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Morphological Variations in Mungbean Treated with Irradiated Guayabano Fruit Juice

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ABSTRACT

Guayabano fruit juices were exposed to gamma ray doses of 0, 1, 2, 3, and 4 KGy, stored for 0, 4 and 8 days, and used for treatment of Vigna radiata *(L.) Wilczek seedlings. The effects on* V. radiata *were evaluated using the following parameters: root and shoot lengths, % survival, % survivors that flowered and formed pods, pod yield, pod length and seedset. Except for plant survival and pod length, all the other characters were significantly affected by irradiated juices. Chlorophyll mutants were not produced. Increased frequency of viable leaf variants was observed in the M_2 seedlings of plants treated with irradiated juice.*

INTRODUCTION

This study was undertaken to determine the wholesomeness of irradiated guayabano (*Anona muricata* L.) fruit juice, in particular (i) to ascertain the effects of irradiated guayabano fruit juice (GFJ) on the growth and morphology of mungbean (*Vigna radiata* [L.] Wilczek), (ii) to know if irradiated GFJ can induce mutations on the M_1 and M_2 mungbean plants, and (iii) to assess the effect of duration of storage on the capability of irradiated juice to induce mutations and

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at the same time determine if the doses used could affect the shelf-life of guayabano juice.

Radiation has been used by geneticists for many years now as an important tool for the production of commercially important plant varieties. In medicine its use has long been recognized, for example in the treatment of cancer. Recently, the possibility of using radiation for food preservation is being evaluated in the hope of reducing the use of chemical preservatives.

Part of the wholesomeness test undertaken on irradiated food is mutagenecity testing, since irradiation is known to cause the production of free radicals. For these tests, both plant and animal systems have been utilized. The use of plants as test organisms has been considered as both fast and cheap and, at the same time, reliable since the DNA is similar in eukaryotes.

Ehrenberg (1) and Meletti et al. (2) were among the investigators who made use of plant systems. Both found a significant increase in the frequency of cholorophyll mutations in seeds and embryos exposed to irradiated glucose and glycine (50 Mrad of gamma) and endosperm (500 rads of X-rays), respectively.

The cytotoxicity of irradiated food was found to be influenced by the duration of storage. Ehrenberg et al. (3) reported that the main fraction of free radicals induced in food has a half-life of 48 hours at 25°. After six months of storage, the radical content was zero or less than 1% of the initial concentration. Kesavan and Swaminathan (4) found that the radiomimetic principle in the gamma irradiated (0.5 Mrad) medium persisted even after 15 days of storage. The same observation was reported by Berry et al. (5) in 1% dextrose/fructose solution irradiated with 2.5 rads of gamma. The toxic product formed in the latter persisted for about six months. The study of Makinen et al. (6) suggests the dose-dependence of detoxification. The higher the dose the longer the detoxification period required.

Irradiated food can also affect the growth of organisms. It may either stimulate or inhibit growth depending on the amount of radiation it received (6–10). In general, food receiving high doses of radiation can reduce growth while doses of 2 KGy and below can stimulate growth.

This study is especially significant now that irradiated food products are increasing and may dominate the market (11, 12). Since the DNA is similar in eukaryotes, whatever the effect of irradiated food on plants may be the effect on humans.

MATERIALS and METHODS

Preparation and irradiation of the guayabano fruit extract

Ripe fruits of guayabano were washed, peeled, and deseeded. The fleshy pulp was homogenized with a fruit blender, and the mixture passed through a strainer. The collected juice was poured into sterile 60-ml prescription bottles. All materials used for the preparation were heat-sterilized.

For the duration of the study, 30 bottles of the juice were prepared and treated at the Gamma Cell Facility of the Philippine Atomic Energy Research Institute in Diliman, Quezon City, using a Cobalt-60 source. Two trials were made with an interval of seven weeks, using doses of 0, 1, 2, 3 and 4 KGy. At three bottles per dose per trial, a total of 30 bottles were used.

Immediately after irradiation, one set each from the irradiated and unirradiated juices was used for treatment and analysis. The other two sets were stored under laboratory conditions and used on the fourth and eighth day of storage. During the study period, the temperature in the laboratory room ranged from 28°C to 30°C.

Treatment of the Test Material and Data Collection

Seeds of mungbean (*Vigna radiata* [L.] Wilczek), variety MG50-10A, were obtained from the Bureau of Plant Industry in Manila. The seeds were thoroughly washed and then soaked in distilled water for 24 hours. The seedcoats were removed with the use of forceps. One day-old seedlings with more or less similar size and growth were selected and dried on blotting paper just before treatment. A total of 200 seedlings was used for each treatment. The seedlings were soaked for 6 hours in 25% aqueous solution of the guayabano extract (100 ml/100 seedlings), then washed in running water for another six hours.

One hundred seedlings from each treatment were placed on moist tissue paper using the blotter-sandwich technique, and exposed under constant flourescent illumination of about 120 footcandles. The blotter-sandwiches were marked to preclude errors in scoring, and arranged in a completely randomized design. After five days, the root and shoot lengths were measured with a celluloid ruler.

The other 100 seedlings from each treatment were planted in the field using a dose-to-row plan. Storage period of the juice was used as a blocking factor.

Planting distances were 10 cm between hills and 20 cm between rows. The plants were grown until maturity for collection of the following data: % survival after 30 days, % of survivors that flowered and formed pods, plant height at maturity, number of days to flowering, number of days to ripening of pods, number of pods per plant, pod length and seedset.

Seeds collected from the M_1 plants were sown in the field, one plot per treatment, and the M_2 plants scored for morphological and chlorophyll mutation seven days after sowing. Observation was continued until the seedlings reached the first trifoliate stage.

RESULTS AND OBSERVATIONS

Shoot and root lengths of mungbean seedlings

The duration of storage and the gamma ray dose received by the guayabano fruit juice (GFJ) were found to significantly affect the results (Appendix A and B). The data (Tables 1 and 2) show that the stored juices inhibited both shoot and root elongation in mungbean. The shoot length decreased linearly with storage time (Fig. 1a). This linear relationship was not observed for the root length (Fig. 2a).

Juices receiving 2, 3, and 4 KGy of gamma radiation stimulated shoot and root growth (Figs. 1b and 2b). Seedlings treated with 1 KGy of gamma radiation did not differ significantly from the control.

The interaction between gamma treatment and storage time was evident (Figs. 1c and 2c) and was found to be significant at 1% level (Appendix B).

Plant survival and percentage of plants that flowered and formed pods under field conditions

Percentage survival decreased with increased storage time (Tables 3a-c, Fig. 3a). The gamma treatment did not produce any remarkable effect on survival (Appendix C, Fig. 3b).

Most of the survivors in the treatment groups GFJ-DO (73.6%) and GFJ-D4 (68%) flowered and formed pods. Only 7.6% did in the treatment group GFJ-D8 (Fig. 4a). A slight increase in the percentage was observed with increase in gamma ray dose (Fig. 4b).

Figs. 3c and 4c show the varying effects of treated juices on the two parameters. The GFJ stored for eight days produced toxic effects on the plants. The very few data obtained for the GFJ-D8 group were actually gathered from the juice treated with 4 KGy of gamma radiation.

Plant height of surviving plants

The mean plant height at maturity of the mungbean plants under field conditions is presented in Table 4. Very few survivors were obtained for GFJ-D8, so this treatment group was not included in the statistical analysis for this parameter and the subsequent ones.

Plant height (Fig. 5a) decreased with increased storage period of the GFJ. Such decrease was not significant as shown by ANOVA (Appendix D). No trend could be deduced at 0–2 KGy (Fig. 5b). At 2-4 KGy levels, a decrease in mean plant height was observed with corresponding increase in dose. The effect of the gamma treatment was highly significant (Appendix D). Interaction between the gamma treatment and the storage time was observed (Fig. 5c).

Dwarf plants that reached the booting stage but did not flower were obtained in the treatment groups GFJ-D0-3KGy and GFJ-DO-4KGy.

Number of days to flowering

In general, flowering time was delayed by the increase in storage time and gamma radiation dose (Figs. 6a & b). A significant delay in flowering time was observed in the group given the 4 KGy treated juice (Table 5). The effect of storage time was significant (Appendix E). The trend for D0 and D4 (Fig. 6c) was similar for gamma ray treatment 0 to 3 KGy. For the 4 KGy treated group, plants subjected to the unstored juice flowered after 46 days while the plants given the juice stored for four days flowered five days earlier. Using the LSD method, no significant difference between the means was observed.

