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Effect of different doses of gamma radiation on avocado buds for produce of new genotypes

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ABSTRACT

This study was carried out during two successive seasons (2016 and 2017) at "El Kanater El Khayreia" Experimental Station at Kalubia Governorate, Egypt. To evaluate the effect of radiation to obtain mutations induced in plants with improved genotypes. Fuerte and Hass avocado bud sticks of about 20 cm of length from the new flushes including 5-7 buds were selected from a mature bearing. These shoots were defoliated, terminal apex was removed and immediately kept in foil paper for irradiation. The Cobalt-60 type is using in this work as gamma cell source. The dose levels were 0 (control),10, 20, 30, 40 and 50 Gy gamma rays, bud sticks grafted on Duck seedlings as rootstock in September 2015 &2016 growing seasons. The influence was evaluated through the response of the different measurements as (survival %, lethality %, internode length (cm), internode number, leaf number, leaf area (cm²), fruit length, fruit diameter, fruit weight, seed weight, oil % as well as, fruit firmness). The obtained results revealed that the survival percentage decreased with the increase of radiation doses. On the contrary, Lethality % percentage increased with the increase of radiation doses. Moreover, in the vegetative growth parameters the greatest decreased were statistically detected by increasing level of dose of gamma rays with one exception in the parameter of internode number. However, fruit physical properties decreased by increasing of radiation doses. Meanwhile, oil content % increased by increasing of radiation doses.

Keywords: Fuerte, Hass, Avocado, radiation, doses, survival and gamma rays

Introduction

In fruit crops, mutagenesis has already been used to introduce many useful traits affecting plant size, blooming time and fruit ripening, fruit color, self-compatibility, self-thinning, and resistance to pathogens (Spiegel, 1990; Masuda *et al.,* 1997 and Sanada and Amano, 1998). Predieri and Govoni (1998) proposed the precise method for the determination of absorbed dose of radiation. A concept of LD50 (lethal dose 50 %) is used to refer the optimum dose to be used in the experiment. By definition LD50 is the dose which causes 50 % lethality in the organism used for irradiation in defined time. Yoshioka *et al.,* (1999) reported that gamma radiation induced mutants which are resistant to black spot disease, caused by *Alternaria alternata* in Japanese pear, is the most important and serious disease in Japanese pear. When the branches (bud sprouts) are vegetatively propagated by clonal techniques, the new phenotype is generally maintained leading to a new variety, often exhibiting only one phenotypic character different from the parent. Among the bud sports, the most common and wide spread mutations are color alterations in the red or purple anthocyanin content of fruit (Walker *et al.,* 2006). Compared with conventional hybridization breeding, bud sport is a consequence of genetic variation of somatic cells leading to the occurrence of qualitative and quantitative phenotypic alteration in plants, which can be observed in many vegetative propagating plants including grapes (Liu *et al.,* 2007). Bud mutants have been widely exploited by vine growers to develop new cultivars of wine grapes and table grapes. Already (Arthur *et al*, 2011) used the gamma radiation in cuttings of different cultivars avocado for propagation of plants and concluded that in the dose of 15 Gy was an increase in the tillering of avocado sprouts without affecting the germinative capacity and the final size of the obtained sprouts, thereby reducing the amount of seed required for propagation. Furthermore, the exposure avocado seeds at high radiation doses to propagation effects is not recommended because it reduces the germination and decreases the size of the germinated plants.

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Kunzang, (2017) reported that mutation is sudden heritable changes in the DNA sequence that are not derived from genetic segregation or recombination. In fruit crops spontaneous bud mutation are more common called as bud sports. The occurrence of a large number of natural bud sports in citrus, mango, grapes etc. made the fruit breeders interested to breed through induced mutation. Dose inducing 25 to 50% lethality (LD25-LD50) among mutated plants will result in the highest mutation rates. The objective of this work was carried out to get production of new genotypes of avocado with distinct fruit traits.

