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PRELIMINARY INVESTIGATION EFFECTS OF PHYSICAL MUTAGEN (X-RAYS) ON GERMINATION AND MORPHOLOGICAL PARAMETERS OF *DENNETTIA TRIPETALA* BAK.F AT EARLY GROWTH

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ABSTRACT

Dennettia tripetala is a slow growing plant species. It has many medicinal uses. Fresh fruits of D. tripetala were collected from a mother tree. The seeds were exposed to different few x-ray doses using medical x-ray device. The same soil medium was used to raise the seedlings. Experiment was arranged in a Completely Randomized Design inside a greenhouse with five replicates. It was carried out for the period of thirteen months. Data on germination percent and morphological parameters such as root length, seedling height, number of leaf (leaf production), leaf area, basal diameter were taken for the statistical analysis. High germination percent was induced by the different doses (1,2,4,6 and 8 MGy) of x-rays. Germination occurred in control seeds and seeds treated with 6 MGy after 19 days of sowing, but it delayed to twenty-three days in other treatments (1, 2, 4 and 8 MGy). The highest mean germination (93.3 %) was recorded among the treated seeds. The least mean germination (72.3 %) was recorded in control. In this preliminary study, it was deduced that the response of each morphological parameter to the doses were varied. Some parameters were stimulated by some doses and some parameters were not stimulated by some doses of X-rays.

Keyword: *Dennettia tripetala*, X-irradiation, Germination, Morphological parameters.

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INTRODUCTION

In Nigeria, many indigenous plants are used as spices, food or medicine. A great number of these plants are traditionally noted for their medicinal and pesticide properties. *Dennettia tripetala* (commonly known as peper fruit) is widely consumed by the inhabitant of West Africa due to its distinctive spicy taste. It is also used traditionally as a remedy for cough, fever, toothache, diabetes and nausea (Sylvia, 2015). The roots, barks, leaves and fruits are the parts that are usually used medicinally because of the following medicinal properties: antioxidant, anti-inflammatory, antibacterial, anti-fungal, antiviral, analgesic, hypoglycemic, mildly laxative, anthelmintic, stimulant, anti-emetic (Aderogba *et al*., 2011, Ogbonna *et al*., 2013, Oyemitan *et al*., 2008). *D. tripetala* grows as a small woody shrub. It can grow to a height of 12-15 m and have an average girth of 0.6 m. The wood is white in colour and soft. The leaves are 3-6 inches long and 1.5-2.5 inches broad. They are elliptic in shape. The fruits are mainly made up of the seeds and a bit of hard spicy flesh. The fruits and seeds are edible and are consumed because of the spicy nature. The wood is used as fuel. The plant usually produces fruit between the months of March and May (Achinewhu *et al.,* 1995). Phytochemical screening of the ethanoic extract of *D. tripetala* revealed the presence of tannins, alkaloids, steroids, flavonoids, cardiac glycosides, saponins, and terpenoids (Elekwa, *et al.*, 2011). These constituents provide a scientific basis for the use of *D. tripetala* in traditional medicine.

