

Gamma radiosensitivity study on rice (*Oryza sativa* L.)

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ABSTRACT

It has been long known that plants are sensitive to ionizing radiations such as beta particles and gamma rays. Seeds or unfertilized flower buds and whole plant or part of it have been irradiated in order to investigate the effects of these radiations on crop plants. Studies have shown that radiation of different types at various intensities can either inhibit growth or kill the plant completely. The radiosensitivities of different plants vary greatly according to source of radiation. In present investigation, nine popular rice varieties cultivated in Andhra Pradesh, were used to examine varietal differences in radio sensitivity to gamma radiation during the wet season of 2012. Dry seeds of Nine rice varieties BPT 5204 (Samba mahsuri), JGL 384 (Polasaprabha), Surekha, Vijetha (MTU- 1001), JGL 1798 (Jagithal Sannalu), NLR 34449, Swarna, MTU 1010 (Cottondora sannalu) and Erramallelu (WGL 20471)) were exposed to gamma radiation ranging from 0.20 kGy to 2.00 kGy using continuous gamma sterilization plant (GC 5000, designed by Board of Radiation & Isotope Technology, Mumbai) with 444 TBq (12000Ci) and Cobalt – 60 source at Quality Control Laboratory, Acharya N. G. Ranga Agricultural University, Rajendranagar, Hyderabad-500 030 with a specific activity of 2.64 kGy hr⁻¹, to determine their response to radiation stress and the effective radiation dose for identification of Lethal dose 50 (LD₅₀). Data on germination parameter was generated as per [10]. Percentage germination was measured on 7th day after germination in irradiated sample. The results indicated that increasing doses of gamma irradiation had significant effect on germination for the first seven days under laboratory conditions. With increase in radiation above 0.80 kGy a reduction in percentage of germination under laboratory conditions was observed in irradiated seeds as compared to control. Increase in gamma ray doses from 0.20kGy to 0.60 kGy had little or no effect on germination percentage as there were no significant differences in germination percentage of irradiated seeds and non-irradiated (control) for all the varieties evaluated. The LD₅₀ values determined from linear regression analysis (Polynomial fit using Origin Pro 8 software) based on percentage of germination ranged from 0.89 kGy (JGL 1798) to 1.88 kGy (Vijetha). The varieties JGL 1798 and BPT 5204 had highest radiosensitivity while Vijetha and Surekha were relatively tolerant. However, the varieties MTU 1010 (1.66kGy), Erramallelu (1.57 kGy), Swarna (1.56 kGy), JGL 384 (1.38 kGy) and NLR 34449 (1.32 kGy) are moderately tolerant. These ranges of LD₅₀ values determined for the different rice varieties could be useful in rice varietal improvement programmes.

Key words: Germination, Lethal Dose 50, Radiation stress.

INTRODUCTION

Rice is a major staple food crop of India ensuring food security to millions of people. The realized yield potential in rice is mainly achieved through genetically stable high yielding varieties. Continuous mono-cropping of rice predominated by few varieties led to narrow genetic base making the rice ecosystem highly vulnerable to external factors like radiation stress. Climate change driven radiation stress on rice not only would result in irreversible genetic changes but also interfere with establishing optimum field population by drastically reducing the germination capability of seed. The study on the effects of radiation in plants is a broad and complex field. Work is being done in many areas on a large number of plant species.

Mutation means a sudden heritable change in the genetic material at the gene or chromosome level [5]. They may be caused by error during cell division or by exposure to the DNA- damaging agents or mutagens in the environment. Radiation has been found to affect the size and weight of plants. In many radiobiological reactions, the effect of a given dose depends on the intensity of radiation or the manner in which the total dose is fractioned. Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical and physiological changes in cells and tissues [9]. Gamma radiation can be useful for the alteration of physiological characters [11]. The biological effect of gamma ray is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals [12]. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose [3]. In view of the potential hazards of natural and artificial gamma radiations, it becomes essential to understand the extent of damage caused to the rice system. Since rice is the most important staple food crop, the ill effects of radiation at germination level need to be thoroughly investigated. The radiation induced growth abnormalities in seedlings were mainly due to cell death and suppression of mitosis at different exposures. The purpose of this work is to study the effects of ionizing radiation of different doses from ^{60}Co on rice (*Oryza sativa* L.), which is major staple food crop of India and other part of the world.

MATERIALS AND METHODS

Fifty (50) seeds of nine rice varieties were subjected to gamma rays from ^{60}Co source using doses from 0.20 to 2.00 kGy (Tables 1). Linear regression analysis from origin pro 8 software package was used to estimate the optimum LD₅₀ doses for the different rice varieties using percentage of germination in laboratory conditions as a standard measure of germination test (Table 3) (Fig.1 to Fig.9).

Germination Evaluation

Fifty (50) seeds by doses and nine varieties (Table 1) of irradiated and non-irradiated rice varieties were taken in 0.1mm thick polythene bags of 15 X 22cm dimension and sealed. The bags were subjected to gamma irradiation by exposing them to doses of 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40, 1.60, 1.80 and 2.00 KGy at a specific activity of 2.64 KGy/hour and sown in three replications per treatment on blotting paper in 9 cm Petri dishes soaked with distilled water to observe germination under laboratory conditions (Temperature $28 \pm 2^\circ\text{C}$). Filter paper was maintained moist with distilled water on need basis. The seeds were then observed daily for a period of seven (7) days. Percentage germination was recorded for each variety for the first seven days.

Samples were irradiated in continuous gamma sterilization plant (GC 5000, designed by Board of Radiation & Isotope Technology, Mumbai) with 444 TBq (12000Ci) and Cobalt – 60 source at Quality Control Laboratory, Acharya N. G. Ranga Agricultural University, Rajendranagar, Hyderabad- 500 030 and were compared with the observations made on non irradiated control. The material for irradiation was placed in an irradiation chamber located in vertical drawer inside the lead flask. Radiation field was provided by a set of stationary Cobalt-60 source placed in a cylindrical cage. The source was doubly encapsulated in corrosion resistant stainless steel pencils and was tested in accordance with international standards. Two access holes of 8 mm diameter were provided of service sleeves for gasses, thermocouple, etc. Mechanism for rotating/stirring samples during irradiation is also incorporated. The lead shield provided around the source was adequate to keep the external radiation field well within permissible limits. The quantity of absorbed dose (KGy) can be defined as the amount of energy absorbed per unit mass of the matter at the point of interest. The experiment was carried out as per completely Randomized Design (CRD). Standard germination test was estimated using dry seeds of all varieties with an average moisture content of 13%. The irradiated seed along with non- irradiated control seeds were sown in petridish in the

laboratory. Data on germination and growth parameters were recorded seven days after sowing under ambient condition. Germination percentage was calculated.

RESULTS AND DISCUSSION

In plant cells, the nucleus is considered the principal site of damage by ionizing radiation. The process leading to radiation damage may be summarized as: the initial physical stage which lasts only a minute fraction of a second; the physico-chemical stage lasting about 10-16s; the chemical stage lasting a few seconds and the biological stage in which the time scale varies from tens of minutes to tens of years depending on the particular symptoms.