Number of days to ripening of pods

The treatment group GFJ-D4 required a longer time for pod maturation (Table 6, Fig. 7a) as compared to those receiving the unstored juices. No apparent trend could be deduced on the effects of irradiated juice (Fig. 7b). However, considering the relatively high standard deviation values and very small sample size (n=3) for treatment groups GFJ-D4-OKGy and GFJ-D4-1KGy, it can be safely stated that juices receiving higher doses of gamma radiation (3 and 4 KGy) retard pod maturation to a significant degree. The same trend is evident in Fig. 7c where the interaction between variables was found to occur at the 4 KGy level. This suggests that whatever inhibitors were present at DO had slowly decreased in amount at day 4, for this particular radiation dose.

Ripening of pods was markedly affected by gamma treatment and period of storage (Appendix F). Interaction between the two variables was found to be significant at 5% level.

Number of pods per plant

The various storage periods and levels of gamma dose received by the juice used for treatment significantly affected plant yield (Appendix G). Stored irradiated juice lowered pod yield to as much as 69% (Table 7, Fig. 8a). A slight increase in yield was observed in GFJ-O KGy and GFJ-2 KGy plants (Fig. 8b). Plant yield was reduced by the juice receiving higher doses.

No interaction was observed between the two variables used (Fig. 8c). There is a similarity in the trend for both D0 and D4. The highest average pod yield was observed at treatment groups GFJ-D0-2KGy and GFJ-D4-3KGy.

Pod length

Plants treated with stored juices produced significantly shorter pods than those soaked in unstored juices (Table 8, Fig. 9a, Appendix H). The average pod length for the groups treated with irradiated and unirradiated juice (Fig. 9b) varied slightly but this was not significant.

In Fig. 9c, the line for D4 is markedly curvilinear suggesting a significant difference in the effects of gamma treatment at that storage period. LSD showed that the GFJ-D4-2KGy group produced significantly longer pods than those receiving GFJ-D4-4KGy treatment.

Seedset

ANOVA showed that period of storage markedly influenced the seedset (Appendix I) but the LSD test was not effective enough to reveal this. A decreasing trend in seedset was observed with period of storage (Table 9, Fig. 10a).

The influence of gamma ray treatment on seedset was found to be highly significant. Plants in the GFJ-3KGy group showed the highest mean seedset of 6.16 ± 2.66, while the GFJ-4KGy group had the lowest with a mean of 5.22+2.63 (Fig. 10b). Within the D0 group, those treated with juice receiving 2 KGy and 3 KGy produced a significantly greater number of seeds per pod. The D0-1KGy and D0-4KGy groups were similar to the control.

Interaction between the two variables is evident (Fig. 10c) but insignificant was shown by ANOVA.

Morphological variations observed in M_2 seedlings

The leaf variations observed in M_2 seedlings are listed in Table 10. Among these variations are those that depart from the normal lanceolated shape such as clover-shaped, cuplike, and orbicular. Seedlings with truncate apices instead of the usual acute form were also scored, as well as those whose leaves were unusually twisted, wrinkled, or undeveloped.

M_2 seedlings from the GFJ-1KGy and GFJ-D0-0KGy groups were also observed to possess three leaves on the seventh day of growth period. This was considered abnormal since, ordinarily, seedlings possess only two leaves until the emergence of the first trifoliate.

A seedling from the GFJ-D0-3KGy group was observed to have a bifurcate leaf. This was not observed in the progenies of plants from the other treatment.

At the first trifoliate stage (approx. 18 days), excessively twisted leaves became flattened and could be distinguished from the normal by their slightly mosaic appearance. Some seedlings with cuplike leaves produced a normal trifoliate leaf (Fig. 11a), while a seedling with morphologically normal leaves produced a trifoliate whose leaflets were all clover-shaped (Fig. 11b).

The frequency of seedlings with abnormalities was relatively higher in the D0 treatment than in the D4 and D8. In the D4 group, the frequency of progenies with deviant character was observed to decrease with subsequent increase in dose. In the other treatment groups, gamma dose did not affect the production of variants to a significant degree.

No chlorophyll mutation was observed.

DISCUSSION

Root and shoot elongations in mungbean seedlings were greatly stimulated by GFJ treated with 2–4 KGy of gamma radiation. Similar observations were obtained by Chopra (6), and Jonard (9), and their co-workers in barley and Jerusalem artichoke explants treated with irradiated juices and glucose solutions, respectively. Growth stimulation has been associated with the expansion of cells as brought about by increased auxin levels in the plant (13) and by the specific effects of radiation on respiratory enzymes resulting in increased activity of catalase and peroxidase (14). At the molecular level, this growth stimulation has been related to the enhancement of gene activity at the transcriptional and translational levels (15).

Growth reduction, on the other hand, as observed in some mungbean plants treated with GFJ irradiated with 3 and 4 KGy, could possibly be attributed to reduction in auxin level (16), alteration of levels of nucleic acid synthesis, and abnormal metabolism of amino acids (17). These foregoing processes may also explain the delay in flowering time, low pod yield, and reduced seedset in some treated plants. Sterility has been pointed out to result from point mutations and small deficiencies in the genome (18, 19).

The 2 and 3 KGy doses significantly stimulated shoot length at the seedling stage, but such stimulation was not carried through to maturity. The plant height at maturity of mungbeans treated with these doses did not significantly deviate from the control. This could be due to exogenous factors (e.g. soil nutrients present) which could have influenced the effect of radiolytic products on the plants' physiology.

The effects of the irradiated juice on plant height, flowering, pod formation and ripening, and seedset could also be attributed to the production of radiolytic substances in the irradiated juice that could have caused the physiological and genetic changes in the plants. Guayabano fruits have been found to be composed primarily of water and carbohydrates, and very minimal amounts of vitamins, minerals, and fats (20). These substances are readily transformed into various products upon exposure to ionizing radiations. Irradiation of carbohydrates can bring about the production of 2-deoxygluconolactone, gluconic acid, hydrogen, carbon dioxide, methane, carbon monoxide, water, and dicarbonyl sugars (21). Dicarbonyl sugars readily convert to α, β-unsaturated carbonyls upon enolization or dehydration and have been found to be so highly cytotoxic (21).

The radiolytic products of water include hydroxyl ions, hydrogen ions, hydrated electrons, and hydrogen peroxide (21). The free radicals, OH and H, can attack the DNA. Specifically, the OH radical can attack the 5–6 double bond of the pyrimidine bases. In the presence of oxygen, a peroxidation of the 5–6 bond is produced. In deoxygenated systems, two OH radicals are fixed at the double bond directly forming a glycol. For cytosine, deamination to form uracil also takes place (22). The free radicals, together with the other radiolytic products of water, can also react with the other components of food to produce other organic free radicals that may cause further changes in the physiological and genetic states of the cells (23).

Leaf variants similar to the ones obtained by Alvarez (24) using single and combined treatments of ethylmethanesulfonate (EMS) and gamma radiation were obtained in this study. The frequency of variants was even observed to increase with the dose received by the juice, suggesting the dose dependence of the production of substances causing such changes. The production of leaf variants rather than chlorophyll mutants may indicate the presence of substances in the GFJ whose radiolytic products act on the genes that control leaf shape rather than on the genes that control chlorophyll formation. Although unexpected, this result opens new possibilities for research.

Even if no chlorophyll mutation was observed as expected in most of the seedlings of EMS-treated plants (25), the earlier proposition by Chopra (6) that the action of irradiated apple and orange juice is similar to that of EMS and myeleran cannot be totally discounted. It is possible that the quantity of mutagens produced in the irradiated medium may be very minimal to induce chlorophyll mutations but strong enough to create genetic changes that bring about the production of viable variants.

The findings in this study vary from those reported by Ehrenberg (1) and Meletti et al. (2), who found a significant increase in the frequency of chlorophyll mutations in seeds and embryos, as well as endosperm exposed to irradiated glucose and glycine. This could be due to the higher dose of gamma radiation used by Ehrenberg (500 Krads) and to the different method used by Meletti. Although Meletti and his co-workers used a lower dose (500 rads) of X-rays, they surgically transplanted the treated endosperms into embryos, thereby insuring their utilization until the plants were already capable of synthesizing their own food.

The period of storage greatly influenced the biological action of both irradiated and unirradiated juices and, in most cases, produced significant interactions with the gamma treatment. The composition of the unstored juices might have changed with storage, as indicated by the fact that while the unstored juices caused a delay in flowering and produced an increase in seedset, pod length, pod yield, and early pod ripening, the reverse was observed in the plants treated with the stored juice. Also, the four-day-old juice did not produce as much leaf variants as the unstored ones. This may indicate that most of the mutagens present in unstored juices are lost with storage.