Apart from the medicinal uses, this plant can also be put to use in the field of biotechnology as scientific evidence abounds for the potential use of this plant in meat preservation (Sylvia, 2015). Despite the usefulness of *D. tripetala*, much attention has not been given to its germination and growth. According to IUCN (2006), *D. tripetala* is one of the threatened species in Nigeria and it is known to be slow growing. One of the limitations in available germplasm of any plant is poor growth and yield (Dhillon, *et al.,* 2014). Interactions between various types of electromagnetic radiation and living organism have attracted the attention of scientist since the introduction of technical devices that operate using electro-magnetic waves (WHO, 2014). Physical mutagen includes various kind of radiation, such as X-rays, gamma rays, beta rays, and neutrons are common ionizing radiations used for inducing mutation (Dipak, 2009). Ionizing radiation has enough energy so that during an interaction with an atom, it can remove tightly bound electrons from the orbit of an atom causing the atom to become charged or ionized. The effect of ionizing radiation on any living organism depends on the amount and the rate of ionizing radiation which was absorbed, the type of ionizing radiation which was absorbed, and the type and number of cells affected (Mozumder and Hatano, 2004; Balashov and Gil, 1997). Dwimahyani and Ishak (2004) induced mutations in *Jatropha curcas* for improvement of agronomic characters with irradiation dose of 10 Gy and identified mutant plant with early maturity, 100 seeds weight (30 % over control) and better branch growth. Mutagenic plants and their role on human food systems is less well known, with one Journalist writing "Though poorly known radiation breeding has produced thousands of world's crops including varieties of rice, wheat, barley, pear, peas, cotton, pepper mint, sunflowers, peanuts, grape fruit, sesame, bananas, cassava and sorghum" (Broad, 2006). During the period 1930 – 2004, gamma rays were employed to develop 64 % of the radiation induced mutant varieties followed by x-rays 22 % (Ahloowali, 2004). The behaviour of numerous plant species under exposure to gamma radiation have been extensively studied (Irfaq and Nawab, 2003, Tuncer and Yanmaz, 2011). In comparison, studies on the effect of x-rays are relatively limited (Norah and Jameel, 2012). In order to improve poor growth of *D. tripetala*, modulating techniques should be engaged. Mutation breeding which is an efficient and much cheaper method than others can play an important role in crop improvement either directly or by supplementing the conventional breeding (Dhillon *et al.,* 2014). Therefore, in the present investigation, mutation induction in *D. tripetala* using x-irradiation was undertaken to examine its effect on the germination and early growth characters.

MATERIALS AND METHODS

Study Area

The study was carried out at the Forestry Research Institute of Nigeria Humid Research Station, Umuahia, Nigeria. The station lies on latitude 5^0 30\` 48\` N to 5^0 35\` 15'' N and longitude $7^0$ 31\` 03'' E to 7^0 31\` 32'' E along Umuahia/Ikot- ekpene road, Umuahia, at an altitude of over 122 m above sea level. The rainfall pattern is bimodal with peaks around June to July and September to October. Annual rainfall is 2238 mm. Minimum and maximum temperature **are** 23 ^{0}C and 3 ^{0}C respectively. Relative humidity is 86.4%. The vegetation has been described as high forest and soil type is sandy loam (Okeke *et al*., 1999; Joseph and Joy, 2015).

Seed collection and preparation

In this study, mature fruits of *D. tripetala* were collected from a mother tree at home garden Umuogele, Isiala-ngwa south, Eastern part of Nigeria in the month of April 2020. Extraction of seeds was carried out a day after plucking the fruits and the seeds were air dried in the laboratory with ambient temperature for 2 days. Seeds of *D. tripetala* were irradiated with different doses of x-rays namely 1, 2, 4, 6 and 8 MGy, using medical X-ray device. Whereas the untreated seeds *were* used as control.

Experimental Design

Each treatment was replicated five times with 15 seeds in each replicate and arranged in Completely Randomized Design with 75 seeds for each treatment. All the seeds were sowed on germination boxes containing sterilized river sand for 31 days in a green house. About 450 seeds were used for the entire experiment. Observation on seed germination were made and recorded regularly. At the age of one month, 15 seedlings were removed from each treatment into polythene-pots of 8x17 cm size, containing a mixture of well sterilized loam soil and sand soil of the same proportion. A month after transplanting the seedlings, morphological parameters were started taking. At the end of 13 months, biomass assessment was carried out. The yield from the study was assessed in form of biomass production. The soil in all experimental units was gently loosed and seedlings were removed. The roots of the seedlings were washed in order to remove the soil particles that attached to them and their fresh weights were then taken. The samples were oven dried at 88 ^{0}C for 24 hours in Carbolite oven. Samples from each treatment were weighed using MP "Citizen Electronic" Weighing Balance with 0.01g sensitivity. The data were subjected to analysis of variance and standard error.