The effect of gamma irradiation on germination of seeds (BPT 5204(Samba mahsuri), JGL 384 (Polasaprabha), Surekha, Vijetha (MTU- 1001), JGL 1798 (Jagithal Sannalu), NLR 34449, Swarna, MTU 1010 (Cottondora sannalu) and Erramallelu (WGL 20471)) was studied, in order to identify the LD50 doses of gamma radiation in some selected seeds. Table 1 shows that germination percentages decreased after gamma irradiation. Increase in doses of gamma irradiation had significant effect on seed germination from 0-7 days. The decrease in germination was not directly proportional to the increase in dosage nor was a definite pattern observed in all the nine rice varieties. Similar results have been reported in rice by [18], [17], [19] and [7]. The effectiveness of gamma irradiation on percentage of germination after 7 days was evident at 0.80 and 1.20 (LD50) and reduces significantly at 1.80 kGy and 2.00 kGy in all the varieties except Vijetha and Surekha (Fig 1).

The germination ranged from 2% (JGL 1798) to 100% in different varieties in the standard germination test. In Surekha and Vijetha a stimulatory effect was observed in germination at dose 1.00kGy and 0.40kGy as compared to control 99.67% and 98.67% respectively. Table 2 shows that the maximum reduction in percentage of germination was observed with a dose 2.00kGy in JGL 1798 (98.005) followed by JGL 384, Erramallelu, Swarna and NLR 34449 (96.00, 93.33, 91.89 and 91.33% respectively). The doses of gamma radiation ranging from 0.20 to 1.00kGy there is no significant different in germination percentage as compared to controls in selected varieties where as the variety JGL 1798 has significant effect in germination was recorded i.e. 65.33 and 19.33% at doses of 0.80 and 1.00kGy respectively.

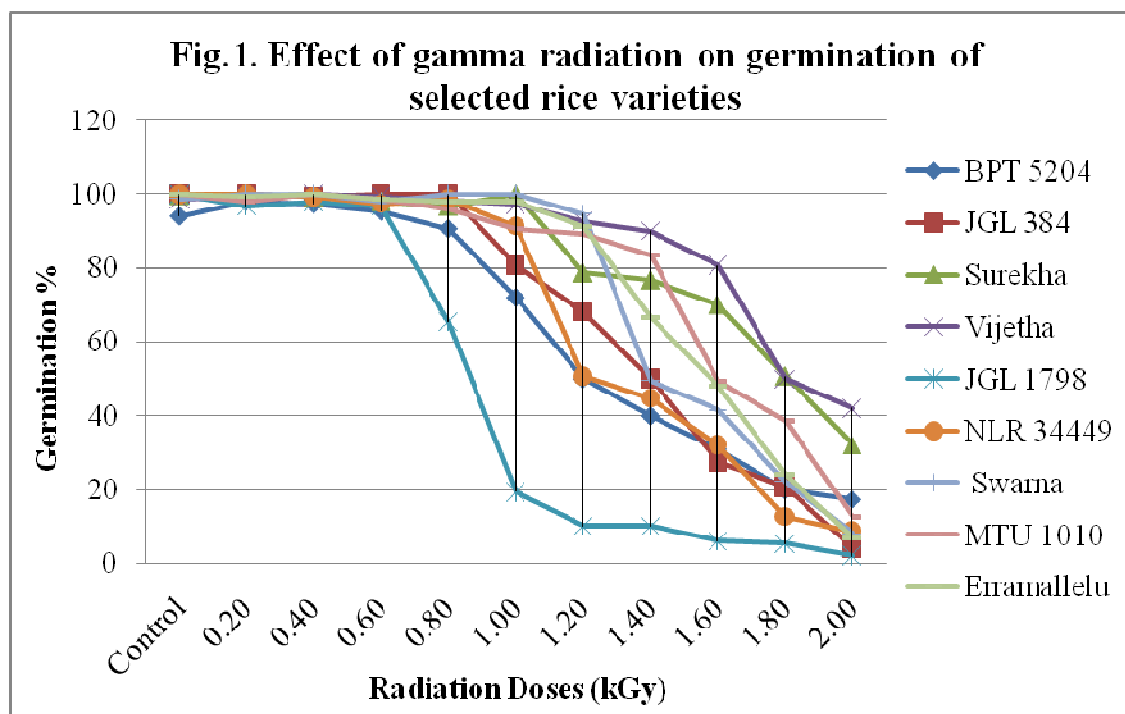


Table 1: Effect of Gamma Radiation on Germination of Selected Rice varieties

	BPT 5204	JGL 384	Surekha	Vijetha	JGL 1798	NLR 34449	Swarna	MTU 1010	Erra mallelu
Control	94.00	100.00	99.67	98.67	100.00	100.00	98.67	100.00	100.00
0.20	98.00	100.00	99.33	99.33	96.67	100.00	100.00	98.00	99.33
0.40	97.33	99.33	99.33	100.00	98.00	99.33	100.00	100.00	100.00
0.60	95.33	100.00	98.00	99.33	96.67	97.33	98.00	98.67	98.67
0.80	90.67	100.00	96.67	98.33	65.33	98.67	100.00	96.00	98.00
1.00	72.00	80.67	100.00	97.33	19.33	91.33	100.00	90.67	98.00
1.20	50.00	68.00	78.67	92.67	10.00	50.67	94.67	89.33	91.33
1.40	40.00	50.00	76.67	90.00	10.00	44.67	49.33	83.33	66.67
1.60	31.00	27.33	70.00	80.67	6.00	32.00	41.33	49.33	48.00
1.80	20.00	20.67	50.67	50.00	5.33	12.67	22.00	38.67	24.00
2.00	17.33	4.00	32.00	42.00	2.00	8.67	8.00	12.67	6.67
GM	64.15	68.18	81.91	86.21	46.30	66.85	66.79	77.88	75.52
SED	1.85	1.50	0.86	1.11	1.06	1.53	1.58	1.66	1.75
CD(0.05)	3.83	3.12	1.79	2.30	2.21	3.17	3.28	3.44	3.63
Cd (0.01)	5.21	4.24	2.44	3.13	3.00	4.32	4.46	4.67	4.94
CV%	3.53	2.70	1.29	1.58	2.81	2.80	2.90	2.61	2.84

Table 2: Percentage reduction in germination by gamma radiation

Radiation Doses (kGy)	BPT 5204	JGL 384	Surekha	Vijetha	JGL 1798	NLR 34449	Swarna	MTU 1010	Erra mallelu
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.20	-4.26	0.00	0.34	-0.67	3.33	0.00	-1.35	2.00	0.67
0.40	-3.55	0.67	0.34	-1.35	2.00	0.67	-1.35	0.00	0.00
0.60	-1.42	0.00	1.68	-0.67	3.33	2.67	0.68	1.33	1.33
0.80	3.55	0.00	3.01	0.34	34.67	1.33	-1.35	4.00	2.00
1.00	23.40	19.33	-0.33	1.35	80.67	8.67	-1.35	9.33	2.00
1.20	46.81	32.00	21.07	6.08	90.00	49.33	4.06	10.67	8.67
1.40	57.45	50.00	23.08	8.79	90.00	55.33	50.00	16.67	33.33
1.60	67.02	72.67	29.77	18.25	94.00	68.00	58.11	50.67	52.00
1.80	78.72	79.33	49.17	49.33	94.67	87.33	77.70	61.33	76.00
2.00	81.56	96.00	67.89	57.43	98.00	91.33	91.89	87.33	93.33

Several workers have studied effect of gamma rays on seed germination of gymnosperms. The higher exposures were usually inhibitory [4]; [16]; [14]. The higher Exposures are usually inhibitor on seed germination of gymnosperm an Angiosperm [23] where as lower exposures are sometimes stimulatory [6]; [21]. The results of [11] have shown that survival of plants to maturity depends on the nature and extent of chromosomal damage. Increasing frequency of chromosomal damage with increasing gamma radiation may be responsible for less germinability and reduction in plant growth and survival. Changes in the germination percentage were found to attribute to gamma rays treatments. The stimulating causes of gamma ray on germination may be certified to the activation of RNA or protein synthesis, which occurred during the early stage of germination after seeds irradiated [1].