There was a very low % survival in the batches treated with the stored juices. Such result could be due essentially to the formation of fermentation products of guayabano, which could also indicate the ineffectiveness of the doses used in prolonging the shelf-life of the juice for eight days. Results showed that the unirradiated juice stored for eight days, was detrimental with not a single survvors. Among those stored for eight days, the number of survivors was observed in the juice that received the highest dose. Free radical formation is correlated with the dosage of irradiation. The higher the dose the more free radicals are formed and, as this study would indicate, the more effective the sterilization is, consequently the more effective it is in retarding fermentation (the results of the follow-up study on this will be given on a separate report gas chromatography analysis of the stored and unstored juices subjected to varying gamma radiation doses). The same interplay of factors (e.g. composition of guayabano juice; presence/absence of microorganisms in the extract, radiation dose, duration of storage, fermentation products formed, etc.) could have also influenced the results for pod length.

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Table 1. Mean length of shoots of mungbean seedlings treated with irradiated and unirradiated guayabano fruit juice (in cm)*

Gamma Ray Dose (KGy)	Total Plants	STORAGE PERIOD Day 0 Mean ± S.D.	Day 4 Mean ± S.D.	Day 8 Mean ± S.D.	Mean ± S.D.
0	100	17.82 ± 3.70	8.26 ± 6.64	5.64 ± 7.28	10.58c ± 6.41
1	100	19.44 ± 2.95	10.03 ± 6.65	3.67 ± 4.57	11.05c ± 7.93
2	100	19.49 ± 3.12	16.08 ± 5.55	15.21 ± 6.25	16.93a ± 2.26
3	100	18.14 ± 3.18	19.28 ± 2.74	15.12 ± 6.52	17.52a ± 2.15
4	100	17.15 ± 2.88	17.85 ± 4.32	12.67 ± 7.23	15.89b ± 2.81
Mean ± S.D.		18.41a ± 3.70	14.30b ± 4.88	10.46c ± 7.28	

*Means followed by the same letter are not significantly different by DMRT.

Table 2. Mean length of roots of mungbean seedlings treated with irradiated and unirradiated guayabano fruit juice (in cm)*

Gamma Ray Dose (KGy)	Total Plants	STORAGE PERIOD Day 0 Mean ± S.D.	Day 4 Mean ± S.D.	Day 8 Mean ± S.D.	Mean ± S.D.
0	100	5.49 ± 2.00	3.11 ± 2.17	2.16 ± 2.55	3.59c ± 1.72
1	100	6.00 ± 1.41	3.53 ± 2.15	1.89 ± 2.27	3.81c ± 2.07
2	100	6.24 ± 1.69	6.69 ± 1.68	6.92 ± 3.40	6.28a ± 0.62
3	100	5.72 ± 2.17	5.34 ± 1.19	7.16 ± 3.71	6.07a ± 0.96
4	100	4.98 ± 2.07	5.20 ± 1.38	6.42 ± 3.12	5.53b ± 2.19
Mean ± S.D.		5.69a ± 0.48	4.57b ± 1.16	4.91b ± 2.65	

*Means followed by the same letter are not significantly different by DMRT.

Table 3a. Plant survival and percentage of survivors that flowered and formed pods (SFFP) under field conditions

Gamma Ray Dose (KGy)	Total Plants	STORAGE PERIOD					
		Day 0		Day 4		Day 8	
		%Surv.	%SFFP	%Surv.	%SFFP	%Surv.	%SFFP
0	100	81.00	71.60	7.00	42.86	0.00	0.00
1	100	77.00	80.52	7.00	42.86	1.00	0.00
2	100	79.00	81.01	29.00	79.31	1.00	0.00
3	100	78.00	69.23	54.00	98.15	1.00	0.00
4	100	73.00	65.75	22.00	77.27	13.00	38.46

Table 3b. Means and standard deviation for plant survival and SFFP for storage time*

Storage period	% Survival	% SFFP
0	77.60a ± 2.97	73.60a ± 6.65
4	23.80b ± 19.41	68.00a ± 24.25
8	3.20c ± 5.50	7.60b ± 16.99

Table 3c. Means and standard deviation for plant survival and SFFP for the gamma doses

Gamma Ray Doses (KGy)	% Survival	% SFFP
0	29.33 ± 44.88	38.30 ± 36.23
1	28.33 ± 42.25	41.00 ± 40.04
2	36.33 ± 39.51	53.30 ± 46.20
3	44.33 ± 39.40	55.70 ± 50.34
4	36.00 ± 32.36	60.30 ± 20.11

*Means followed by the same letter are not significantly different by DMRT.

Table 4. Mean plant height of mungbeans at maturity (in cm)*

Gamma Ray Dose (KGy)	STORAGE PERIOD Day 0 Mean ± S.D.	Day 4 Mean ± S.D.	Mean ± S.D.
0	28.96a ± 13.89	18.20bc ± 11.25	23.59ab ± 7.61
1	22.83b ± 9.81	13.47c ± 6.72	18.15c ± 6.62
2	23.31b + 9.98	29.81a + 7.94	26.46a + 4.46
3	22.93b ± 8.63	22.89b ± 9.22	22.91b ± 0.03
4	23.38b ± 6.80	13.40c ± 6.17	20.88bc ± 7.06
Mean ± S.D.	24.28 ± 2.63	20.51 ± 6.87	

*Means followed by the same letter are not significantly different by LSD.

Table 5. Mean number of days to flowering of mungbean plants under field conditions*

Gamma Ray Dose (KGy)	STORAGE PERIOD Day 0 Mean ± S.D.	Day 4 Mean ± S.D.	Mean ± S.D.
0	37.66cd ± 6.34	40.33abcd ± 1.16	38.99b ± 1.89
1	37.74cd ± 5.77	41.00abcd ± 0.00	39.37ab ± 2.30
2	36.94d ± 5.94	39.52bcd ± 4.87	38.23b ± 1.83
3	38.50cd ± 5.77	41.83b ± 4.41	40.16b ± 2.36
4	46.23a ± 11.56	40.94bc ± 2.58	43.58a ± 3.74
Mean ± S.D.	39.41 ± 3.85	40.72 ± 0.86	

*Means followed by the same letter are not significantly different by LSD.

Table 6. Mean number of days from flowering to ripening of pods of mungbean plants under field conditions*

Gamma Ray Dose (KGy)	STORAGE PERIOD Day 0 Mean ± S.D.	Day 4 Mean ± S.D.	Mean ± S.D.
0	27.21b ± 2.69	29.33ab ± 2.89	28.27ab ± 1.50
1	27.58b ± 1.94	31.00a ± 0.00	29.29ab ± 2.42
2	26.91b ± 2.05	27.17bb ± 3.27	27.04b ± 0.19
3	27.70b ± 1.88	29.32a ± 2.19	28.51a ± 1.14
4	29.52a ± 2.99	29.06a ± 2.36	29.29ab ± 0.33
Mean ± S.D.	27.78b ± 1.02	29.18a ± 1.36	

*Means followed by the same letter are not significantly different by LSD.

Table 7. Mean number of pods per mungbean plant*

Gamma Ray Dose (KGy)	STORAGE PERIOD Day 0 Mean ± S.D.	Day 4 Mean ± S.D.	Mean ± S.D.
0	11.67bc ± 11.34	1.67d ± 0.58	6.67ab ± 7.08
1	16.21a ± 16.42	2.00cd ± 0.00	9.10ab ± 10.05
2	14.25ab ± 12.00	6.74d ± 4.99	10.50a ± 5.31
3	8.07cd ± 5.46	4.58d ± 4.76	6.33b ± 2.47
4	6.25 d ± 5.56	2.59d ± 1.68	4.42b ± 2.59
Mean ± S.D.	11.29a ± 4.15	3.52b ± 2.13	

*Means followed by the same letter are not significantly different by LSD.

Table 8. Mean pod length of mungbean plants after treatment with irradiated guayabano fruit juice*

Gamma Ray Dose (KGy)	STORAGE PERIOD Day 0 Mean ± S.D.	Day 4 Mean ± S.D.	Mean ± S.D.
0	7.65a ± 0.78	6.93abc ± 0.35	7.33 ± 0.16
1	7.26a ± 1.04	6.67abc ± 0.93	6.95 ± 0.16
2	7.42a ± 1.32	7.20ab ± 1.26	7.22 ± 0.13
3	7.35a ± 0.86	6.75bc ± 1.51	7.05 ± 0.11
4	7.37a ± 1.21	6.18c ± 1.20	6.91 ± 0.15
Mean ± S.D.	7.41a ± 0.14	6.75b ± 0.38	

*Means followed by the same letter are not significantly different by LSD.