RESULTS

Mutation breeding is another form of traditional breeding method. It involves the treatment of viable seeds or plant part with adequate dosage of ionizing radiation such as X-rays or gamma rays .to generate mutants (Wiel *et al*., 2010). Such treatments can result in single base-pair changes, which could either be substitution, insertion or deletion

The results of this study, that is, the effect of x-rays on the seeds germination and morphological parameter of *D. tripetala* were presented in Table 1 and Figures 1-6. The *D. tripetala* seeds responded variously to ionizing radiation (x-ray). The germination trend was between 72.3 % and 93.3 % across the treated seeds and control. The highest mean germination (93.3 %) was obtained from the seeds exposed to 6 MGy followed by 90.3% which obtained from the seeds exposed to 1 MGy. The least germination percent was recorded from untreated seeds (control) with germination mean of 72.3% (Table 1). Ionizing radiation from x-rays significantly influenced the germination of the seeds at probability level ($P \leq 0.05$). The mutagen used showed no significant effect on the root length of *D. tripetala.* The longest mean root length was recorded in seeds treated with 4 MGy and 6 MGy with the mean root length of 13.08 cm and 13.04 cm respectively. The least mean root length was observed from the seeds treated with 8 MGy with the mean root length of 10.73 cm. (Table1).

Table1: Effect of x-irradiation on percent germination and growth parameters of *D. tripetala*

Treatment	Germination %	Root length (cm)	Plant height (cm)	Leaf production	Leaf area (cm^2)	Basal Diameter (mm)	Fresh weight (g)	Dry weight (g)
1MGy	90.37±4.970	11.205±0.745	9.396±0.745	5.375±0.625	11.605±2.419	7.925±0.622	1.738±0.160	0.792±0.110
2 MGy	81.7±3.850	12.046±0.686	8.016±0.686	5.000±0.567	9.147±1.394	9.325±0.275	2.827±0.11	1.130±0.125
4 MGy	86.7±2.360	13.081±0.587	7.730±0.587	5.500±0.845	8.396±1.059	6.787±0.123	2.731±0.338	1.091±0.135
6 MGy	93.3±6.140	13.047±0.509	9.875±0.509	5.500±0.567	8.356±1.516	7.350±0.298	2.302±0.212	0.920±0.085
8 MGy	90.0±4.97	10.733±0.604	8.049±0.604	6.125±0.693	10.171±1.836	7.650±0.423	2.456±0.345	0.982±0.138
Control	72.3±7.06	12.253±0.619	9.697±0.619	5.375±0.625	12.831±2.018	8.487±0.665	2.392±0.428	1.038±0.219
P≤0.05	**7.3800**	**3.5103**	**2.3400**	**0.3070**	**1.0670**	**4.0200**	**1.5270**	**0.7640**

Key MGy = X-ray dose

The mean seedling height for the ionizing radiation were between 7.730 and 9.875 cm with the highest mean height obtained from seedlings germinated from the seeds exposed to 6 MGy with mean of 9.875cm while 4 MGy gave the least mean height (7.730cm). There was no significant difference ($P \leq 0.05$) in the height growth of the seedlings germinated from irradiated seeds of *D. tripetala (*Table, 1 Figure 1*).*

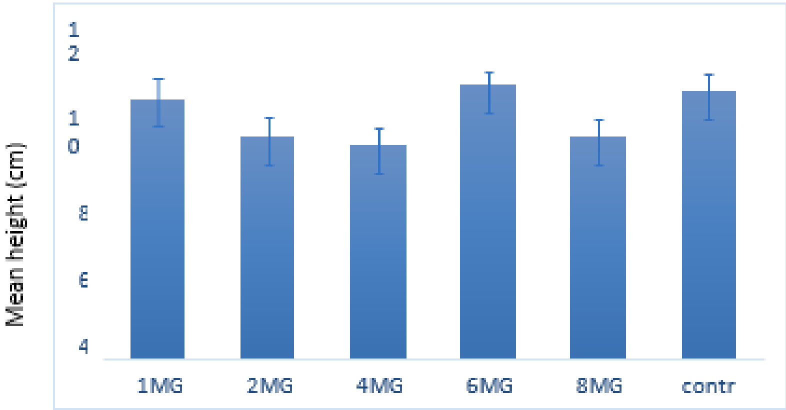

Figure1: Effect of X-irradiation on height growth of *D. tripetala* seedlings.