Table 3: Summary of LD50 values for the Selected Rice varieties (Dry Seeds) in Lab conditions:

Sample ID	Variety	Regression Equation $Y = mx + c$	LD50 Dose (kGy)
I	BPT 5204	$47.19 = (-76.45) x + 146.16$	1.29
II	JGL 384	$50.00 = (-89.90) x + 174.39$	1.38
III	Surekha	$49.63 = (-85.13) x + 203.77$	1.81
IV	Vijetha	$48.95 = (-81.82) x + 203.08$	1.88
V	JGL 1798	$49.12 = (-143.24) x + 176.78$	0.89
VI	NLR 34449	$50.45 = (-97.02) x + 179.07$	1.32
VII	Swarna	$49.87 = (-120.36) x + 238.15$	1.56
VIII	MTU 1010	$49.72 = (-111.48) x + 235.79$	1.66
IX	Erramallelu	$50.05 = (-122.7) x + 243.64$	1.57

Fig.1: Lethal Dose 50 of BPT – 5204 (Samba Mahsuri)

Fig. 1.1: Polynomial fit to graph

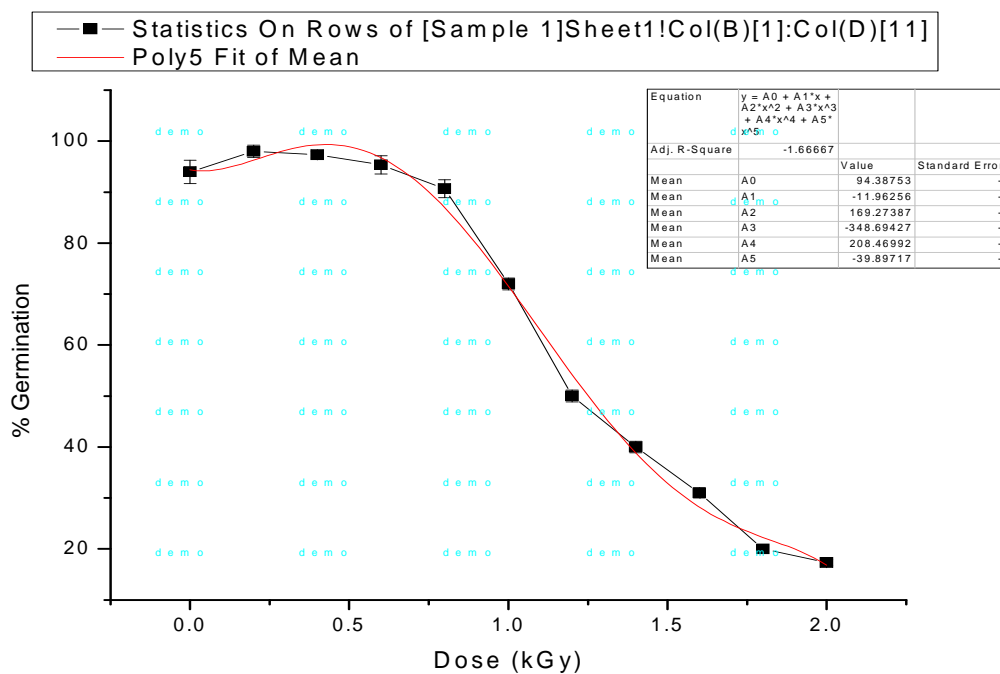
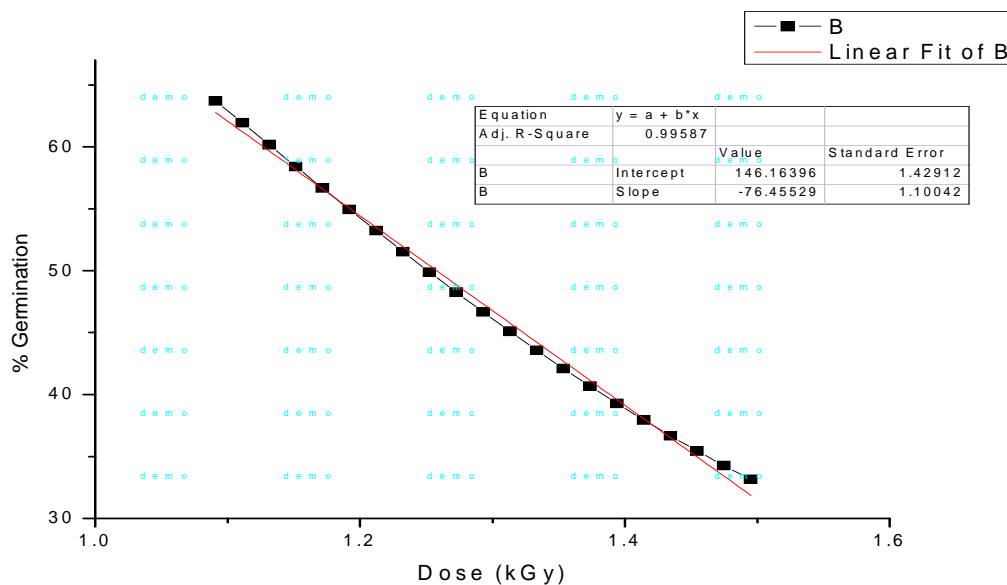


Fig.1.2: Linear fit of data selected around assumed LD50



LD50 for Samba Mahsuri (BPT 5204) = 1.29 kGy

Fig.2: Lethal Dose 50 of JGL 384 (Polasaprabha)