Table 9. Mean number of seeds per pod of mungbean treated with guayabano fruit juice exposed to gamma radiation*

Gamma Ray Dose (KGy)	STORAGE PERIOD Day 0 Mean ± S.D.	Day 4 Mean ± S.D.	Mean ± S.D.
0	6.02c ± 3.13	7.17abc ± 0.58	6.60ab ± 0.81
1	6.40c ± 8.82	5.33abc ± 2.89	6.11ab ± 0.75
2	7.62a ± 2.56	5.81c ± 2.01	6.72ab ± 1.28
3	7.26ab ± 2.35	6.16c ± 2.66	6.71a ± 0.76
4	6.25bc ± 2.77	5.22c ± 2.63	5.74b ± 0.73
Mean ± S.D.	6.71 ± 0.69	6.04 ± 0.78	

*Means followed by the same letter are not significantly different by LSD.

Table 10. Types and frequency of leaf variants observed in M_1 seedlings of mungbeans treated with irradiated and unirradiated guayabano fruit juice (GFJ)

GFJ Treatment	Total Number Scored	Types of Viable Mutations*														Total	%
		A	B	C	D	E	F	G	H	I	J	K	L	M	N		
DAY 0																	
0	3600	–	58	45	6	15	26	5	39	14	–	2	–	–	–	210	5.83
100 krads	4550	–	59	13	2	23	60	2	65	18	–	2	2	–	–	306	6.72
200 krads	4700	–	65	80	–	29	72	4	68	16	–	3	1	–	–	388	7.19
300 krads	1400	1	13	12	–	11	30	7	29	8	1	4	3	–	1	120	8.57
400 krads	1200	–	29	10	–	15	24	8	25	5	–	3	3	1	–	123	10.25
DAY 4																	
0	30	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1	3.33
100 krads	30	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	3.33
200 krads	290	–	–	1	–	–	2	–	3	2	–	–	–	–	–	8	2.76
300 krads	395	–	1	1	–	2	2	–	1	2	–	–	–	–	–	9	2.28
400 krads	195	–	–	–	–	1	2	–	1	–	–	–	–	–	–	4	2.05

*A-clover-shaped leaves; B-cuplike leaves; C-twisted leaves; D- with three leaves; E-leaf ends not acuminate; F-1 leaf undeveloped/smaller; G-1 leaf wrinkled; H-minute leaves; I- rosette-like leaves; J-1 leaf bifurcated; K-1 leaf orbicular in shape, the other smaller and cuplike; L-apices somehow truncate with scalloped margin, shape somewhat circular, margin near the base is entire; M-first two leaves cuplike but 1st trifoliate is normal; and N-first two leaves normal but 1st trifoliate leaflets are clover-shaped.

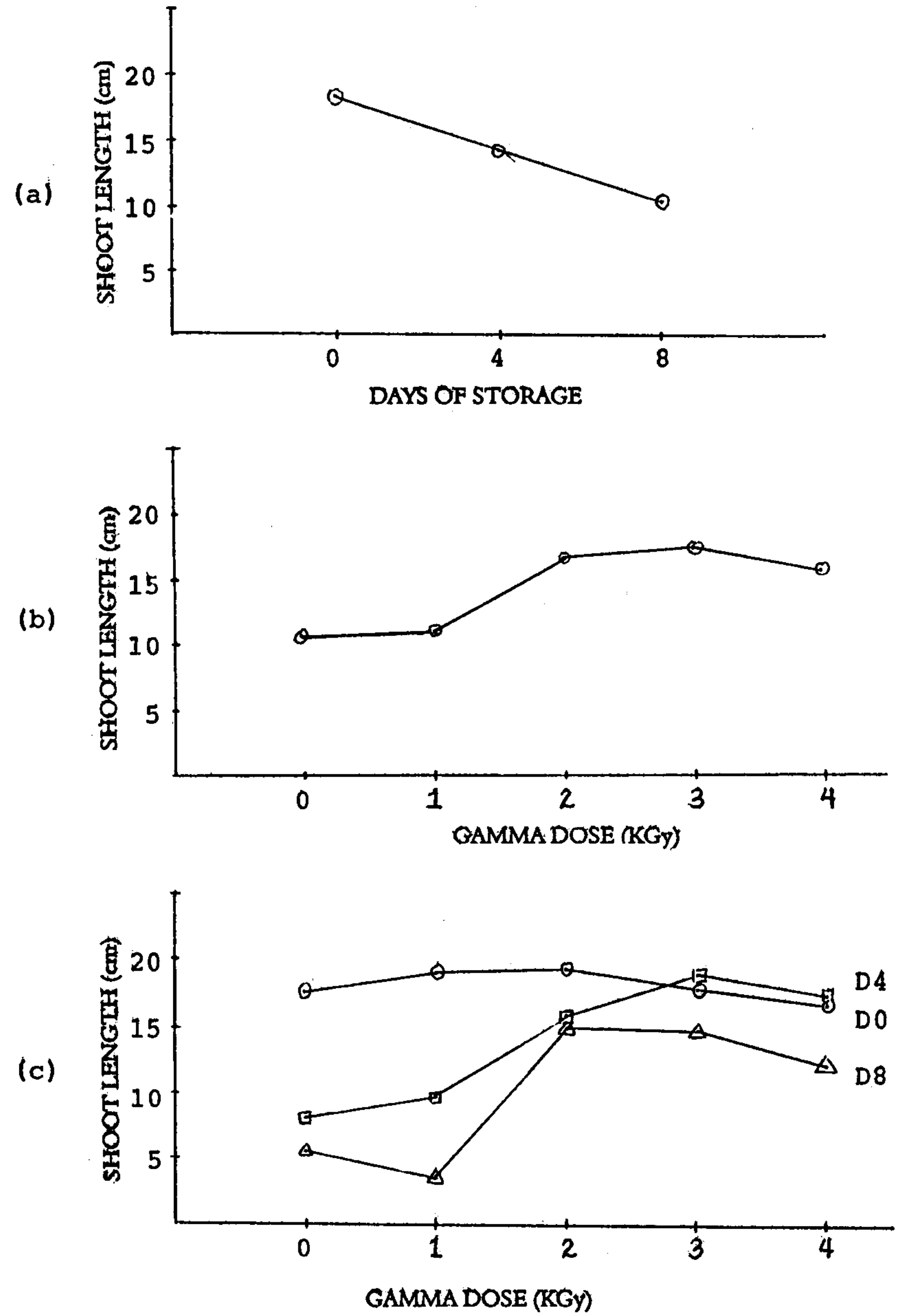


Figure 1. Effect of treated and untreated guayabano fruit juice on the mean shoot length of M_1 mungbean seedlings.