Materials and Methods

This field work was carried out in "El Kanater El Khayreia" Experimental Station at Kalubia Governorate, Egypt, to evaluate the effects of radiation to obtain mutations induced in plants with improved genotypes. Fuerte and Hass avocado bud sticks of about 20 cm of length from the new flushes including 5-7 buds were selected from a mature bearing. These shoots were defoliated, terminal apex was removed and immediately kept in foil paper for irradiation. The Cobalt-60 type is using in this work as gamma cell source. Dose levels were 0 (control),10, 20, 30, 40 and 50 Gy gamma rays, bud sticks grafted on Duck seedlings as rootstock in September 2015 &2016 growing seasons. All the bud sticks were tightly covered with a transparent polythene sheet to avoid desiccation of the buds. Grafted buds were continuously observed and survival data recorded after 6 weeks of budding and green color was considered as sign of success while a blackish color was recorded as failure of the grafting. Data on the percentages of survival of bud woods were recorded and LD50 determined for the bud wood experiment.
The LD50 (The dose at which 50% of the population killed).

Vegetative growth of bud wood subjected to gamma rays:

Some of vegetative growth of bud wood were calculated: internode length (cm), internode number, leaf number and leaf area (cm²). To measure the actual leaf area, a digital planimeter can be used. The leaf width (W) and length (L) of the leaves recorded and then the leaf area (cm²) calculated as following equation by Uzun and Çelik (1999):
$LA = -50.63 - 1.353\ L/W + 5.347\ W + 0.6\ W^2 + 5.489\ L$
Whereas, leaf area (LA), length (L), width (W), width square (W^2) and length/width (L/W).

Fruit physical parameters*:*

Samples of five fruits from each treated branch and untreated (control) were collected at maturity stage to estimate some properties i.e. fruit damnation (length and diameter, cm), fruit weight (gm), seed weight (gm) and percentages of flesh were calculated.

Fruit chemical characters:

Fruit firmness: was determined using Ametek pressure tester, fitted with an 8 mm hemispherical probe (probe penetration 2 mm). Firmness of 5 fruits from each replicate was measured at two opposite points on the equator of each fruit after removing a thin slice of skin from each site Meir *et al.,* (1995).

The oil content: the percentage of oil content in the dried flesh samples was extracted by means of soxhelt fat-extraction apparatus using hexane (40-60oC boiling point) for the extraction, which continued about 6 hours according to (A.O.A.C., 1980).

Statistical analysis:

All data obtained during both seasons were subjected to analysis of variance according to Snedecor and Cochran (1989). In addition, significant differences among means were differentiated according to the Duncan, multiple test range (Duncan, 1955) where capital letters were used for distinguishing means of different treatments for each investigated characteristic.

Results and Discussion

Survival and Lethality %:

With regard to the response of Survival % to the differential doses of gamma rays Table (1) and Fig. (1) shows obviously some considerable variations in this respect. Herein, the greatest values of Survival % were significantly coupled with 0 Gy during 2016 and 2017 seasons. Moreover, 10 Gy and 20 Gy ranked statistically second and third during 1st and 2nd seasons, respectively. On the contrary, the lowest values Survival % usually in concomitant to 40 Gy which ranked statistically last during both 2016 and 2017 seasons, respectively. Generally, the survival percentage decreased with the increase of radiation doses. On the contrary, Lethality % percentage increased with the increase of radiation doses. Hence, the highest values of lethality % were usually in concomitant to 40 Gy during both seasons of study. Such trend was true during both 2016 & 2017 experimental seasons with both cultivars (Fuerte and Hass).

Table 1: Statistics related to survival and lethality percentages of avocado cvs. Fuerte and Hass bud wood subjected to gamma radiation during 2016 and 2017 experimental seasons

Parameters / Treatments	Fuerte				Hass			
	Survival %		Lethality %		Survival %		Lethality %	
	2016	2017	2016	2017	2016	2017	2016	2017
0 Gy	93.00 a	96.80 a	7.00 e	3.20 d	84.60 a	80.80 a	15.40 d	19.20 d
10 Gy	88.20 a	90.00 ab	11.80 d	10.00 cd	72.80 b	71.20 b	27.20 c	28.80 c
20 Gy	81.40 b	84.00 b	18.60 c	16.00 c	60.00 c	53.60 c	40.00 b	46.60 b
30 Gy	72.80 c	68.80 c	27.20 b	31.20 b	41.00 d	46.00 d	59.00 a	54.00 a
40 Gy	48.00 d	43.60 d	52.00 a	56.40 a	0.00	0.00	0.00	0.00
50 Gy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Means of each column followed by the same letter/s during every season are not significantly differ at 5% level