Fig. 2.1: Polynomial fit to graph

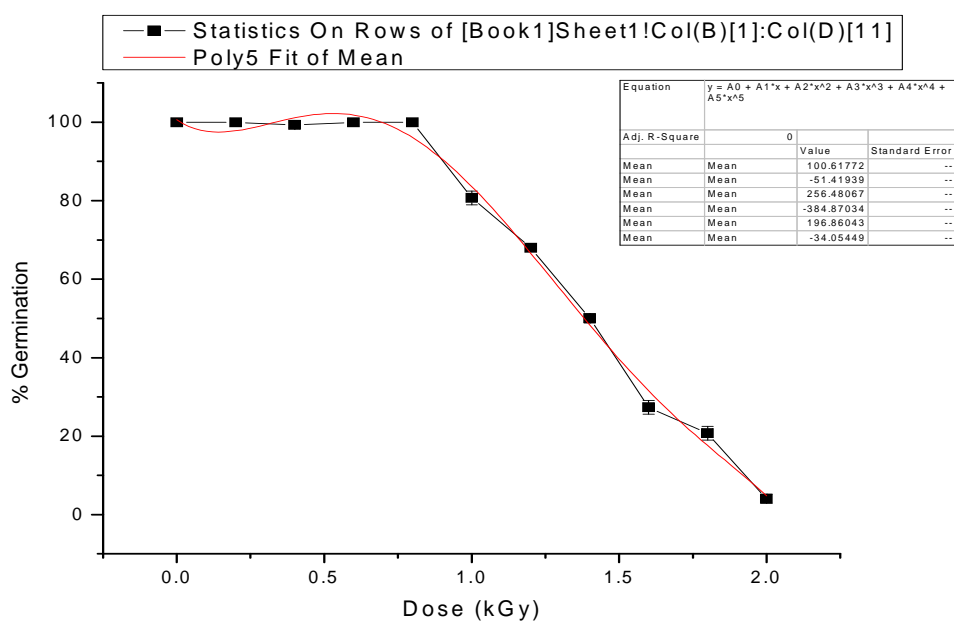
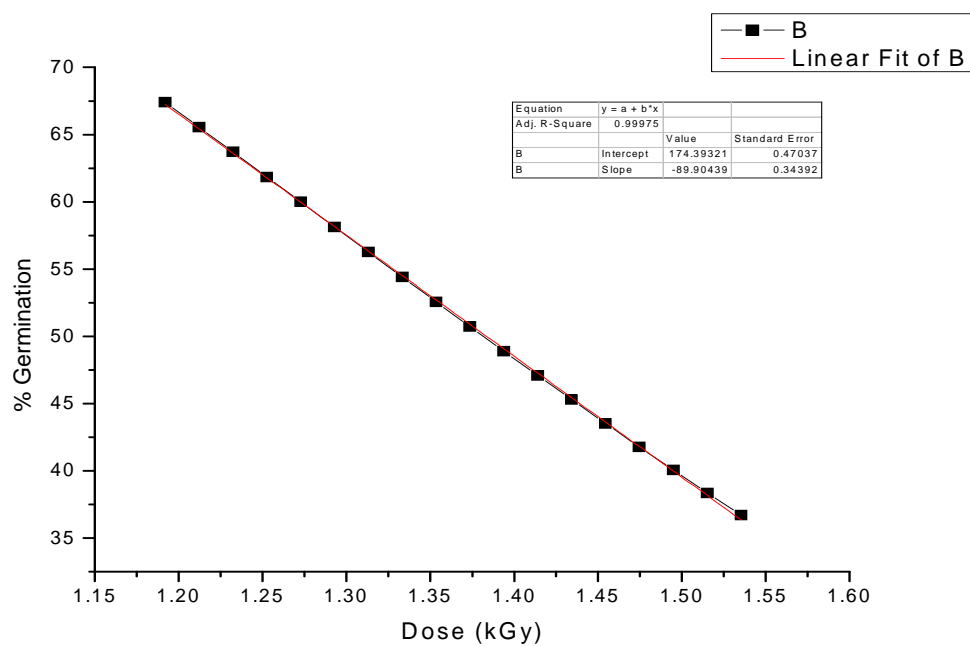


Fig. 2.2: Linear fit of data selected around LD50



LD50 for JGL 384 (Polasaprabha) = 1.38 kGy

Fig.3: Lethal Dose 50 of Surekha

Fig. 3.1: Polynomial fit to graph

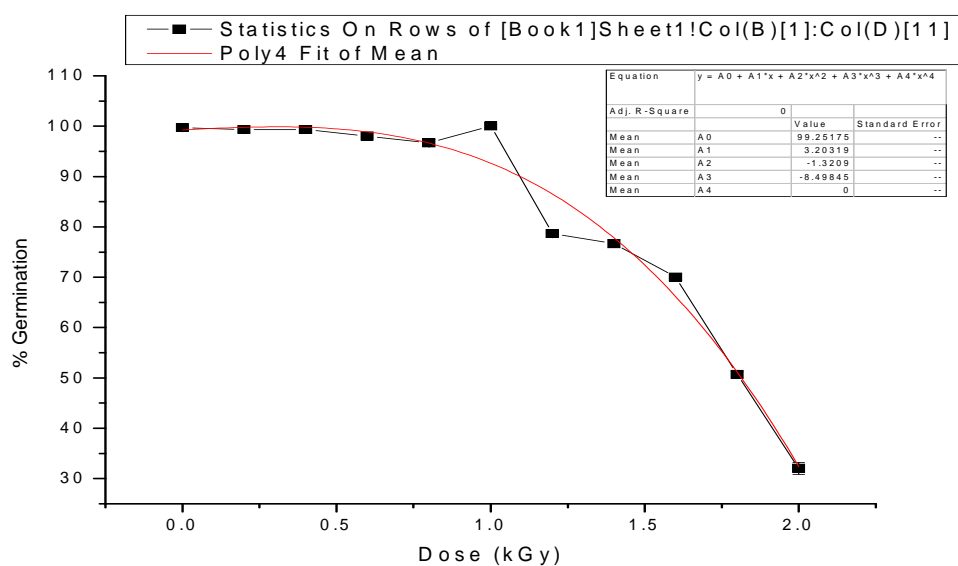
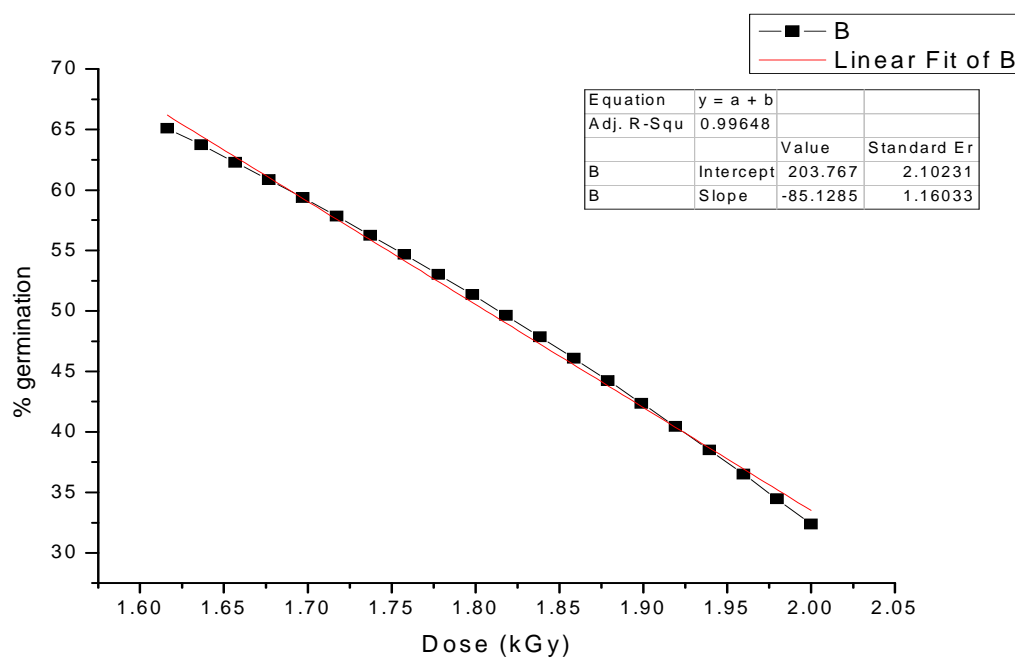


Fig.3.2: Linear fit of data selected around LD50



LD50 for Surekha = 1.81 kGy

Fig.4: Lethal Dose 50 of Vijetha (MTU 1001)