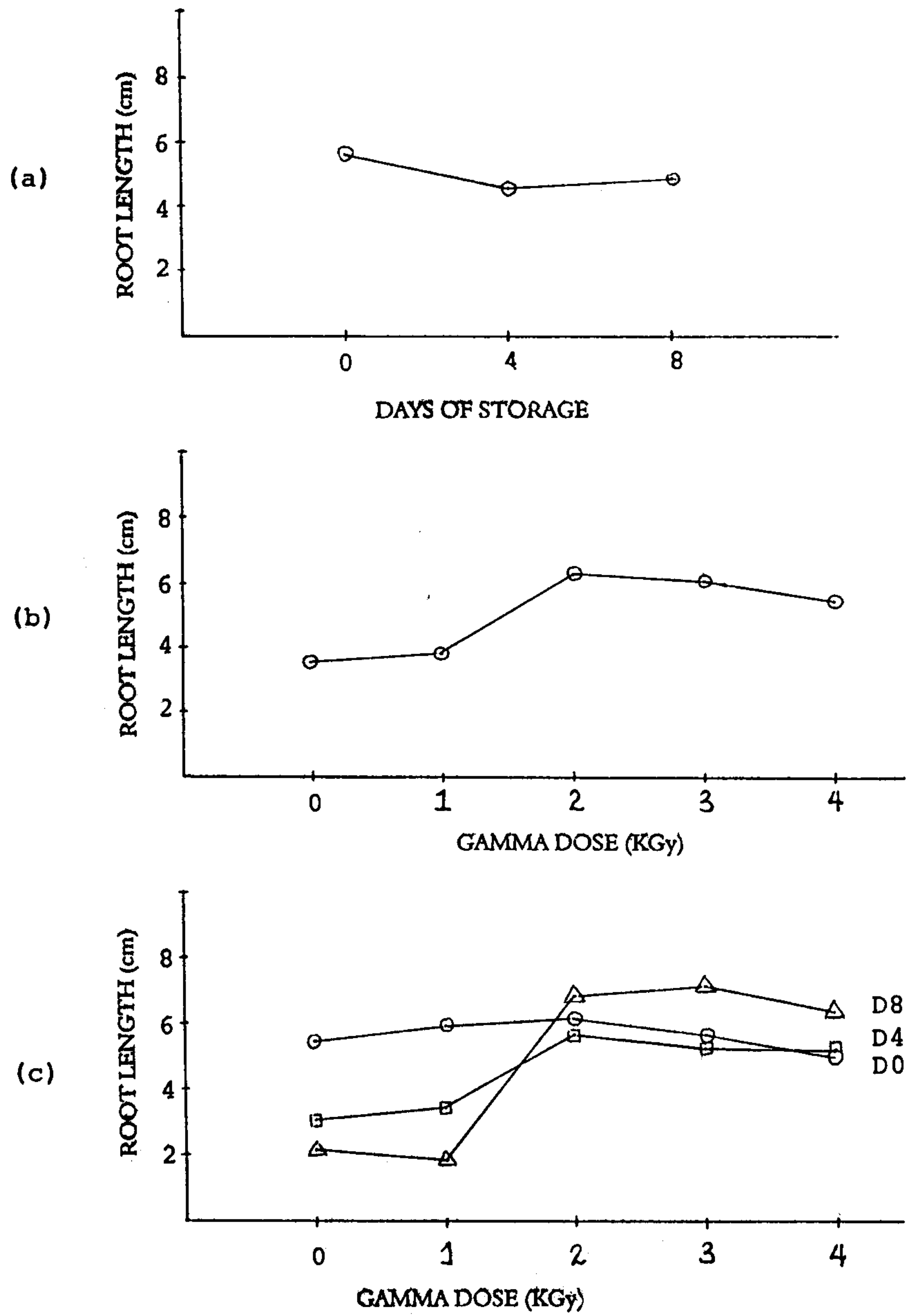


Figure 2. Effect of treated and untreated guayabano fruit juice on the mean root length of M_1 mungbean seedlings.

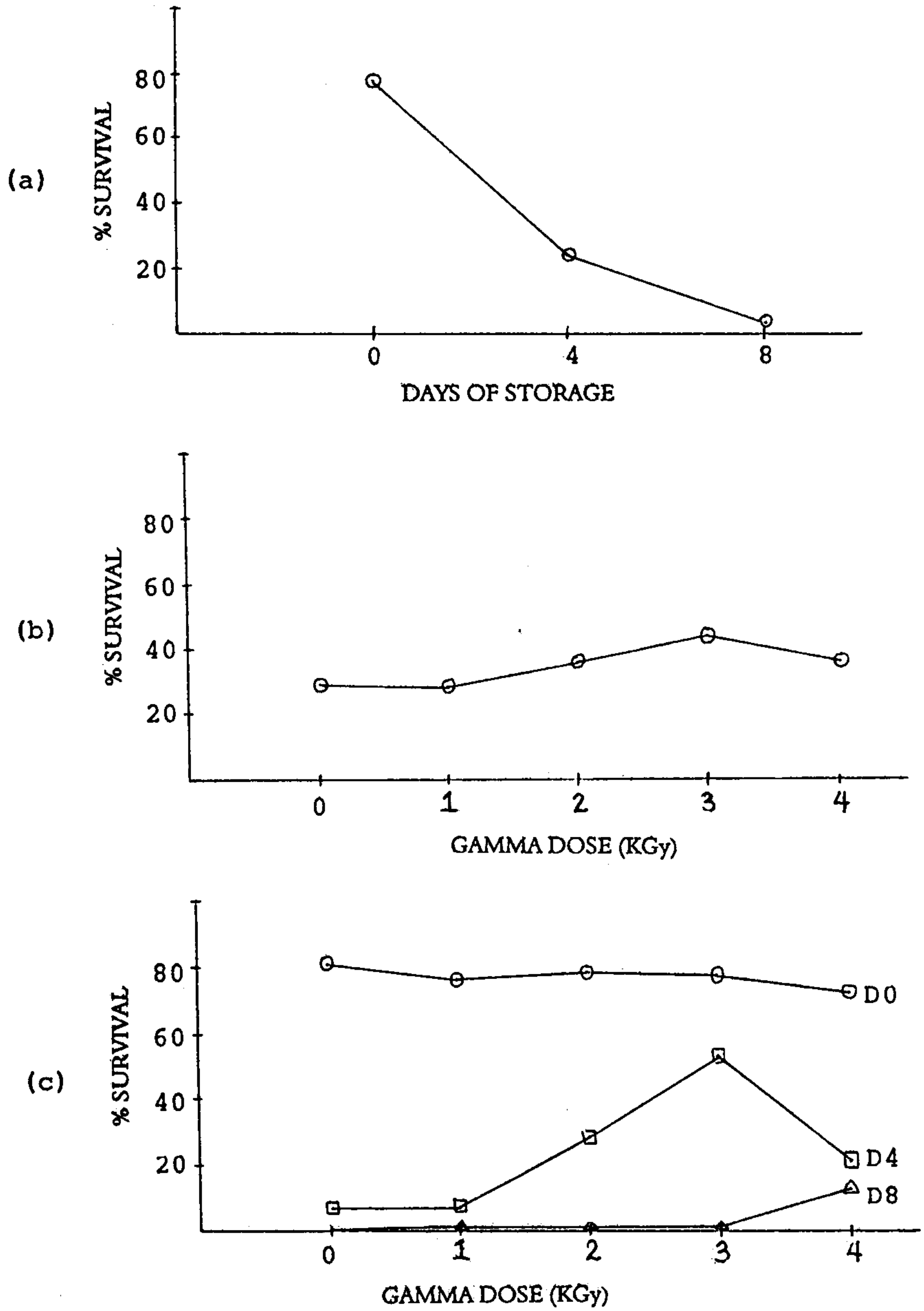


Figure 3. Effect of treated and untreated guayabano fruit juice on the survival of mungbean plants under field conditions.

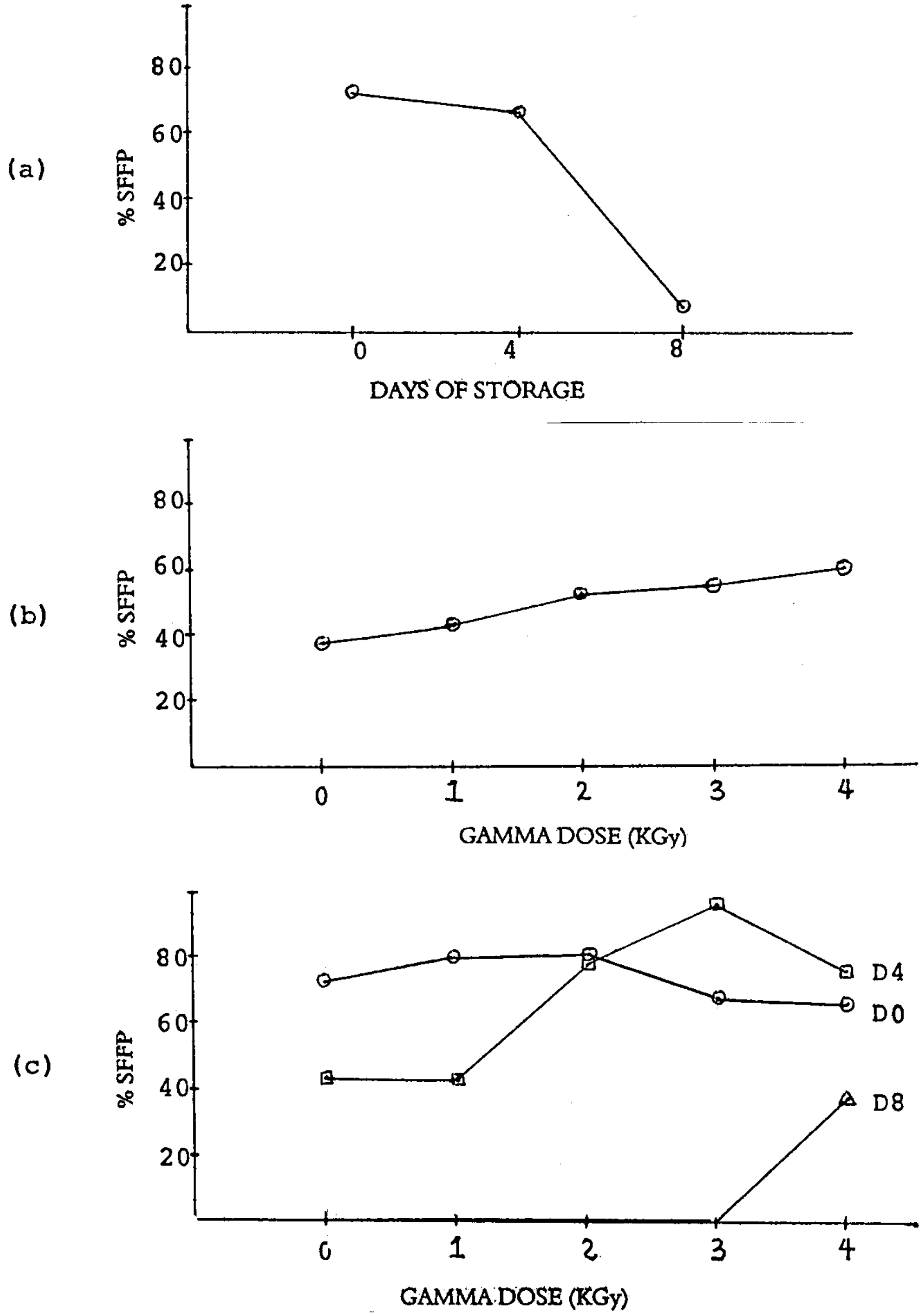


Figure 4. Effect of treated and untreated guayabano fruit juice on the percentage of survivors that flowered and formed pods (%SFFP).