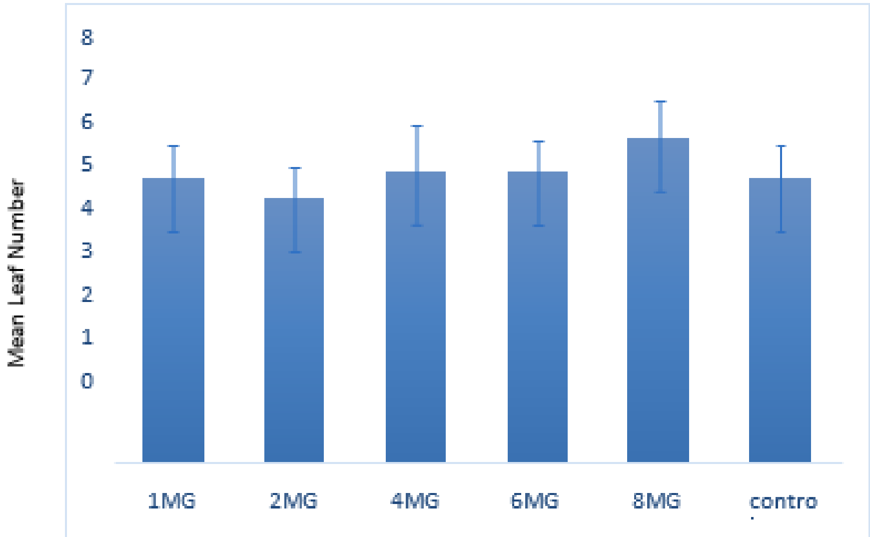

Figure 2: Effect of X-irradiation on leaf production of *D. tripetala* seedlings.

The mean number of leaves produced varied between 5.00 and 6.12 with the highest mean leaf production (6.12) recorded from the seedlings germinated from the seeds treated with 8 MGy dose of x-ray (Table 1, Figure2). This was followed by 1MGy and control which gave mean leaf production of 5.37. At the probability level of $P \leq 0.05$ there was no significant difference from the analysis of variance carried out.

Effect of x-irradiation on the seeds of *D. tripetala* before sowing, not significantly influenced leaf expansion of the seedlings. The largest leaf area was recorded from the control with leaf area of 12.83 cm^2. This was followed by seedlings germinated from the seeds exposed to 1 MGy of x-exposure. The least leaf expansion was recorded in the seedlings germinated from the seeds treated with 4 MGy (Table 1, Figure 3).

The mean basal diameter growth ranged from 6.78 to 9.32 mm (Table 1, figure 4). The highest mean diameter (9.32 mm) was recorded in the seeds exposed to 2 MGy and the least diameter growth (6.78 mm) was recorded from the seedlings germinated from seeds exposed to 4 MGy. Analysis of variance showed that x-ray had significant influence on mean diameter growth of the seedlings ($p \leq 0.05$).