Fig. 4.1: Polynomial fit to graph

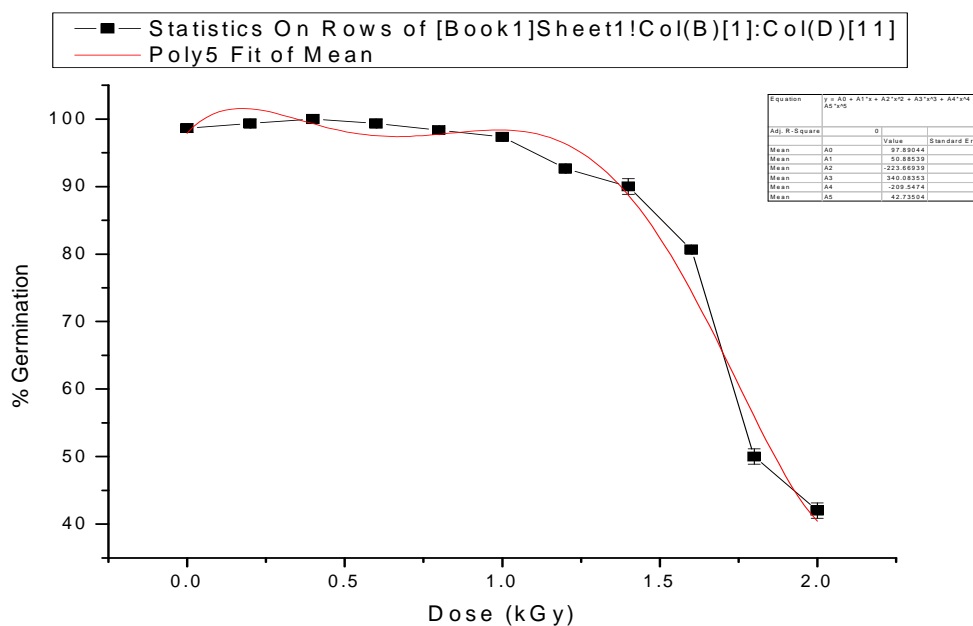
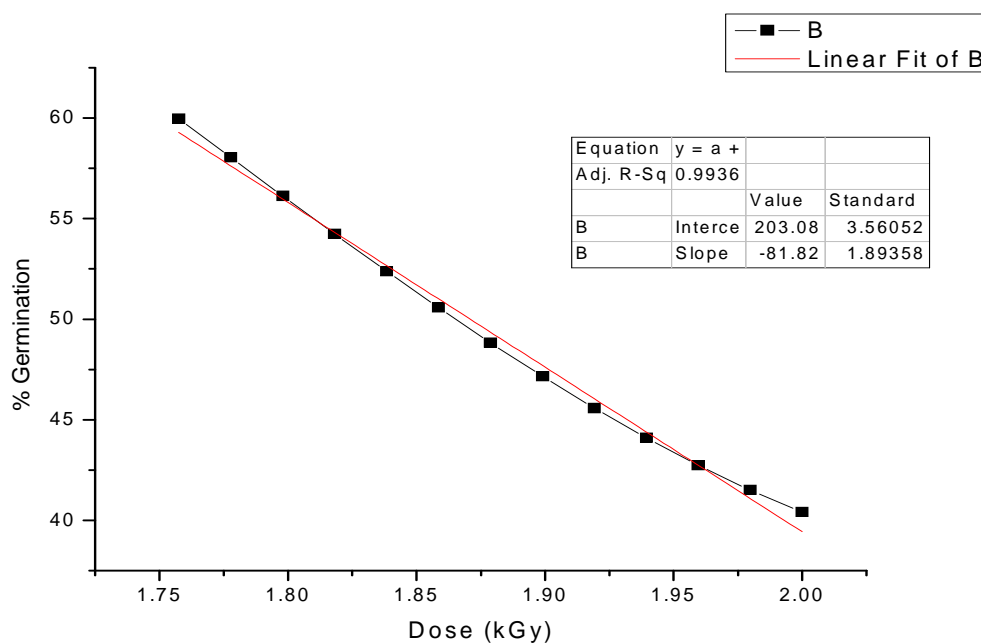


Fig. 4.2: Linear fit of data selected around assumed LD50 value



LD50 for Vijetha (MTU 1001) = 1.88 kGy

Fig.5: Lethal Dose 50 of JGL 1798 (Jagithal Sannalu)

Fig.5.1: Polynomial fit to graph

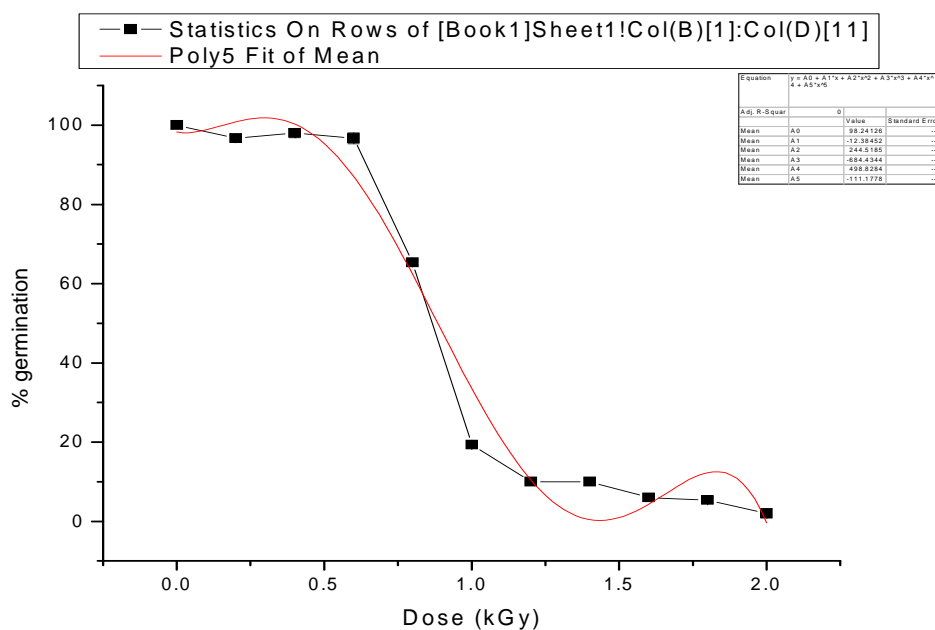
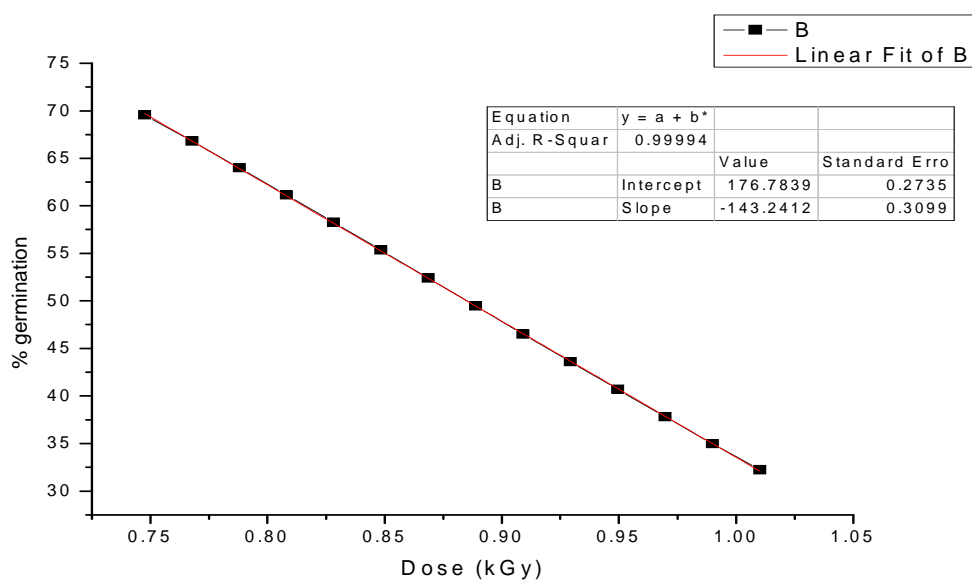


Fig.5.2: Linear fit of data selected around assumed LD50 value



LD50 for JGL 1798 (Jagithal Sannalu) = 0.89 kGy

Fig.6: Lethal Dose 50 of NLR 34449 (Nellore Mahsuri)

