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DETERMINATION OF LD_{50}, SEED GERMINATION, MORPHOLOGICAL MUTANTS FOR INDUCED MUTAGENESIS THROUGH GAMMA RADIATION *ANDROGRAPHIS PANICULATA* (BURM. F.) NEES

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Abstract

The present study was conducted during 2018th June –August to determine the lethality dose LD_{50} of physical mutagens on *A. paniculata*. The Gamma radiation dose ranging from 5,10,15, 20, 25, 30, 35, 40, 45 and 50KR. The objective of this study the LD_{50} observed in Gamma rays 25 KR. The current study investigated the effect of gamma rays germination percentage, days to first flower, leaf breadth, shoot length, fresh weight of root, dry weight of root and morphological mutants were also observed in *A. paniculata*. The study revealed that gamma radiation significantly affected on germination percentage. However other parameters show declining tendency with increasing doses of gamma rays.

Key words: *Andrographis paniculata*, Gamma rays, $LD_{50,}$ Morphological mutants.

Introduction

A. paniculata a well known herb belonging to the family Acanthaceae is grown widely in tropical areas of Asia like India, Pakistan and Sri Lanka and commonly known as "Kalmegh" reported Vijaykumar. K *et al.*, 2007. The plant grows well in all types of soil which explains its wide distribution. It grows in soil types where almost no other plant can be cultivated, particularly 'Serpentine soil', which is relatively high in metals such as aluminum, copper and zinc Samantaray S *et al.*, 2001. The best harvesting period of *A. paniculata* leaves Anonymous, 2000. It is at 3-5 months old or at 50% blossom as a result of which the highest quantity of active lactone compound was found followed by final harvesting after next 2-3 months, with an yield of 2-3 ton per hectare (fresh weight) or 0.5-1 tons per hectare (dried weight). The plant is a predominant ingredient in at least 26 Ayurvedic formulations and in several polyherbal preparations of Indian systems of medicine used as a hepatoprotective agent. MishraSk, *et al.*, 2007. Mutation breeding has been widely used for the improvement of plant characters in various crops. It is a powerful and effective tool in the hands of plant breeders especially for autogamous crops having slender genetic base (Micke, 1988). The role of mutation breeding increases the genetic variability for the desired traits in various crop plants and have been proved beyond doubt by a number of scientists (Tah, 2006; Adamu and Aliyu, 2007; Khan and Goyal, 2009; Kozgar *et al.*, 2011; Mostafa, 2011). Several factors such as properties of mutagens, duration of treatment, Ph-pre and post treatment, temperature and oxygen concentration etc. influence the effect of mutagens.

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Physical Mutagen (Gamma radiation)

Gamma radiation can be useful for the alteration of physiological characters (Kiong *et al.*, 2008). The biological effect of gamma-rays is based on the interact with atoms or molecules in the cell. The radicals induced can damage or modify important components of plant cells and have been reported to affect the seed germination, morphology, anatomy, physiology, biochemical and photochemical characters of plants differentially depending upon the level of irradiation. The effects include changes in cellular structure and metabolism of the plants. Irradiation it is on considered that among the physical mutagen, gamma rays stand first in its effectiveness in the induction of mutations. Kharkwal, 2012. reported that mutation breeding has contributed significantly to the global agriculture by

producing more than 3000 mutant varieties with enhanced production and productivity in about 175 plant species. 2012. These effects include changes in the cellular structure and metabolism of the plants e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the anti- oxidative system and accumulation of phenolic compounds (Kim *et al.,* 13, 2004; Kovacs and Keresztes14, 2002; Wi *et al.,* 15, 2005).

Materials and Methods

Seed collection and Germination test in Petri dish

The conduct experiment was conducted during January to February 2018. The dry and dormant seeds variety CIM Megha Collected in the month of December 2017 from the Research form of Central Institute of Medicinal and Aromatic Plants Resource Centre, Hyderabad, India. The *A. paniculata* seeds were treated with Gamma rays, treatment were packed in moist germination paper were selected for each treatment in the gamma chamber at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50KR dose of gamma rays in CO_{60} source. Eleven petridishes in which 100 seeds per treatment and control were planted and germination percentage for each treatment compared to control were subsequently determined. The gamma irradiation was carried out at Indira Gandhi Centre for Aromatic Research (IGCAR) at Kalpakkam. After the finishing point of the treatment the treated seeds were sown directly in the field along with their relevant controls to raise the M_1 Generation. In field the morphological and growth parameters observed such as days to germination, germination percentage, survival percentage, shoot length /plant, root length /plant, number of branches / plant, number of leaves /plant, days to first flower and morphological mutants as chlorophyll mutants observed on 30days and recorded.