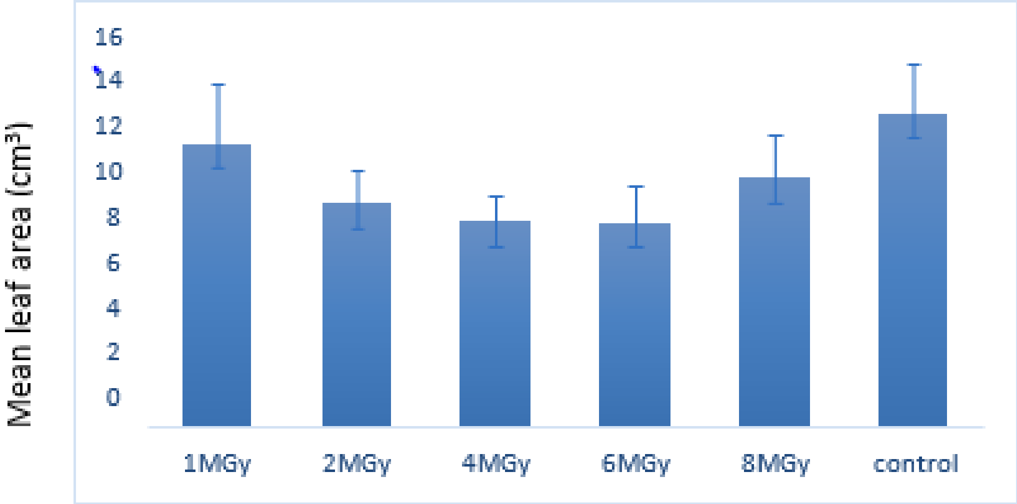

Figure 3: Effect of X-irradiation on leaf area of *D. tripetala* seedlings

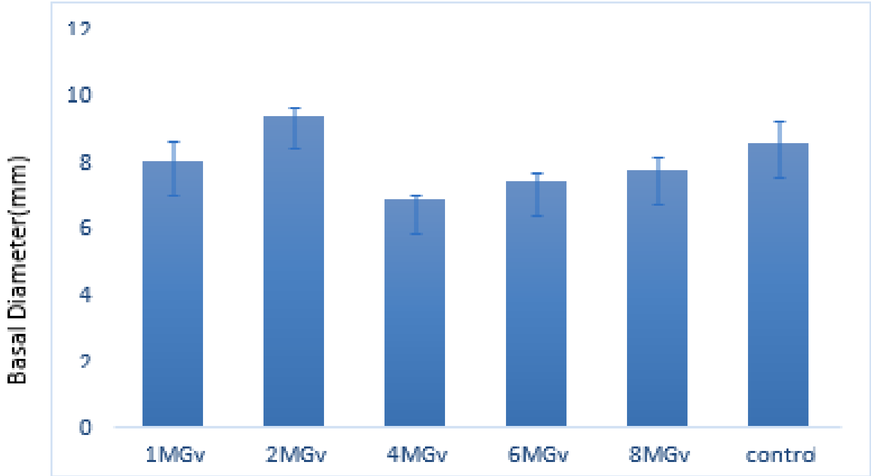

Figure 4: Effect of X-irradiation on basal diameter of *D. tripetala* seedlings

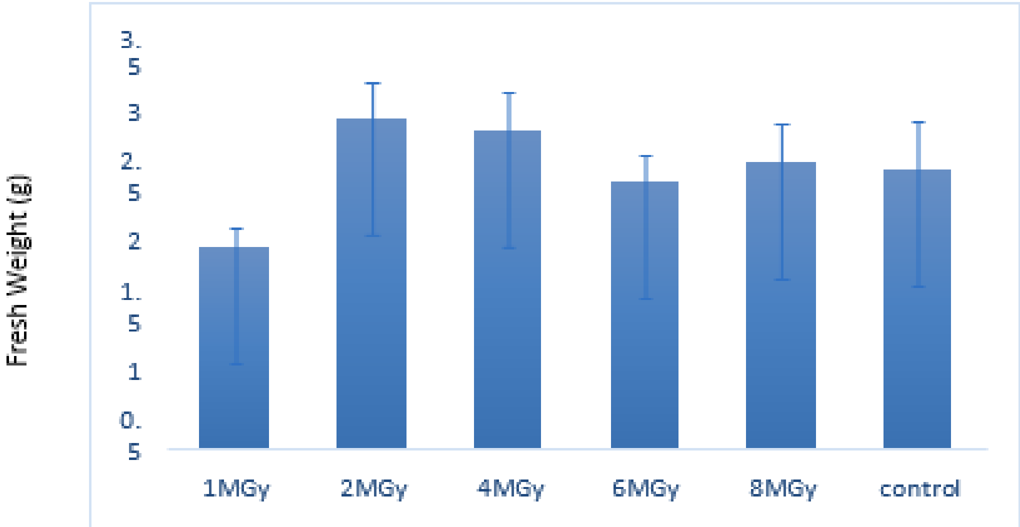

Figure 5: Effect of X-irradiation on fresh weight of *D. tripetala* seedlings

The result of ionizing radiation (x-ray) on the fresh weight of the seedling of D. tripetala germinated from seeds exposed to different doses of x-ray showed no significant effect on the fresh weight of the seedlings ($p \leq 0.05$) The maximum weight was recorded from the seeds exposed to 2 MGy with mean of 2.82 g followed by 4 MGy which produced seedlings with 2.73 g fresh weight. The least fresh weight was recorded in seedlings emanated from seeds exposed to 1 MGy with weight mean of 1.73 g (Table 1, Figure 5).

