61250-13-3.

Carvalho, N.M. and J. Nakagawa, 2000. Seeds: Science, technology and production. FUNEP, São Paulo, ISBN: 85-87632-01-9.

Cuero, R.G., J.E. Smith and J. Lacey, 1986. The influence of gamma irradiation and sodium hypochlorite sterilization on maize seed microflora and germination. Food Microbiol., 3: 107-113.

Delouche, J.C., 1986. Observaciones sobre deterioracion de semillas. Semillas, 1: 8-11.

Demir, I. and K. Mavi, 2008. Seed vigour evaluation of cucumber (*Cucumis sativus* L.) seeds in relation to seedling emergence. Res. J. Seed Sci., 1: 19-25.

Demirbas, A., 2005. Biodiesel impacts on compression ignition engine (CIE): Analysis of air pollution issues relating to exhaust emissions. Energy Sour., 27: 549-558.

Devendra, C. and G.V. Raghavan, 1978. Agricultural by-products in South East Asia: Availability, utilization and potential value. World Rev. Anim. Prod., 14: 11-27.

Dusan, R., 2004. Twenty years of radiation sterilization in Croatia. Radiat. Phys. Chem., 71: 597-602.

El-Bazza, Z.E.M., A.F. Hala, M.E.D.Z. EL-Fouty and S.Y.M. El-Tablawy, 2001. Inhibitory effect of gamma radiation and *Nigella sativa* seeds oil on growth, spore germination and toxin production of fungi. Radiat. Phys. Chem., 60: 181-189.

Elmer, W.H., 2001. Seeds as vehicles for pathogen importation. Biol. Invasions, 3: 263-271.

Emam, O.A., S.A. Farag and N.H. Aziz, 1995. Comparative effects of gamma and microwave irradiation on the quality of black paper. Zeitschrift Lebensmitteluntersuchung Forschung A, 201: 557-561.

Fanan, S., 2008. Physiological and sanity quality of castor bean seeds related to clime and harvest conditions. http://www.iac.br/PosIAC/pdf/pb1212506.pdf.

Finney, D.J., 1971. Probit Analysis. 3rd Edn., Cambridge University Press, Cambridge, UK.

Fonseca, N.R., M.L. Myczkowski, M. Prior, R.O. Sá, J. Nakagawa, C. Cavariani and M.D. Zanotto, 2004. Assessment of viability and germination of castor bean seeds. Proceedings of 1st Brazilian Symposium on Castor Bean Plant, Nov. 23-26, Campina Grande, Brazil, pp: 7-12.

Fricke, H. and E.J. Hart, 1966. Chemical Dosimetry. In: Radiation Dosimetry, Attix, F.H. and W.C. Raesch (Eds.). Vol. 2. Academic Press, New York.

Gaydou, A.M., L. Menet, G. Ravelojaona and P. Geneste, 1982. Vegetable energy sources in Madagascar: Ethyl alcohol and oil seeds (French). Oleagineux, 37: 135-141.

Hoffmaster, A.L., K. Fujimura, M.B. McDonald and M.A. Bennett, 2003. An automated system for vigour testing three-day-old soybean seedlings. Seed Sci. Technol., 31: 701-713.

Hyun-Pa, S., K. Dong-Ho, J. Cheorun, L. Cheol-Ho, K. Kyong-Soo and B. Myung-Woo, 2006. Effect of gamma irradiation on the microbiological quality and antioxidant activity of fresh vegetable juice. Food Microbiol., 23: 372-378.

IBGE, 2009. Levantamento sistemático da produção agrícola brasileira. http://www.ibge.gov.br/home/estatistica/indicadores/agropecuaria/lspa/lspa_200901_4.shtm.

Ingram, M. and J. Farkas, 1977. Microbiology of foods pasteurised by ionising radiation. Acta Alimentaria, 6: 123-185.

Ivanov, A.S., V.P. Ovchinnikov, M.P. Svinin, N.G. Tolstun and S.L. Bogart, 2001. 4 MeV high-voltage accelerator with 500kW electron beam for radiation sterilization. Vacuum, 62: 225-231.

Johansen, E., M.C. Edwards and R.O. Hampton, 1994. Seed transmission of viruses: Current perspectives. Annu. Rev. Phytopathol., 32: 363-386.

Kimati, H., 1980. Castor Plant Diseases. In: Phytopathology Manual, Galli, F. (Ed.). Cornell University, São Paulo, ISBN: 85-318-0006-4, pp: 347-351.

Kumagai, J., H. Katoh, T. Kumada, A. Tanaka, S. Tano and T. Miyazakit, 2000. Strong resistance of *Arabidopsis thaliana* and *Raphanus sativus* seeds for ionizing radiation as studing by ESR, ENDOR, ESE spectroscopy and germination measurement: Effect of long lived and spur-lived radicals. Radiat. Phys. Chem., 57: 75-83.

Limonard, T., 1966. A modified blotter test for seed health. Eur. J. Plant Pathol., 72: 319-321.

Maity, J.P., S. Chanda, A. Chakraborty and S.C. Santra, 2008. Effect of gamma radiation on growth and survival of common seed-borne fungi in India. Radiat. Phys. Chem., 77: 907-912.

Maity, J.P., S. Kar, S. Banerjee, A. Chakraborty and S.C. Santra, 2009. Effects of gamma irradiation on long-storage seeds of *Oryza sativa* (cv. 2233) and their surface inecting fungal diversity. Radiat. Phys. Chem.,

Malik, A.R. and G.S. Shamet, 2009. Storage of *Pinus gerardiana* seeds: biochemical changes and its applicability as vigour test. Res. J. Seed Sci., 2: 48-55.