Fig.6.1: Polynomial fit to graph

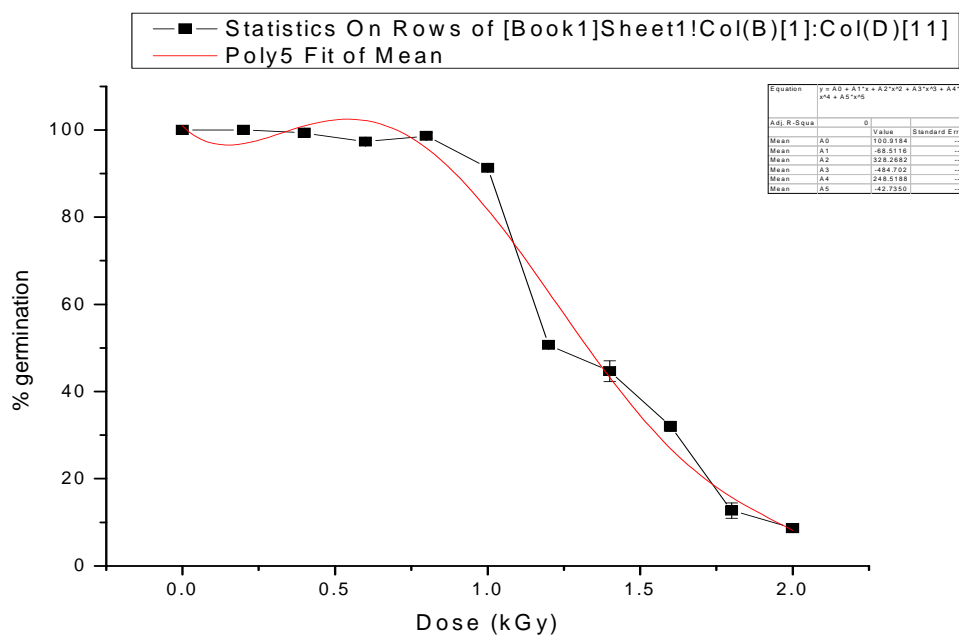
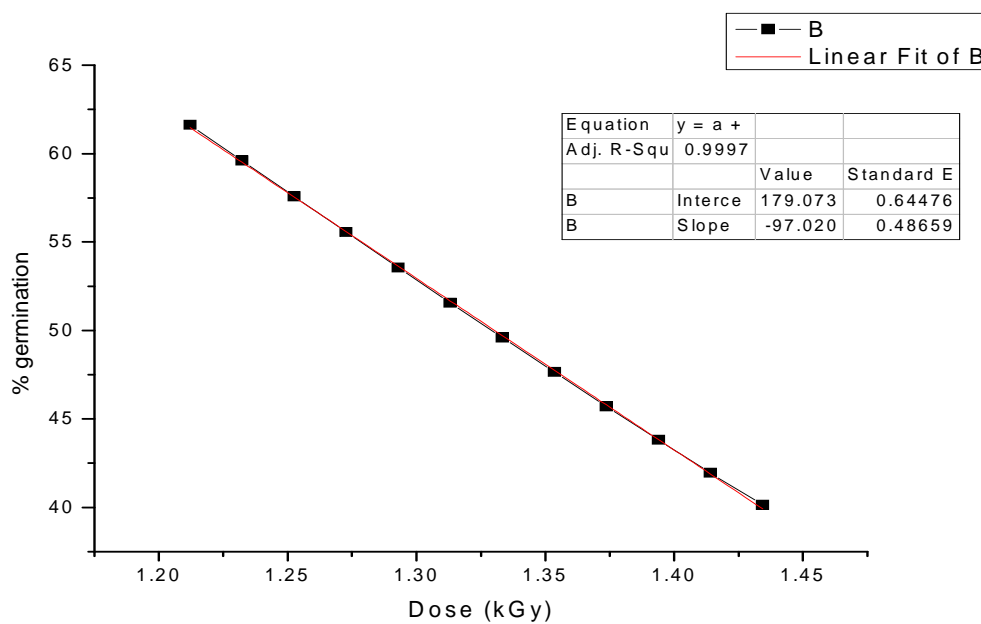


Fig.6.2: Linear fit of data selected around assumed LD50 value



LD50 for Sample VII = 1.32 kGy

Fig.7: Lethal Dose 50 of Swarna

Fig. 7.1: Polynomial fit to graph

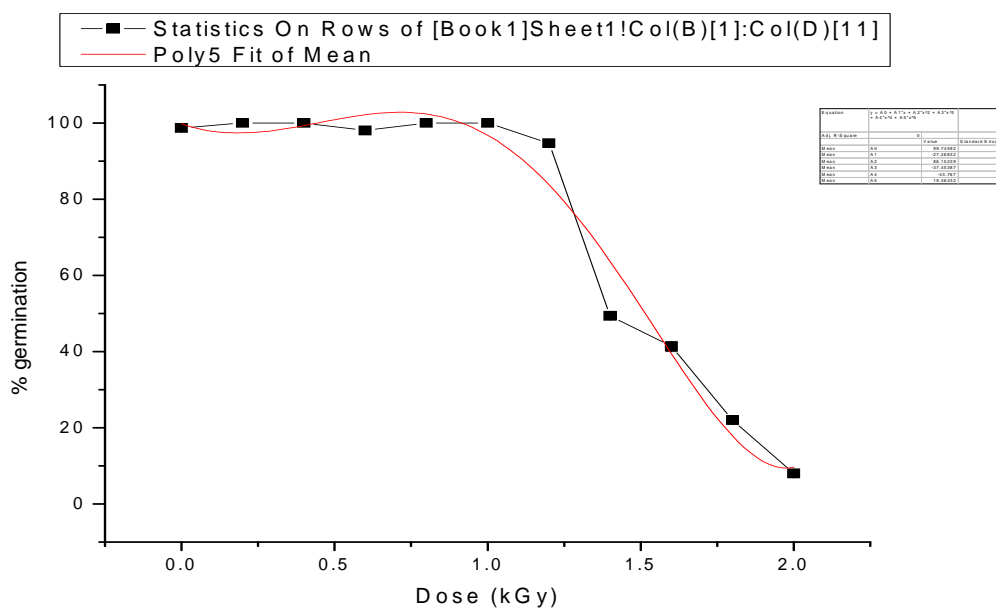
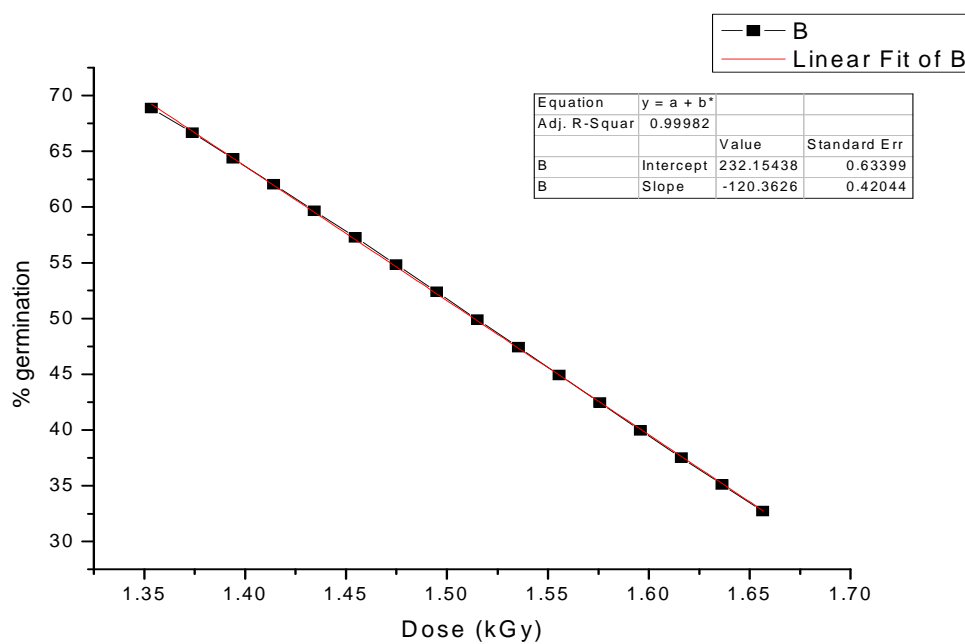