The yield obtained inform of dry weight from the seedlings were not significant. The mean dry weights were between 0.79 and 1.13g. The highest dry weight was recorded from seedlings germinated from seeds exposed to 2 MGy while 1 MGy gave the least dry weight of 0.79 g. The dose 4 MGy also gave yield that closer to that of 2MGy (Table 1, Figure 6).

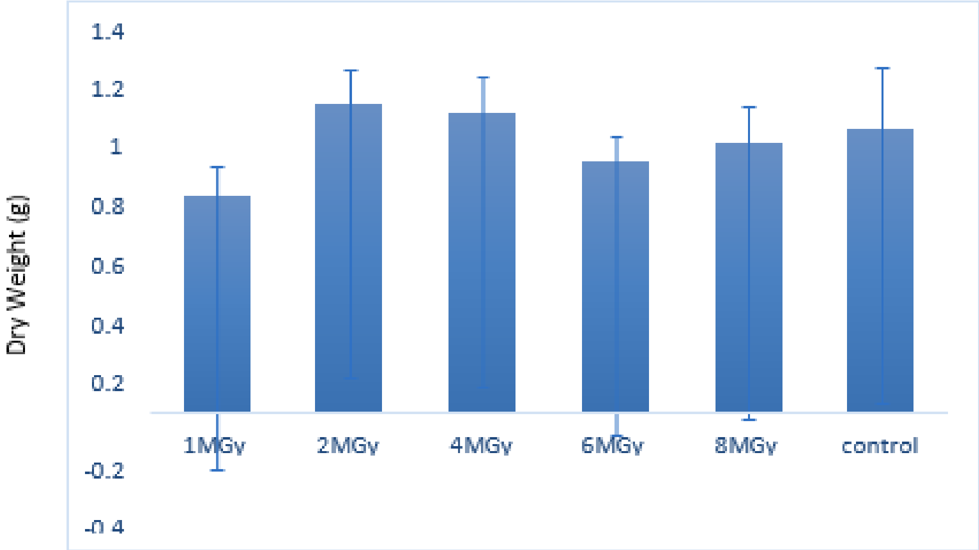

Figure 6: Effect of X-irradiation dry weight of *D. tripetala* seedlings

DISCUSSION

Mutation breeding is another form of traditional breeding method. It involves the treatment of viable seeds or plant part with adequate dosage of ionizing radiation such as X-rays or gamma rays .to generate mutants (Wiel *et al*., 2010). Such treatments can result in single base-pair changes, which could either be substitution, insertion or deletion (Shittu and Mgbeze, 2013)