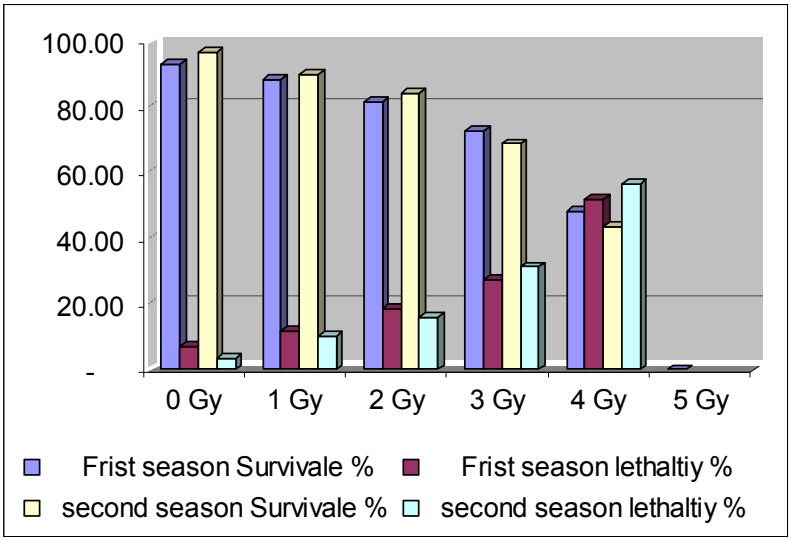

Fig. 1. (a) : Variability of survival and lethality percentage of bud wood avocado cv. Fuerte subjected to gamma rays.

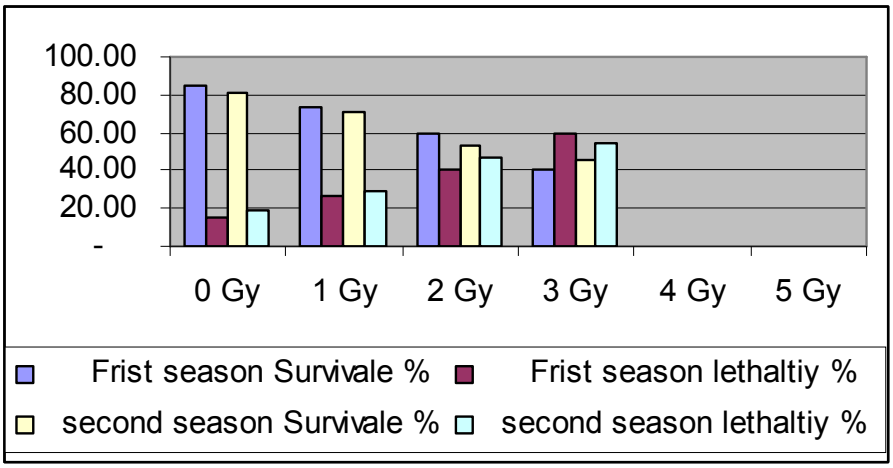

Fig. 1. (b) : Variability of survival and lethality percentage of bud wood avocado cv. Hass subjected to gamma rays.

Anyhow, these results are in agreement, Zamir *et al.,* (2009) studying that the effect of different doses of gamma rays on survival percentage of bud woods of guava cv. Safeda found that the LD50 of bud's survival was exactly 60 Gy. There was a gradual decrease in survival percentage as compared to the control, with 100Gy cussing 86.67% lethality. Zheng *et al.,* (2013) reported that the 30-day- old plantlets irradiated at a dose of 5, 10, 15, or 20 Gy were able to survive completely with on apparent effect of irradiation. Above 25 Gy dose, the rate of seedling survival decreased with increasing irradiation dose. On the other hand, one-fourth seedling died when irradiated at 40 Gy dose. Therefore, 25-40 Gy irradiation dose could be used to induce the mutation of jujube seedlings. Sandra *et al.,* (2015) found that values of median lethal dose, defined as the dose at which 50 % of full sprouts obtained was estimated at 28 to 27 grays (Gy) respectively for 'Duke-7' and 'Hass' cultivars. Moreover, it was demonstrated that 'Hass' behaves as more sensitive to high doses of radiation. These results have immediate use in generating possible populations of mutants may be useful in enhancing the induction of mutations cultivars of avocado.