Table 1: Determination of LD_{50} for Gamma rays on *Andrographis paniculata.*

Treatment of Dose. Gamma rays	Seed germination Percentage(%)	Percent over control	Percent of reduction Over Control
0.0	94.00	100.00	0.00
05	86.07	92.57	7.41
10	75.08	89.34	10.66
15	62.33	77.60	22.04
20	56.90	65.41	34.55
25	50.12	63.85	36.34
30	43.53	57.86	42.14
35	35.67	43.24	56.76
40	28.00	36.45	63.55
45	18.43	25.08	72.92
50	09.03	11.93	90.07

Methodology followed to grow M1 generation

A. paniculata seeds harvested from individual M1 plants were grown as generation in factorial randomized block design (FRBD). 100 seedlings were sown in each plot at spacing 10×15 as well as for observation parameters such as leaf length, root length, days to first flower, plant height, fresh weight of root, dry weight of root. All recommended package of practices were followed during growth period of the crop. Observation on LD_{50} value were recorded on randomly selected plants from each plot each treatment.

Statistical Methods.

The results were calculated using National Pulses Research Centre (NPRC).

Results and Discussion

Seed germination and Seedling survival

Generally, gradual reduction in germination percentage observed from lower to higher dosage of gamma ray treatments. 5KR treated seeds showed the highest germination percentage was observed in lower dosage and lowest germination was observed in higher doses.25KR of gamma rays treated seeds showed on 50 per cent of germination was observed in (50.12) per cent over control (63.85) per cent of *A. paniculata* as given in (Table- 1).In respect of gamma rays treatment, there was a percentage- ate reduction in germination of *A. paniculata* with increased doses of gamma rays and similar result were also reported in black-gram (Ramaswamy 16, 1973), soybean (Bal- akrishnan17, 1991), rice (Ramesh *et al.,* 18, 2002) and Cowpea (Gnanamurthy *et al.,* 19, 2012). The seeds treated with higher gamma radiation i.e. 40KR, 45KR, 50KR show toxic effect on germination. The irradiated seeds of 40KR,

Table 2: Effect of induced mutagenesis on seed germination and seedling survival of M1 generation.

TreatmentDose	Seed germination (%) 15th day			Seedling survival (%) 30th day		
ofGamma rays	Mean ± SE	Percent overcontrol	Per cent reduction over control	Mean ± SE	Percent over control	Percent reduction over control
Control	91.88±0.75	100.0	-	88.55±0.92	100.00	-
20kr	54.77±1.06	56.00	44	54.55±0.76	65.41	36.34
25kr	45.33±1.01	50.00	50	46.22±0.59	57.86	42.14
30kr	35.11±080	43.00	57	37.33±0.91	43.24	56.76

Table 3: Effect of gamma rays on Morphological characters of *Andrographis paniculata* (Burm. f.)in M1 generation.

Treatment dose ofGamma rays	Days to first flower (days)	Plant height (cm)	Number of leaves per plant	Shoot length per plant (gm)	Root length per plant (cm)
Control	112.3±0.85	11.07±0.07	62.2±0.71	16.11±0.15	7.56±0.24
20kr	152.1±1.77	10.07±0.12	16.37±1.01	10.62±0.07	5.24±0.07
25kr	164.2±0.84	5.98± 0.15	14.88±0.85	9.53±0.08	5.28±0.05
30kr	172.3±0.59	4.74± 0.14	13±0.57	7.77±0.42	4.54±0.09

45KR, 50KR are germinated.

Spectrum of Chlorophyll mutations

The spectrum of chlorophyll mutations obtained in the present study induced different types, *viz.*, chlorine, xandha and viridis was observed. These types mutations observed in maize M1 generation slightly.

Fig. 1: Seedling Height Of *Andrographis Paniculata* in Gamma Rays Treatment.