Fig.7.2: Linear fit of data selected around assumed LD50 value



LD50 for Swarna = 1.56 kGy

Fig.8: Lethal Dose 50 of MTU 1010 (Cotondora sannalu)

Fig. 8.1: Polynomial fit to graph

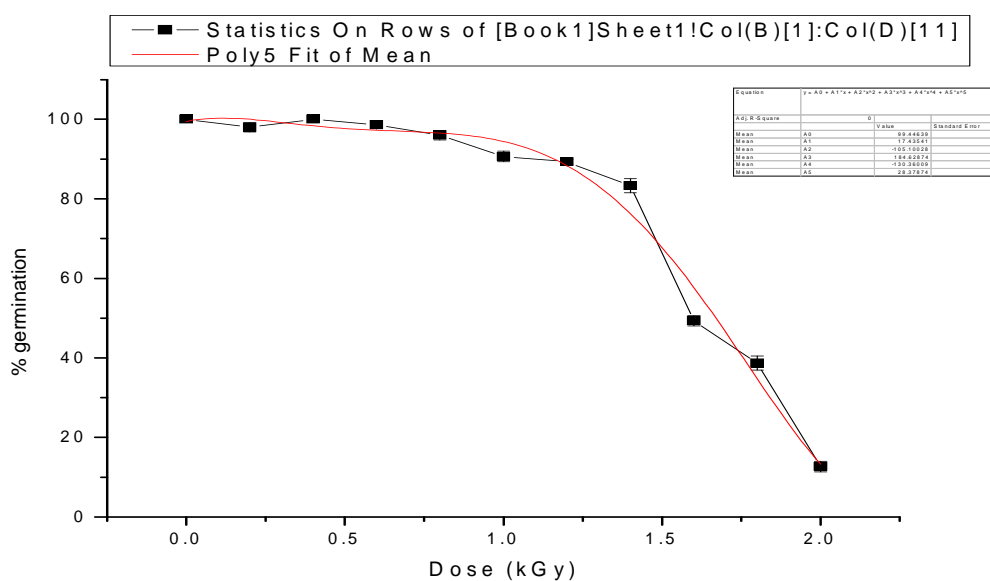
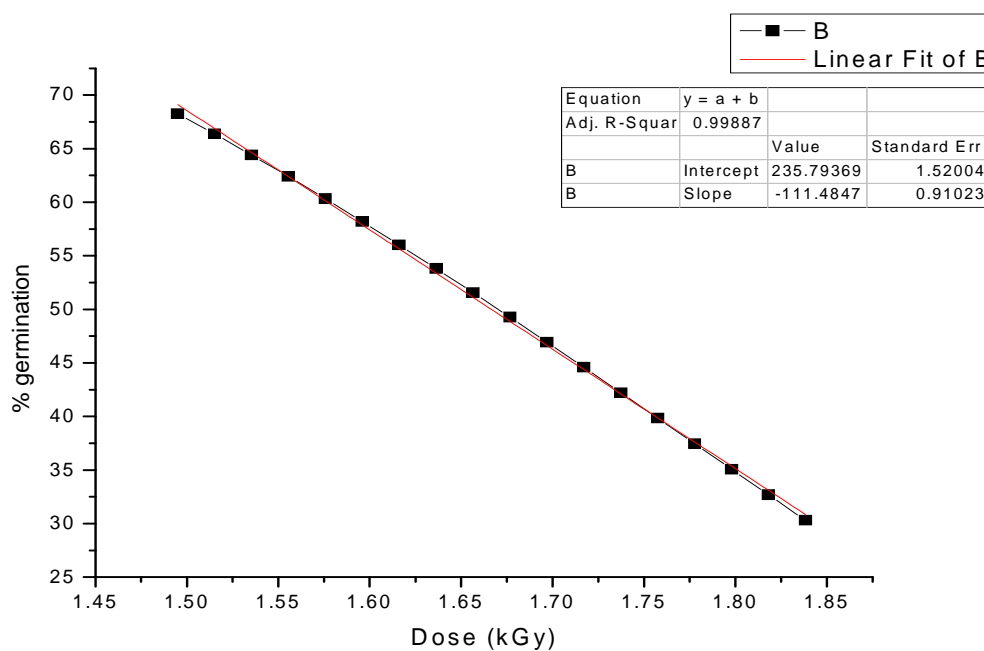


Fig.8.2: Linear fit of data selected around assumed LD50 value



LD50 for MTU 1010 (Cotondora sannalu)= 1.66 kGy

Fig.9: Lethal Dose 50 of Erramallelu (WGL 20471)

Fig. 9.1: Polynomial fit to graph

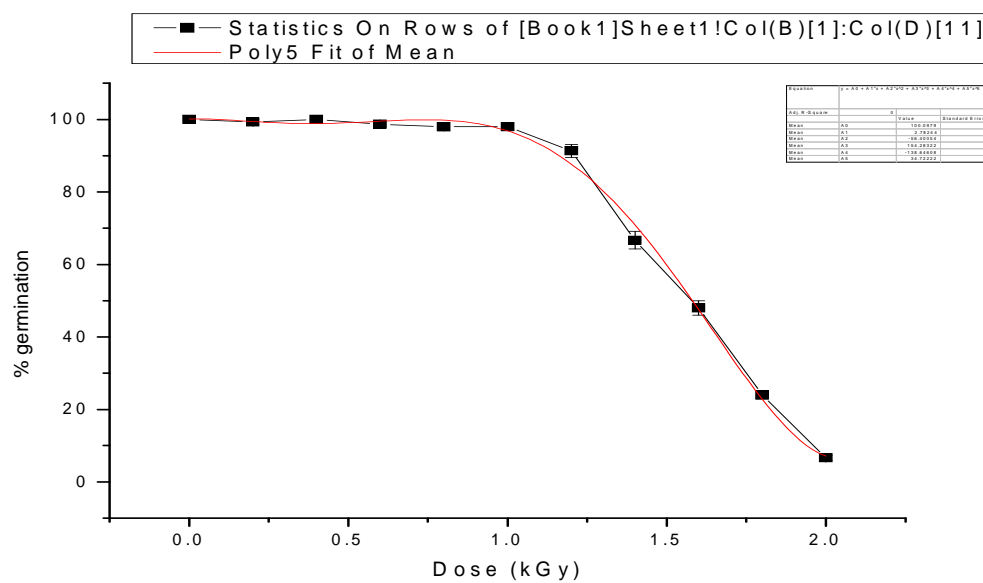
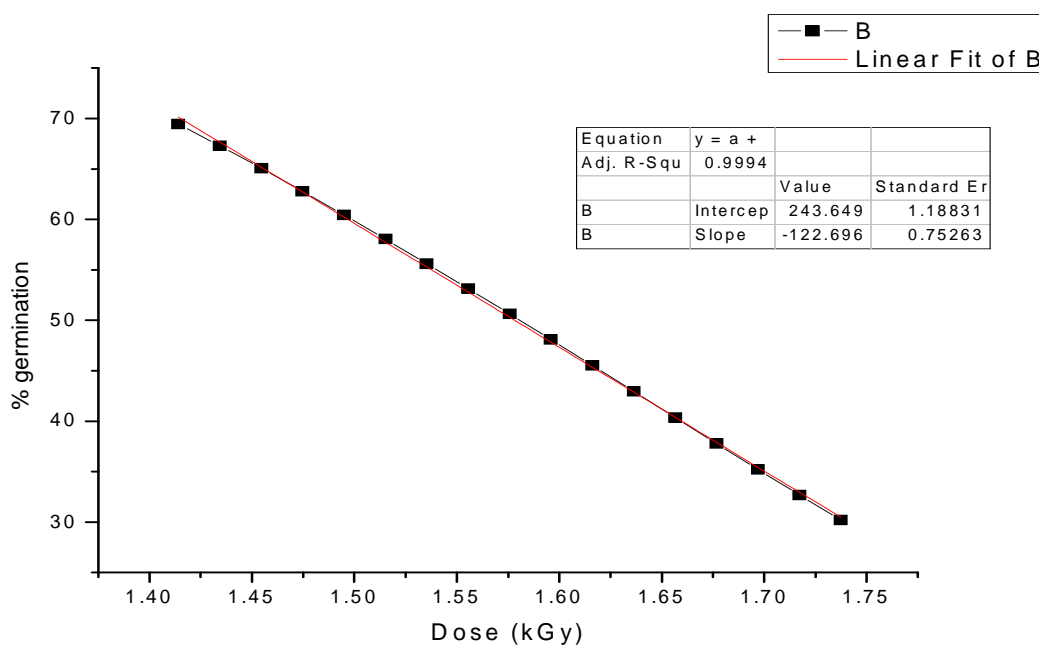