Marcos-Filho, J., M.A. Bennett, M.B. McDonald, A.F. Evans and E.M. Grassbaugh, 2006. Assessment of melon seed vigour by an automated computer imaging system compared to traditional procedures. Seed Sci. Technol., 34: 485-497.

Massola, N.S. and I.P. Bedendo, 2005. *Ricinus communis* L. Diseases. In: Phytopathology Manual: Diseases from Major Crops, Kimati, H., L. Amorim, J.AM. Rezende, A.B. Filho and L.E.A. Camargo (Eds.). Agronômica Ceres, São Paulo, ISBN: 85-318-0043-9, pp: 497-500.

McCormac, A.C., P.D. Keefe and S.R. Draper, 1990. Automated vigour testing of field vegetables using image analysis. Seed Sci. Technol., 18: 103-112.

McGee, D.C., 1995. Epidemiological approach to disease management through seed technology. Annu. Rev. Phytopathol., 33: 445-466.

Nakagawa, J., 1994. Testes de Vigor Baseados na Avaliação Das Plântulas [Seed Vigour Tests Based on Seedling Characteristics]. In: Testes de Vigor de Sementes [Seed Vigour Testing], Vieira, R.D. and N.M. Carvalho (Eds.). FUNEP, Jaboticabal, pp: 49-85.

Nylund, N.O., P. Aakko-Saksa and K. Sipilä, 2008. Status and outlook for biofuels, other alternative fuels and new vehicles. Espoo 2008. VTT Tiedotteita. Research Notes 2426. https://www.senternovem.nl/mmfiles/VTT%202008%20-%20Status%20and%20outlook%20for%20biofuels%20%20other%20alternative%20fuels%20and%20new%20vehicles1_tcm24-280289.pdf.

Rajkowski, K.T. and D.W.L. Thayer, 2001. Alfalfa seed germination and yield ratio and alfalfa sprout microbial keeping quality following irradiation of seeds and sprouts. J. Food Port., 64: 1988-1995.

Reddy, B.S. and A. Chakraverty, 2004. Equilibrium moisture characteristics of raw and parboiled paddy, brown rice and bran. Drying Technol., 22: 837-851.

Robertson, J.L., R.M. Russell, H.K. Preisler and E. Savin, 2007. Bioassays with Arthropods. 2nd Rev. Edn., CRC Press, Boca Ratone, FL.

Roskilly, A.P, S.K. Nanda, Y.D. Wang and J. Chirkowski, 2008. The performance and the gaseous emissions of two small marine craft diesel engines fuelled with biodiesel. Appl. Thermal Eng., 28: 872-880.

Sako, Y., M.B. McDonald, K. Fujimura, A.F. Evans and M.A. Bennett, 2001. A system for automated seed vigour assessment. Seed Sci. Technol., 29: 625-636.

Saleh, Y.G., M.S. Mayo and D.G. Ahearn, 1988. Resistance of some common fungi to gamma irradiation. Appl. Environ. Microbiol., 54: 2134-2135.

Sameh, A.S.A., A.P. Kumar, B.S.M. Rao and R.P. Singh, 2006. Biodegradation of g-sterilised biomedical polyolefins under composting and fungal culture environments. Polymer Degradation Stability, 91: 1105-1116.

Silverman, G.J., 1983. Sterilization by Ionizing Radiation. In: Disinfection, Sterilization and Preservation, Block, S. (Ed.). 3rd Edn., Lea and Febiger, Philadelphia.

Sokal, R.R., 1981. Biometry. 2nd Edn., W.H. Freeman, San Francisco, California, USA.

Souza, L.A., M.L.M. Carvalho, V.Y. Kataoka and J.A. Oliveira, 2009. Teste de condutividade elétrica para avaliação da qualidade fisiológica em sementes de mamona. Revista Brasileira Sementes 31: 60-66.

Urbain, W.M., 1986. Food Irradiation. Academic Press Inc., New York, ISBN-13: 978-0127093703.

WHO, 1981. Wholesomeness of Irradiated Food. World Health Organization, Geneva, ISBN-10: 9241206594.

WHO, 1991. Food Irradiation: A Technique for Preserving and Improving the Safety of Food. World Health Organization, Geneva, pp: 19.

WHO, 1999. High-Dose Irradiation: Wholesomeness of Food Irradiated with Doses Above 10 kGy. World Health Organization, Geneva.

WHO/FAO, 1988. Food Irradiation: A Technique for Preserving and Improving the Safety of Food. World Health Organization, Geneva.

Weiss, E.A., 1971. Castor, Sesame and Safflower. Leonard Hill, London, ISBN: 0-85954-137-1.

Yang, Z., X.W. Luo and C.Y. Li, 2003. Studies on dynamic equilibrium moisture content of wheat grain. J. South China Agric. Univ., 24: 74-76.

Younis, A.E. and M.A. Hammouda, 1960. Pre-sowing gamma irradiation of cotton seeds from a point source of Cs137 and its effect on emergence, growth and yield. Plant and Soil, 13: 311-321.

Yu, Y. and J. Wang, 2005. Effect of c irradiation pre-treatment on drying characteristics and qualities of rice. Radiat. Phys. Chem., 74: 378-383.

Yu, Y. and J. Wang, 2007. Effect of gamma-ray irradiation on modeling equilibrium moisture content of wheat. J. Food Sci., 72: 405-411.

Zarela, G.C.N.Z., B. Bueno, S.D.A. Silva and A.C. Gomes, 2004. Fungos associados às sementes de mamoneira (*Ricinus communis* L.) cultivadas na região de Pelotas, Brasil. Proceedings of 1st Brazilian Symposium on Castor Bean Plant, Nov. 23-26, Campina Grande, Brazil, pp: 1-4.