2- Vegetative growth of bud wood subjected to gamma rays:

In this regard, some vegetative growth of bud wood was evaluated in response to different doses gamma rays i.e., internode length (cm), internode number, leaf number and leaf area (cm²) during 2016 and 2017 experimental seasons are presented in Table (2). It is quite evident that the greatest decreased in the above-mentioned measurements were statistically detected by increasing level of dose of gamma rays with one exception in the parameter of internode number. Herein, the highest internode number were recorded by 30 Gy with Fuerte cv. While, the highest internode number were recorded by 20 Gy with Hass cv. during 2016 and 2017 experimental seasons. Such trend was true during both seasons of study. These results are agreement with many authors i.e, the maximum variability of the evaluated characters; internode length, scion circumference and flowering percentage is found at 15 Gy so this dose is recommended for further radio induced mutation breeding programmers in avocado Hass cv. De La Cruz, et.al (1995). Sezgin and Hüseyin (1999) found that the variation in leaf area values was depened on the selected parameters (length, width, upper leaf lobe length and lower leaflet length). The variations explained by the parameters were 98.3% for avocado cv. Several leaf area prediction models have been produced in previous studies for fruit species such as avocado and peach (Uzun and Çelik 1999). Many studies have been carried out with linear leaf measurements that are highly correlated with leaf area in fruit trees such as apple (Silva *et al.,* 2004), Sweet cherry (Cittadini and Peri 2006) and Kiwifruit (Mendoza, *et al*., 2007). Arthur, *et al*. (2015) observed that the values of the plant height of avocado plants of the Quintal variety from sprouting of the grafting after 2,4,6 and 8 months, showed statistical significance in the dose of 20 & 30 Gy between repetitions. Also the plants in the dose 30 Gy was a smaller plant

and showed morphological mutation fasciations.With regard to plant height in the dose of 10 Gy the gamma radiation induced a stimulation of the Quintal avocado cultivate grafts the which made with that the develop of the plants be larger reaching a greater height in centimeters in relation to other treatments.

Table 2: Statistics related to vegetative growth of avocado cvs. Fuerte and Hass bud wood subjected to gamma radiation during 2016 and 2017 experimental seasons.

Doses level	Fuerte				Hass			
	Internode length (cm)	Internode No.	Leaf area (cm²)	Leaf No.	Internode length (cm)	Internode No.	Leaf area (cm²)	Leaf No.
2016								
0 Gy	11.80 a	23.0 d	95.35 a	16.80 a	8.18 a	19.0 c	76.44 a	9.80 a
10 Gy	10.50 b	27.0 b	89.43 ab	15.00 ab	7.46 b	24.0 b	67.06 b	9.00 ab
20 Gy	9.84 c	25.0 c	80.18 bc	13.20 bc	5.82 c	30.0 a	56.36 c	7.60 bc
30 Gy	8.40 d	32.0 a	98.54 a	11.40 c	3.48 d	15.0 d	36.80 d	6.20 c
40 Gy	5.86 e	20.0 e	76.45 c	7.80 d	0.00	0.00	0.00	0.00
50 Gy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2017								
0 Gy	13.10 a	26.0 c	107.9 b	18.80 a	7.14 a	17.0 c	85.49 a	10.40 a
10 Gy	11.10 b	26.0 c	114.2 ab	16.00 b	6.70 a	27.0 b	71.82 b	9.60 ab
20 Gy	8.30 d	31.0 b	73.67 c	10.00 d	5.54 b	33.0 a	62.13 c	8.20 b
30 Gy	9.80 c	34.0 a	119.5 a	12.00 c	3.78 c	12.0d	33.14 d	5.60 c
40 Gy	4.38 e	15.0 d	53.88 d	3.00 e	0.00	0.00	0.00	0.00
50 Gy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Means of each column followed by the same letter/s during every season are not significantly differ at 5% level

The effect of gamma radiation on some fruit physical properties (fruit length, fruit diameter, fruit weight, seed weight and flesh percentage) of Fuerte and Hass avocado cvs.

Fruit dimensions (diameter and length cm):

The diameter and length of Fuerte and Hass avocado fruits were the investigated two fruit dimensions regarding their subjected to gamma radiation treatments. Table (3) showed obviously that the different doses of gamma rays decreased significantly the fruit dimensions with Fuerte avocado fruits during 2016 and 2017 experimental seasons. Whereas, (0 & 10 Gy) gave the highest significantly fruit dimensions with both cultivars fruits during both seasons of study. Generally, there was a gradual decrease in fruit dimensions with increase in radiation dose in both two cultivars.