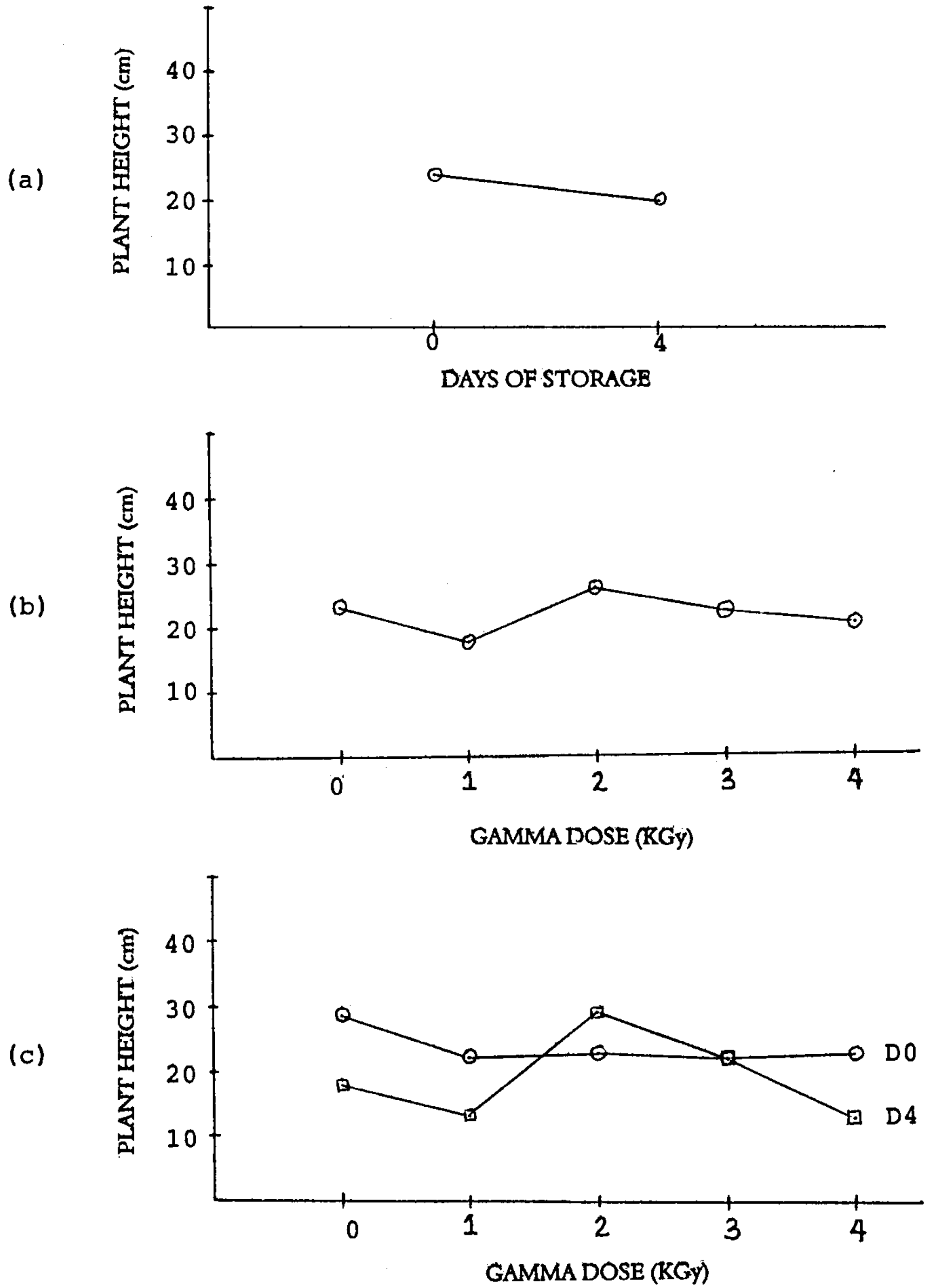


Figure 5. Effect of treated and untreated guayabano fruit juice on plant height of mungbean grown to maturity.

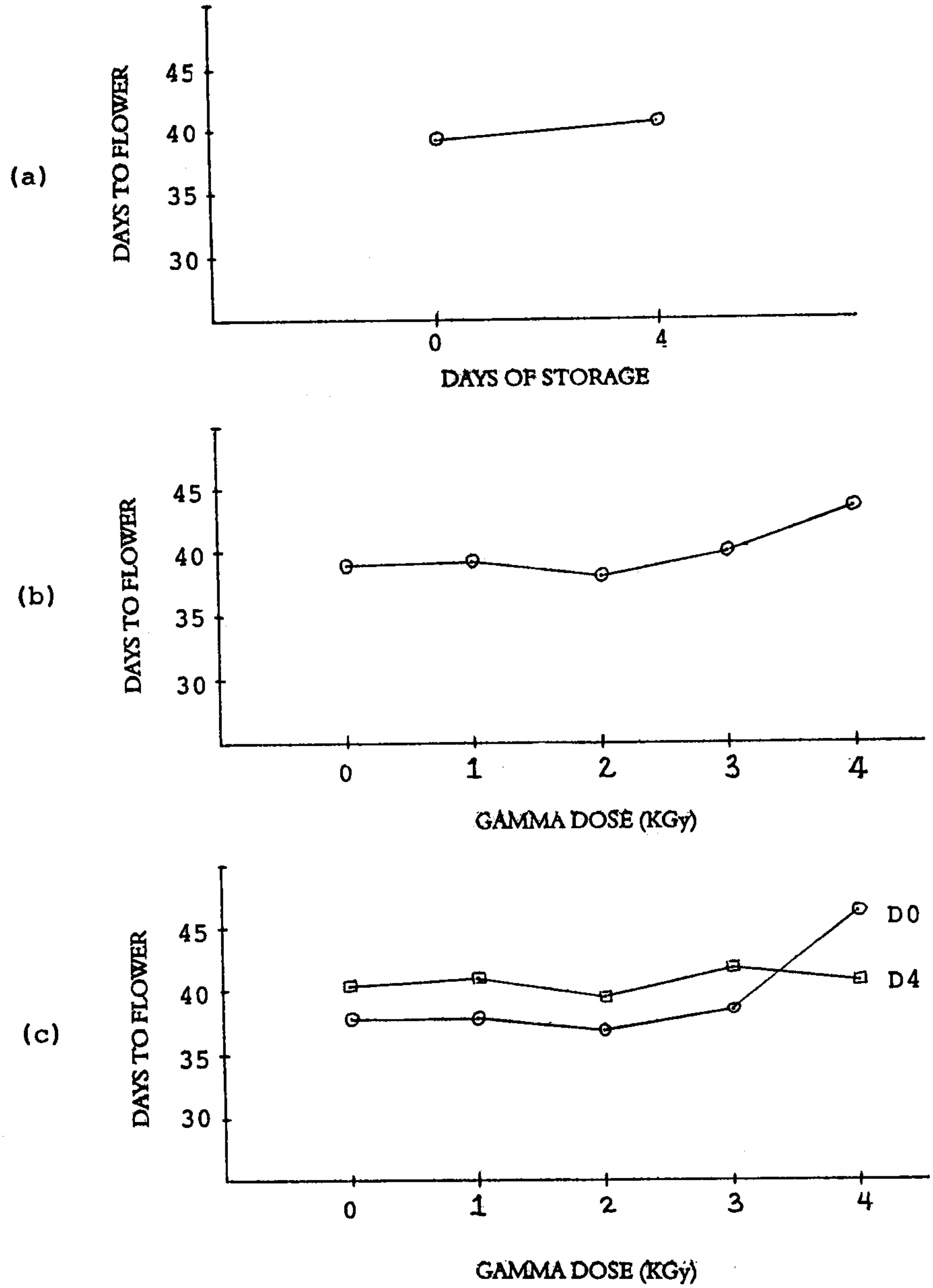
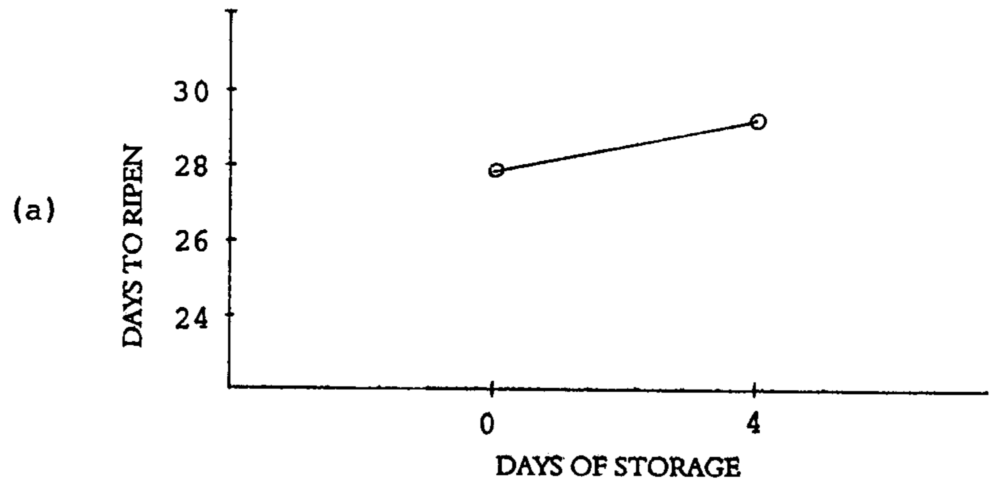
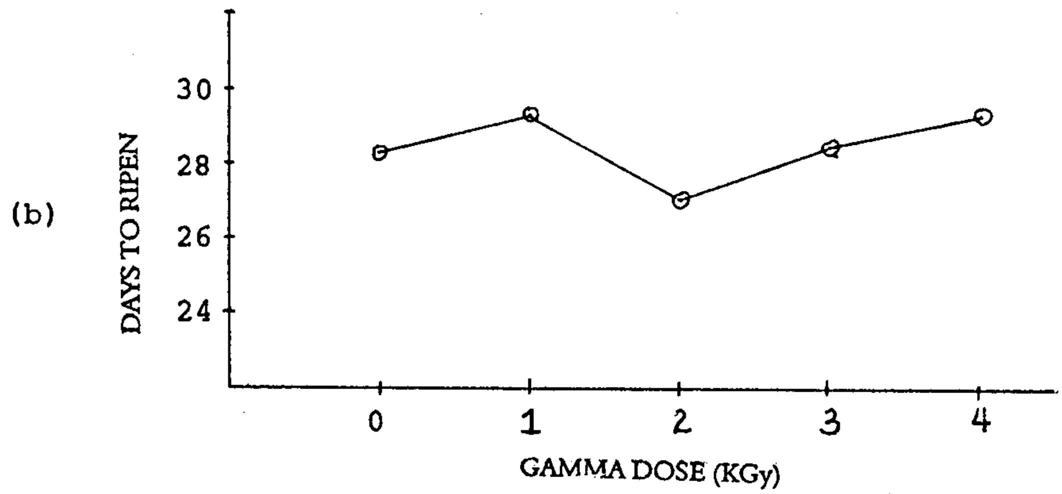