From this study, seed germination in the untreated seeds and seeds treated with 6 Mgy started nineteen days of sowing, but in other treatment, (1, 2, 4 and 8 Mgy) germination was delayed till twenty-three days after sowing. Significant variation was observed in seed germination percent of both irradiated and untreated seeds. The least germination percent recorded among the treated seeds was 81.7% and untreated seeds gave 72.3% germination. There were stimulation effects from x-rays. The reasons for these stimulations may be due to the accelerations in cell division rates as well as activation of auxins according to Gunkel and Sparrow, (1991). Variation (reduction and stimulation) in percentage germination among the treated seeds might have been due to the effect of mutagen on meristematic tissues of the seed (Dhillon, *et al.,* 2014). The results obtained among the treatments is in line with the observation of Mozumder *et al*., 2004, Balashov and Gil (1997) that "the effect of ionizing radiation on any living organism depend, on the amount and the rate of ionizing radiation which was absorbed, the type of ionizing radiation which was absorbed, and the type and number of cells affected". The results obtained in terms of seed germination is also in accordance with the earlier reports on *Acacia leucophloea*, *Albizia lebbeck and Zizyphus mauritiana.* (Selvaraja and Raja, 2010). Profound effect of x-rays was observed on the root length of *D. tripetala.* The highest dose (8 Mgy) gave the least root length (10.73 cm). The stunted effect may due to the negative effect of x-rays on the cells responsible for the growth of the radicle. Reduced growth in the root length of the seedlings may also be attributed to the increase in destruction on growth inhibitors, drop in the auxin level or inhibition of auxin synthesis as reported by Roychodhury and Tah (2011) and Mostafa *et al.* (2014). The root length (12.04 cm) obtained from 2 Mgy almost the same with that of control (12.25 cm). This simply means that the mutation which occurred by 2 Mgy in the seeds was minute (point mutation) as the substitution of a single nucleotide pair in the DNA occurs (Gurcharan, 2004). Similar results also observed from 4 Mgy and 6 Mgy with mean root length 13.08 and 13.04 cm respectively, which were the highest root length. The stimulatory effect of 4 Mgy and 6 Mgy may be attributed to the increase in the rate of cell division or cell elongation as reported by Joshi *et al.,* (2011). Retardation results were observed on the shoot growth of *D. tripetale.* Most of the doses investigated gave stunted effect to the seedlings except 6 Mgy dose which produced seedlings with 0.18 cm difference from untreated seeds. This stunted growth may be due to physiological damage resulted in the alteration from normal to dwarf growth (El-Maksoud and El-Mahrouk, 1993). Joshi *et al.* (2011) explained the dwarfed growth to auxin destruction, changes in biochemical disturbance. The impact of x-rays on the basal diameter of *D. tripetala* was significant. The result showed that 2 Mgy dose have a stimulatory effect on basal diameter (9.32 mm) whereas other doses had retardation effects on the diameter The favourable effect produced by dose 2 Mgy is in line with findings of previous studies of Akshatta *et al.* (2013) who observed stimulatory effect of ionizing radiation on the stem diameter of *Terminalia arjuna* using Gamma rays. The results of other four doses (1, 4, 6, and 8 Mgy) were not stimulatory and similar result was obtained by El-Torky (1992) that all treatments decreased stem diameter, fresh and dry weight of vegetative growth of *Euonymus japonicus.* This reduction on diameter growth by 1, 4, 6 and 8 Mgy might be attributed to the physiological damage to radial cells which responsible for the growth of diameter. The leaf production was only influenced positively by 8 Mgy which gave positive result over other doses and control. The auxin which responsible for leaf production might be on increase as a result of ionizing impact from 8 Mgy. The unfavorable effect from other four doses may be attributed to the drop in the auxin level or inhibition of auxin synthesis as reported by Roychodhury and Tah (2011); Mostafa *et al.* (2014). None of the x-ray's doses examined had better performance than the control in terms of leaf area. This simply means that the radial cells and other cells responsible for leaf elongation and expansion were affected negatively by x-rays. In fresh weight (biomass) observation, it was only 2 and 4 Mgy that produced stimulatory effect over untreated seeds. This is in line with the report made by Jamil and Khan (2002), Moussa (2011) and Akshatha (2013). While the other three doses (1, 6 and 8 Mgy) gave rise to the seedlings within the same range of fresh weight. Dose 1 and 6 Mgy indeed caused reduction in fresh weight. Similar report was made by Tah (2011). Roy *et al.* (1972) reported reduced synthesis of DNA, RNA and protein was detected in x-radiation of cotyledon of stone pine (*Pinuspinea).* The dry biomass of *D. tripetala* investigated gave results in similar trend with that of fresh weight. In broad bean (*Vicia faba)* transpiration rate and stomata opening were affected following irradiation resulting in decrease fresh and dry weights as well as water content of root and leaves (Roy, 1974).

Similarly, x-rays decreased fresh and dry weight and affected nucleic acid and protein metabolism in barley (Joshi and Ledoux, 1970). The doses which gave result lower than that of control in both fresh and dry weights might have caused reduction in stomata efficiency of the leaves. A study by Francis (1934) on wheat seedling showed that high doses of X-rays (563-13560 rad) caused retardation of fresh weight and dry weight production of the growing parts of linear growth of the coleoptiles, leaf, primary root and the lateral roots.

CONCLUSION AND RECOMMENDATIONS

Although small number of different doses were used for this study, some useful results were obtained most especially on germination of the seeds. The result of this study indicates that exposing *D. tripetala*s seeds to X-rays before sowing would affect some morphological characters of seedling positively and it would affect some characters negatively. Further exploration of this technology may prove beneficial for growth strategies of *D. tripetala* and other economic trees.

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