Fruit weight:

As shown from Table (3) that the average fruit weight of Fuerte and Hass avocado trees was significantly affected by increasing doses of gamma rays. The fruit weight increased with the increase in radiation dose up to 20 Gy and then decreased from 30 Gy in both two cultivars of avocado. There was a gradual decrease in fruit weight with increase in radiation dose during 2016 and 2017 experimental seasons.

Seed weight (g):

Concerning the seed weight of Fuerte and Hass avocado cvs. as influenced by the different of gamma radiation doses, data obtained during 2016 and 2017 experimental seasons are presented in Table (3). It is quite evident that seed weight was significantly affected by different doses of gamma rays. The highest seed weight was recorded in the control treatment (0 Gy) the trend was true in cultivars of avocado during both seasons of study. While, the lowest values of seed weight were recorded by 40 and 30 Gy treatments in Fuerte and Hass avocado cvs., respectively.

Flesh percentages %:

Data obtained in Table (3) showed obviously some considerable variations in this respect. Herein, the greatest flesh percentages (81.16 & 83.59) were recorded in the bud wood treated with 10 Gy consecutively in the two studied seasons with Fuerte cv. Whereas, the maximum flesh percentages were (88.08 %) recorded by 20 Gy in 1st season and (87.75 %), recorded by 0 Gy (control treatment) in 2nd season with Hass cv.

Our present results are in agreement with those of previous studies by Zamir *et al.,* (2009) finding that the fruit and seed weight of guava cv. Safeda were significantly affected by different dose of gamma rays. The highest fruit weight 128.38g and seed weight 2.29g were recorded in 20 Gy treatments while, the lowest fruit weight 56.23g and seed weight 1.56g were recorded in 30 Gy treatments. Moreover, the effect of gamma irradiation on fruit weight is especially important with regard to small mandarins. In addition, the reduction in seed number resulted in reduced production of gibberellic acid and, perhaps, also of other growth regulators that are required to promote normal fruit growth (Bermejo *et al*., 2011&2012). On the hand, the reduction in seed numbers of mandarin varieties, also directly contributes to the overall decrease in fruit weight. Zheng *et al.,* (2013) finding that seven types of fruit shape mutations were observed and different ripening timing were record on the orchard on jujube putatively mutated mature trees. All the fruits were drupe, varying from round to oval. Fruits diameters also varied from 0.9 to 2.1 cm, in comparison non-irradiated jujube fruits varied from 1.2 to 1.3 cm in diameter. In their studding Livnat *et al*., (2014) found that the differences in fruit weight of seven irradiated mandarin varieties ranged from just 6.5 g in irradiated "Michal" fruits to 40 - 41 g in "Kedem" and "Or" fruits. The average difference between the fruit weights of all these seven irradiated varieties and that of fruits from unirradiated trees was ~23 g.

Table 3: Statistics related to character growth fruits of avocado cvs. Fuerte and Hass bud wood subjected to gamma radiation during 2016 and 2017 experimental seasons.

Doses level	Fuerte					Hass				
	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Seed Weight (g)	Flesh percentage %	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Seed Weight (g)	Flesh percentage %
2016										
0 Gy	15.80 a	18.78 a	219.6 a	44.23 a	79.88 ab	11.10 a	10.16 a	120.8 a	17.85 a	85.28 c
10 Gy	14.26 b	17.13 b	171.0 b	32.16 b	81.16 a	10.09 b	8.88 b	106.6 b	16.11 b	84.59 c
20 Gy	12.50 c	15.98 bc	142.2 c	26.62 c	80.90 a	8.90 c	7.27 c	93.14 c	11.38 c	88.08 a
30 Gy	10.35 d	14.83 c	103.2 d	22.86 d	77.88 b	7.63 d	5.81 d	76.51 d	9.83 d	87.13 b
40 Gy	8.39 e	11.73 d	93.39 d	19.14 e	79.39 ab	0.00	0.00	0.00	0.00	0.00
50 Gy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2017										
0 Gy	16.20 a	19.38 a	182.7 a	46.62 a	79.68 b	10.98 a	9.66 a	136.5 a	15.91 a	87.75 a
10 Gy	15.21 b	17.70 b	153.3 b	29.76 b	83.59 a	8.94 b	7.48 b	110.7 b	11.56 b	85.55 b
20 Gy	13.73 c	16.80 b	103.8 c	28.62 b	77.83 b	7.88 c	6.16 c	75.72 c	11.56 b	84.65 b
30 Gy	11.45 d	14.08 c	73.62 d	25.66 c	74.08 c	7.75 d	4.8 d	63.78 d	10.43 c	83.44 c
40 Gy	9.49 e	12.48 d	64.65	22.74 d	0.00	0.00	0.00	0.00	0.00	0.00
50 Gy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Means of each column followed by the same letter/s during every season are not significantly differ at 5% level