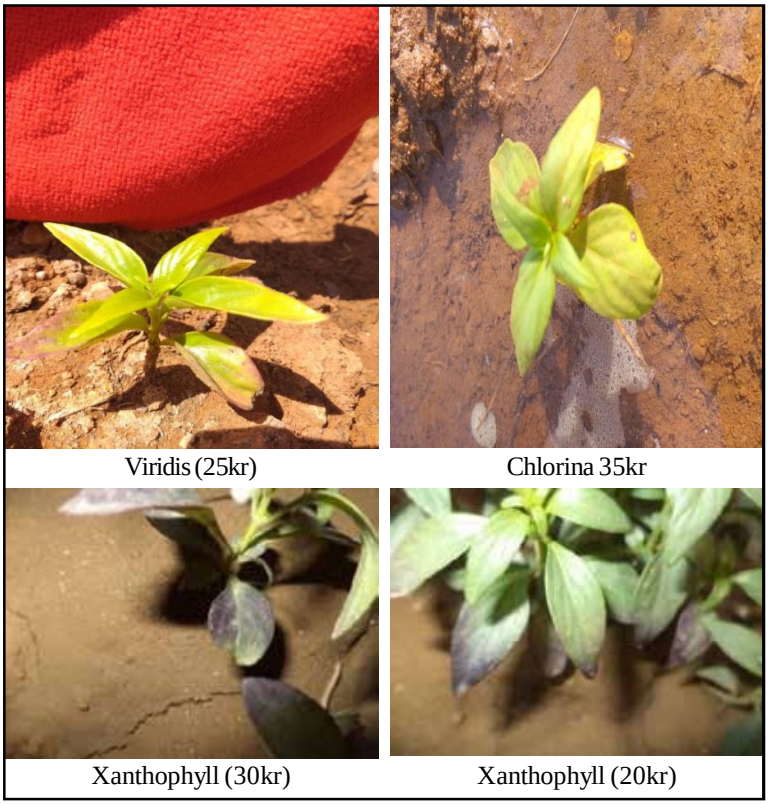

Fig. 2: Morphological mutants of Andrographis paniculata in Gamma rays treatment (30day).

Albino

These seedlings were characterized by their dull white color and were devoid of chlorophyll, carotenoide and other pigments. Albino seedlings are smaller in height and survive to a maximum of 20 days after germination and then die.

Viridis

The seedlings are dark green in the early stages of development and turn normal green in the later stages. The mutants produce normal looking flowers and also set seeds.

Xantha

Colors of the mutants vary from deep yellow to yellowish white. Growth of mutants is retarded and most of them die within 17 to 20 days after emergence.

Chlorina

Normally chlorine mutants do not survive. These mutant seedlings have light ellowish/ yellowish green leaves and culm with yellowish cobs. The mutants breed true for the altered characters.

Days to germination of *A. paniculata* were significantly delayed by higher doses of gamma irradiation. In the present experiment percentage of germination also show decreasing trend when compared to the control. The seedling survival reduced with increase in doses of gamma rays. Similar results have been obtained in soybean (Yamashita and Kawai20, 1987; Bal- akrishnan17, 1991). At 20KR very low survivability are seen. The irradiated seeds 30KR, 40KR, 50KR, 60KR and 70KR are not survived, after germination Cotyledonary leaves are seen but, they get die within 1-2 week.

In the present investigation , Shoot length plant, Root length per plant, no of branches per plant, no of leaves per plant (Table 3). Showed declining trend with increasing doses of gamma rays compared to the Control and days to first flower increases with increasing doses of

gamma rays.

Days to first flower of *A. paniculata.*

The minimum days to first flower was observed in lower dose of gamma rays. While the maximum days flowering stage observed in higher dosage of gamma rays. A minimum day was observed in control (112.3±0.85) and maximum days was observed in (172.3±0.59) 30KR Physical mutagen gamma ray treatments.

Plant height (cm) *A. paniculata*

In general, all the mutagenic treatments caused a reduction in the plant height compared with control. The effect of gamma radiation, there was an increase in the plant height at lower doses of gamma rays. A maximum plant height was observed in Control (11.07±0.07). The minimum plant height was observed in (4.74 ± 0.14) at 35KR of gamma rays. The plant height increased in lower dose of gamma ray treatments, while plant height decreased with higher dose of gamma rays treatment.

Number of leaves per Plant

In *A. paniculata* leaves more level of chemical compound present. A gradual reduction of leaves per plant was observed in high to lower dose of gamma ray treatment, when compared with control. The maximum number of leaves was observed in (62.2±0.70) at in Control. The minimum number of leaves was observed in 30 kr of gamma rays treatments (13±0.57).

Shoot length per plant

In *A. paniculata* Shoot more level of chemical compound such as secondary metabolites also present. A gradual reduction of shoot per plant was observed in high to lower dose of gamma ray treatment, when compared with Control. The Optimum number of shoot length was observed in Control (16.11±0.15) at lower doses. The minimum number of shoot length was observed in 30 KR of gamma rays treatments (7.73±0.42).

Root length per plant (gm) (Fresh weight)

The root length per plant was measured in cm. There was a gradual decrease in root length when the dose of gamma rays increases. The maximum yield was observed in (7.56±0.24). The minimum yield was observed in 50KR (4.54±0.09) as given in (Table 3).

Conclusion

Andrographis paniculata subjected to different doses of gamma irradiation (05KR-50KR) and various morphological mutants as chlorophyll mutants and growth parameters were recorded. The effect of gamma irradiation the seed germination, seedling survival, days to first flower, plant height, number of branches per plant, number of leaves per plant, number of berries per plant, seed yield per plant (gm) and root yield per plant (fresh weight (gm) were gradually decreased from lower to higher doses of gamma rays treatment. Then particular dose was gradually decreased especially 20, 25 and 30KR of gamma rays. The highest germination percentage and seedling survival were observed at lower dose of gamma rays when compared to other doses. The LD_{50} value for 50 percent reduction of seed germination, (LD_{50} lethal dosage), seedling survival was observed in 25KR of gamma rays treatments.

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Abbreviation

Gy- Gamma Rays

KR- Kilo Radiation

IGCAR- Indira Gandhi Centre for Atomic Research.

LD- Lethality Dosage

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