Fig.9.2: Linear fit of data selected around assumed LD50 value



LD50 for Erramallelu = 1.57 kGy

There are many possible reasons for germination inhibition as reported by different authors. Gamma irradiation generates free radicals in plant produce that may bring metabolic disorders in the seeds leading to growth retardation. It is also affects enzyme activity since higher seed vigour is related to higher germination efficiency. It is also reported that high α - amylase activity increased metabolic activity leading to enhanced seed vigour. Hence it may be infused that gamma irradiation may decrease α -amylase activity [13]; [20]; [2]. In some reports decreased lipase activity with increasing gamma irradiation dose in castor seeds resulted in germination inhibition where as some studies associated increased membrane permeability with seed vigour loss and concluded that gamma irradiation and seed membrane permeability are directly related, hence increased radiation doses increases membrane permeability and reduces seed germination [22]; [15]; [8]; [24].

Mean squares from Table 1 show that the differences for various doses of gamma irradiation were reached the level of significance. Statistically maximum germination was recorded at 0.20 and 0.40kGy in all the varieties.

LD 50 values (Table 3) determined based on the percentage of germination ranged from 0.89 kGy for JGL 1798 to 1.88 kGy for Vijetha. The varieties JGL 1798 and BPT 5204 had highest radiosensitivity while Vijetha and Surekha were most tolerant.

CONCLUSION

The research carried out during the course of this investigation clearly revealed the sensitivity of rice crop to gamma radiation stress beyond 0.80 KGy which can be accepted as a threshold level for radiation stress. Moreover high irradiation doses completely inhibit germination. Further, the study also determined the LD₅₀ value for nine rice varieties where in 50% reduction was observed in respect of the germination percentage. LD 50 values (Table 3) determined based on the percentage of germination ranged from 0.89 kGy for JGL 1798 to 1.88 kGy for Vijetha.

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REFERENCES

- [1] Abdel-Hady, M.S., E.M. Okasha, S.S.A. Soliman and M. Talaat. *Australian Journal of Basic Applications*. **2008**, 2(3): 401-405.
- [2] Afzal, I., T. M. Shah, B. M. Atta, M. A. Haq and H. Sayed. *Pakistan Journal of Botany*. **2008**, 40:1033-1041.
- [3] Ashraf, M., A.A. Cheema, M. Rasheed and Z. Qamar. *Pakistan Journal of Botany*. **2003**, 35(5): 791-795.
- [4] Bora, K.C. Relative biological efficiencies of ionizing radiation on the induction of cytogenetic effect in plants. In: Proceeding of the Symposium on the effect of ionizing radiation on seed and their significance for crop improvement, **1961**, pp.345-357.
- [5] Chahal, G.S. an S.S. Gosal, Principles and Procedures of Plant Breeding. Oxford: Alpha Science International Ltd. **2002**, pp. 399-412.
- [6] Chauhan, Y.S. and R.P Singh. *Journal of Indian Botanical Society*. **1980**, 59: 170-172.
- [7] Cheema, A.A., Atta, B. M. Radiosensitivity studies in Basmati Rice. *Pakistan Journal of Botany*. **2003**, 35(2):197-207.
- [8] Golovina, E.A., A. N. Tikhonov and F. A. Hoekstra. *Plant Physiology*. **1997**, 114:383-389.
- [9] Gunckel, J.E. and A.H. Sparrow. Ionizing radiation: Biochemical, Physiological and Morphological aspects of their effects on plants. In: External Factors Affecting Growth and Development, Georg, M. Ed.) Springer-Verlag, Berlin, **1961**, pp. 555-611.
- [10] ISTA. International Rules for Seed Testing. Proceeding International Seed Test Association. 1985, 13: 255-299.
- [11] Kiong, A., A., Ling Pick, S.H. Grace Lai and A.R. Harun. *American-Eurasian journal of Sustainable Agriculture*. **2008**, 2(2):135-149.
- [12] Kovacs, E. and A. Keresztes. *Micron*. **2002**, 33: 199-210.
- [13] Kumagai, J., H. Katoh, T. Kumada, A. Tano, S.Tano and T. Miyazakit. *Radiation Physics and Chemistry*. **2000**, 57: 75-83.
- [14] Kumari, R. and Y. Singh. *Neo Botanica* **1996**, 4(1): 25-29.
- [15] Parera, C.A., D. T. Cantliffe, D.R. McCarty and L.C. Hannah. *Journal of American Society of Horticultural Science*. **1996**, 121: 1069-1075.

- [16] Radhadevi, D.S. and N.K. Nayar. *Geobios*, **1996**, 23 (2-3): 88-93.
- [17] Sanjeev, S., A. K. Richharia and A. K. Joshi. *Indian journal of genetics*. **1998**, 58:455-463.
- [18] Sareen, S. and A. K. Koul. *Indian journal of genetics*. **1999**, 59: 337-344.
- [19] Sarwgi, A.K. and D.K. Soni. *Advances in plant Science*. **1993**, 6: 24-33.
- [20] Stoeva, N., Z. Zlatev and Z. Bineva. *Journal of environmental protection and ecology*. **2001**, 2:304-308
- [21] Taylor, F.G. *Radiation Botony*. **1968**, 8: 67-70.
- [22] Thanki, R. J., K.C. Patel and R.D. Patel. *Earth Environmental Science*. **2007**, 10: 211-214.
- [23] Thapa, C.B. *Botanica Orientalis Journal of Plant science*. **1999**, Pp.120-121.
- [24] Wang, Z. and R.L. You. *Environmental and Experimental Botany*. **2000**, 43: 219-225.