Oil content %:

It is quite evident as shown from tabulated data in Table (4) that oil content % of Fuerte and Hass avocado fruits influenced by gamma radiation treatments. The maximum oil content % were recorded by (40 Gy) treatment with Fuerte cv. and by (30 Gy) treatment with Hass cv. during 2016 and 2017 experimental seasons. On the contrary, the lowest values of oil % were recorded by control treatment (0 Gy) in both cultivars during two seasons of study.

Firmness (lb/inch^2):

It is quite clear as shown from tabulated data in Table (4) that firmness of fruits decreased significantly by increased doses of gamma rays in both cultivars during two seasons of study. Herein, the highest values in this concern was exhibited significantly by control treatment (0Gy) in both cultivars during two seasons of study. The reverse was true with 40 Gy and 30Gy in Fuerte and Hass fruits, respectively. Such trend was true during 2016 and 2017 experimental seasons. In general there are contrasting of oil contents between the varieties avocado by different regions, Venezuelan avocados seem to have relatively low oil contents in comparison to maximal values reported from Brazil (25.5%) (Tango *et al*., 1969); Colombia (15.01%) (Salazar *et al*., 1971); South Africa (40.0%) (Pearson, 1975); Chile (25.8%) (Olaeta *et al*., 1986); and Texas (20.1%) (Rouse & Knight Junior, 1991). Colquhoun *et al.* (1992) used avocados with average weights of 200-500g, and found about 23 % total oil content of which and 54 % was oleic acid. The pulp oil contents of the Fuerte variety was similar to that reported by Gómez-López (1999&2002). In published by Ahmet, *et al*. (2009) Fruit Flesh Firmness (FFF) was different by days after full bloom (DAFB) in verities avocado. In Fuerte avocado, FFF was decreased from 156.02 N to 67.66 N and in Zutano avocado from 95.52 N to 51.58 N during period 245th to 275th DAFB. Oil content was increased from 13.84% to 15.84% of Fuerte avocado and from 8.64% to 14.34% of Bacon avocado during period 125th to 245th DAFB. Abd El-Mongy *et al*. (2009) found that changes in fruit firmness of Hass avocado, control fruits kept at 20oC resulted fruit firmness 3.93 and 4.15 after 15 days in the 1st and 2ed seasons respectively. Aml and Minar (2010), reported that changes in fruit firmness (N) of Fuerte avocado fruits due to different harvest dates and ripening at 20oC for 7 days from the 1st Nov. to 1st Jan. Meanwhile, decrease of fruit firmness percentage (54.28 to 8.03 N) was higher at ripening stage compared with harvest dates.

Table 4: Statistics related to Oil content and Firmness percentages of avocado cvs. Fuerte and Hass fruits bud wood subjected to gamma radiation during 2016 and 2017 experimental seasons.

Parameters / Treatments	Fuerte				Hass			
	Oil content % (DW)		Firmness (Ib/inch2)		Oil content % (DW)		Firmness (Ib/inch2)	
	2016	2017	2016	2017	2016	2017	2016	2017
0 Gy	12.88 d	11.77 e	33.08 a	30.35 a	27.91 c	29.28 d	19.90 a	18.03 a
10 Gy	18.61 c	16.28 d	27.63 b	25.54 b	31.93 b	34.97 c	17.07 b	15.18 b
20 Gy	18.68 c	19.00 c	24.48 c	21.41 c	38.91 a	38.40 b	14.28 c	12.47 c
30 Gy	23.92 b	20.93 b	19.95 d	16.88 d	40.44 a	43.33 a	11.37 d	9.73 d
40 Gy	25.65 a	22.81 a	14.22 e	12.37 e	0.00	0.00	0.00	0.00
50 Gy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Means of each column followed by the same letter/s during every season are not significantly differ at 5% level

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