Figure 6. Effect of treated and untreated guayabano fruit juice on the mean number of days to flowering of mungbean plants grown to maturity.





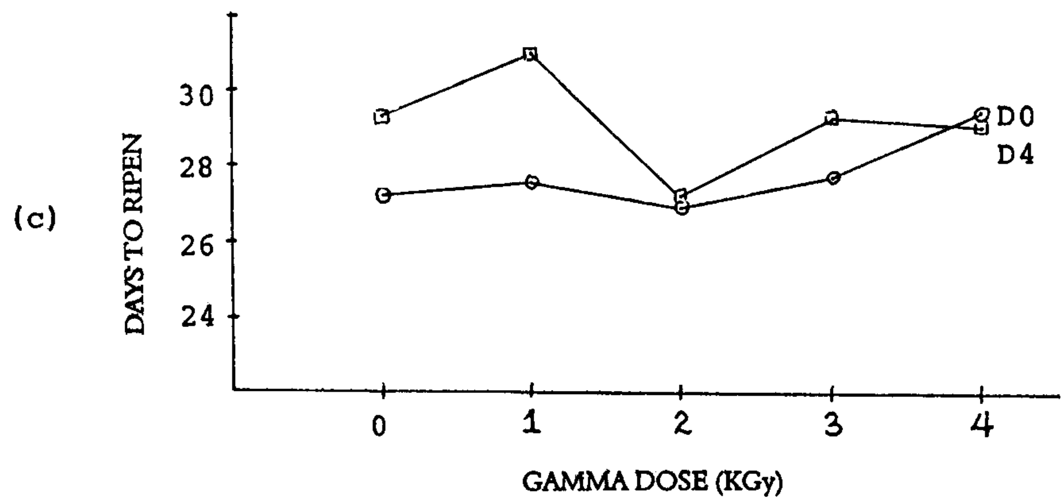


Figure 7. Effect of treated and untreated guayabano fruit juice on the mean number of days from flowering to ripening of pods of mungbean grown under field conditions.

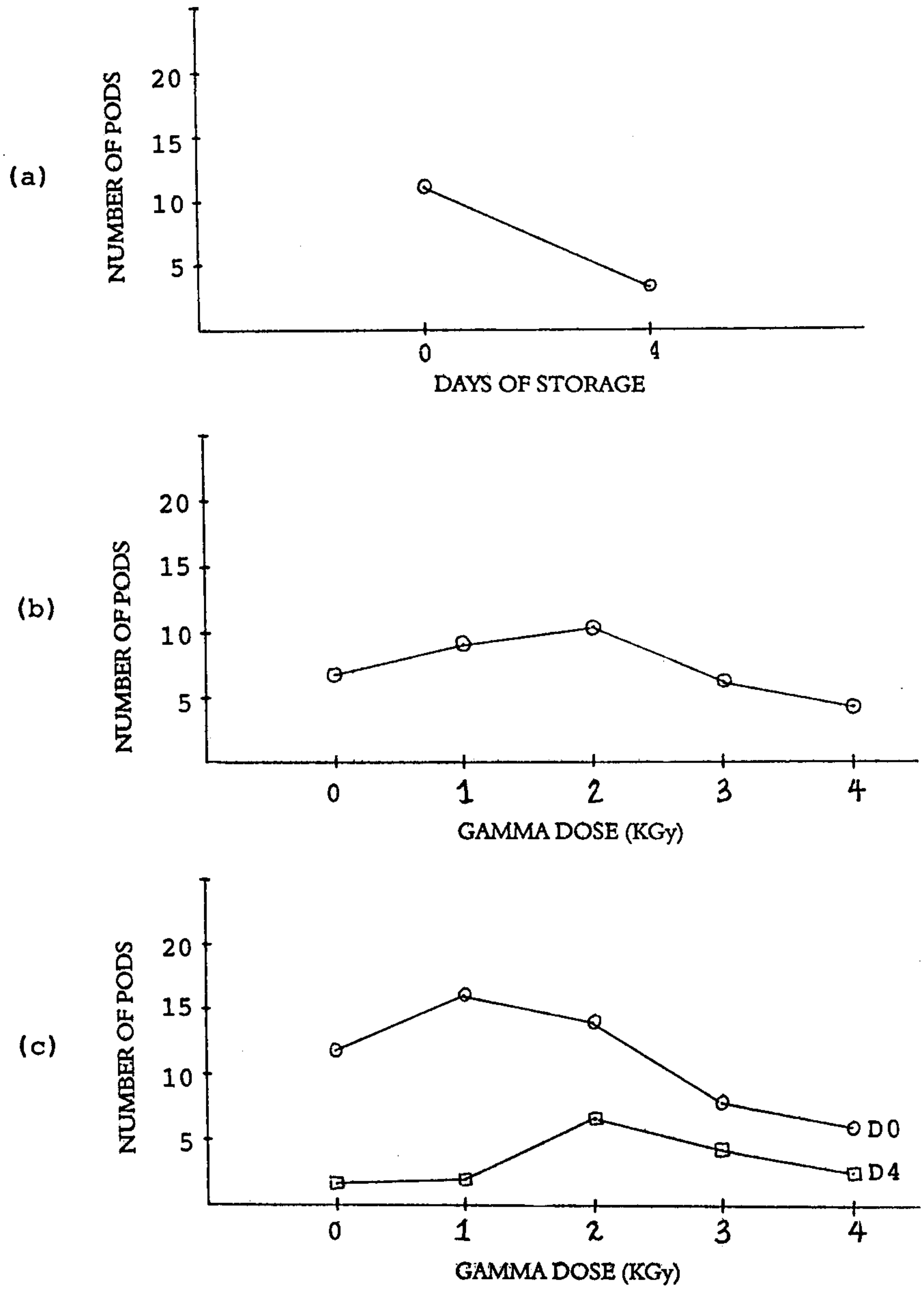


Figure 8. Effect of treated and untreated guayabano fruit juice on the mean number of pods per plant of mungbean grown under field conditions.

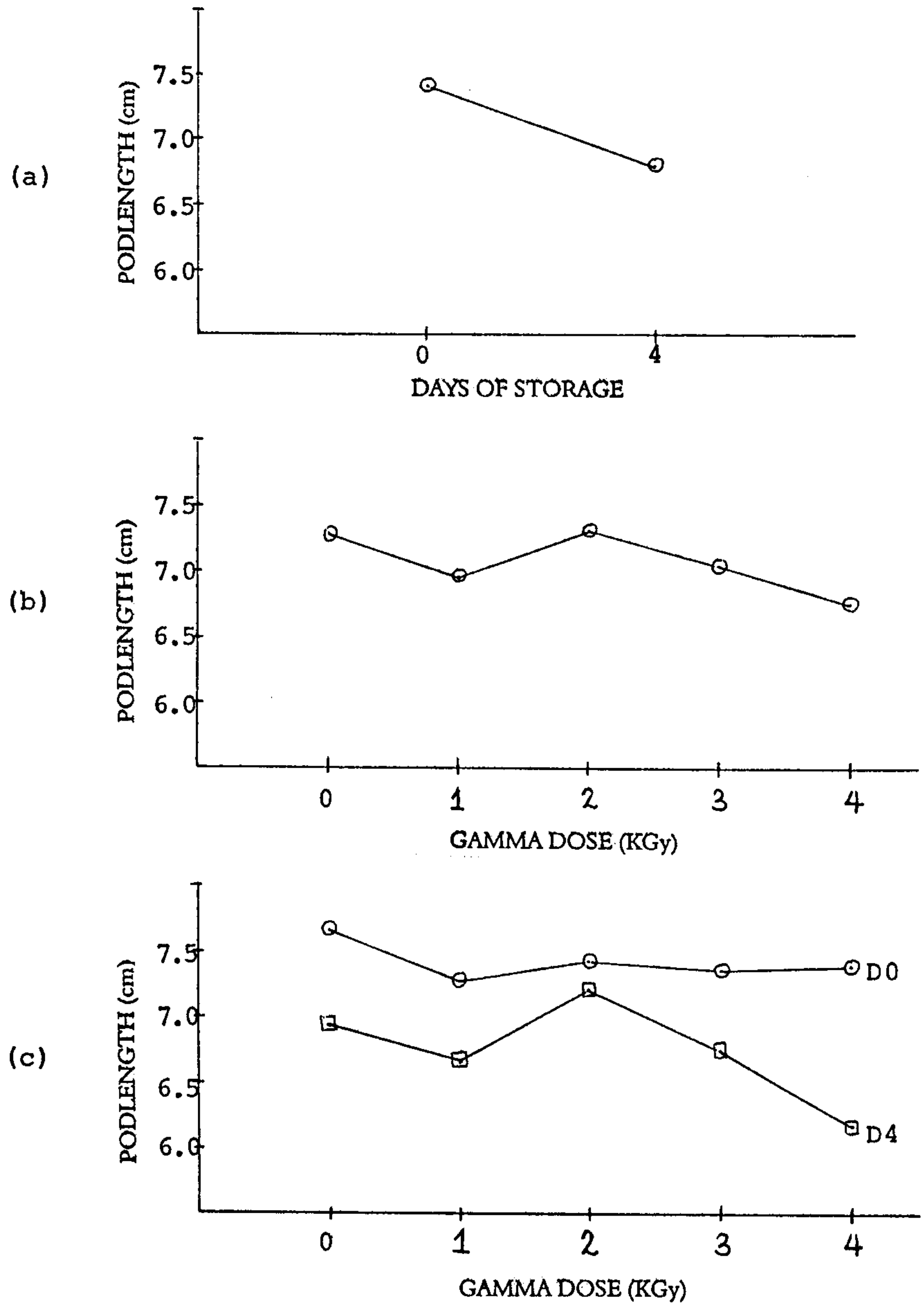


Figure 9. Effect of treated and untreated guayabano fruit juice on the mean pod length of mungbean plants grown under field conditions.

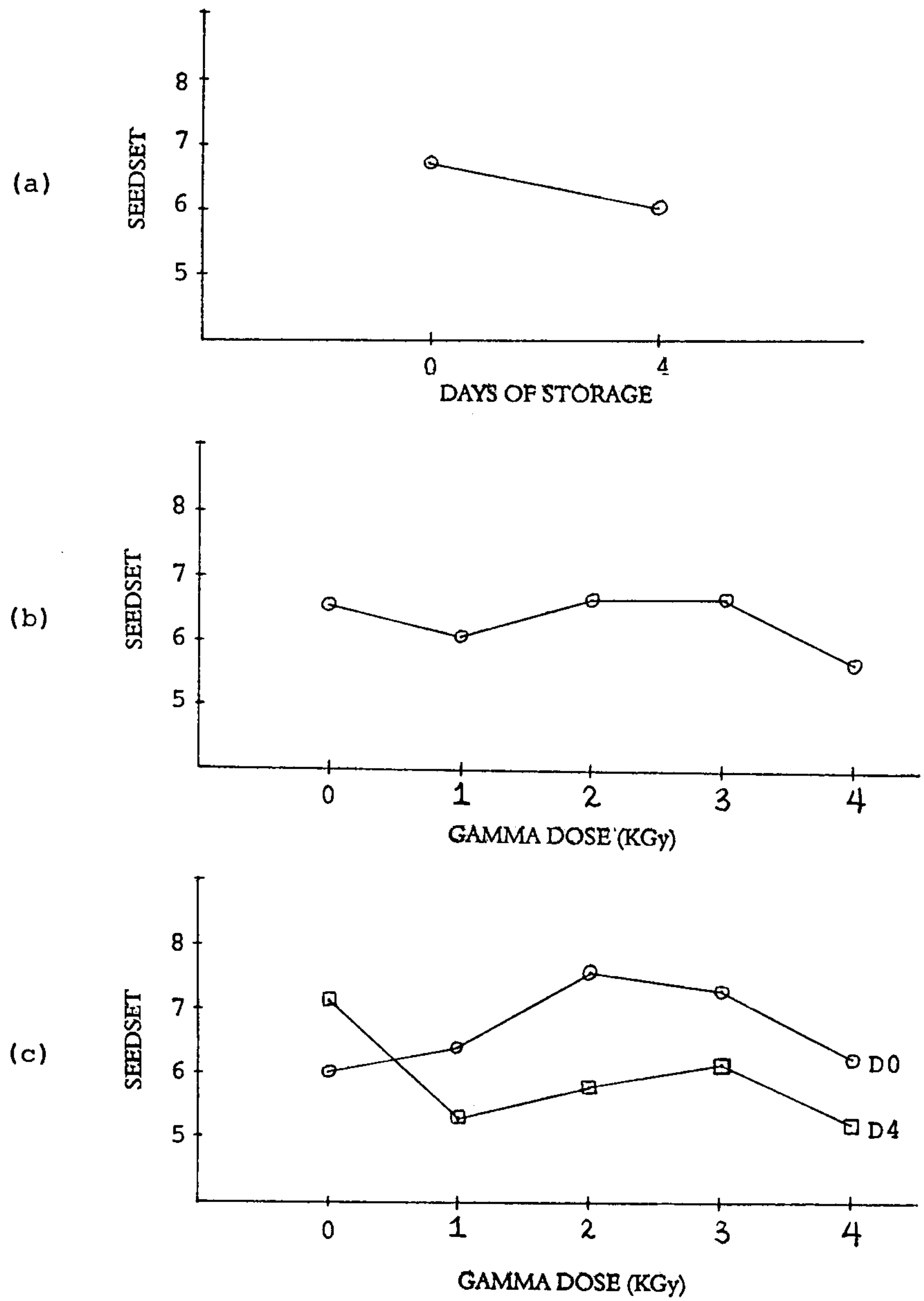


Figure 10. Effect of treated and untreated guayabano fruit juice on the mean seedset of mungbean plants grown under field conditions.

Figure 11. Seedlings at the first trifoliate stage: (A) with cuplike leaves but the first trifoliate is normal; and (B) with morphologically normal leaves but the leaflets of the first trifoliate are clover-shaped.