# Influence of X-rays on *Leucaena lecocephala* L

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**Abstract:** *Leucaena leucocephala* is a new introduction into Pakistan, as a fast growing tree with many properties of economic importance. In this it was decided to produce some mutants of the species with the help of X-rays radiations, with the object that, (a) any of the mutants may prove to be more suitable and useful than the original parents and (b) the mutants thus raised may provide breeding material to the future breeders. With this object, young seedlings, moist seeds and dry seeds of the species were subjected to the X-rays treatment. The young seedlings died after the treatment, the moist seeds failed to germinate; in both these cases X-rays were lethal enough to cause the death. However, in the third lot where treatment was given to the dry seeds 61% of the seeds germinated, but only 36% finally survived. The surviving 36% exhibited certain morphological anatomical and physiological variations, indicating occurrence of some degree mutation of their genetic material. If these mutants survived, then, in the year to come more variation will appear in them as they will reach maturity. At this stage, it will be quite premature to speculate about their values of economic importance and usefulness, except that they may probably provide genetic material to any of the interested plant breeders in future.
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## 1. Introduction

Various forms of radiations such as X-rays, ultra-violet radiations, gamma rays, alpha and beta particles exert their influence on the living beings which are exposed to them. This influence may be slight enough to be not easily detectable and also may be quite harmless; or it may be drastic penetrating, noticeable and heavily damaging. The influence of various radiations on the living beings depends on the following factors: (a) Kind of radiation (b) Dose of the radiation (c) Distance and duration of radiation (d) Influence of temperature (e) Physiological and physical state of the organism. Radiation with in a limited range exert no significant influence on the living organism because of the reason that living organisms are on the face of earth since geological ages and are well adopted to the back ground radation which in an inseparable part of our environment. As such, small amount of radations are not at all harmful for the living organisms even though some radioactive elements may enter into the body of the organisms to become a part of protoplasm and emit various radiations. However a higher dose is very harmful to living organism (Kulsum Z 1985).

Work of the Muller (1928), Alterberg (1928) and Stadler (1928) established the fact that ionizing radiation such as X-rays exert their influence on the genetic material. Later on it was also confirmed, that there exist a direct correlation between exposure to X-rays and production of mutant off-spring. Work of Sax (1940) revealed the fact that correlation exists between the dosage of X-rays and degree of chromosomal damage.

Olson and Gilbertin (1928) treated tobacco plants with different radiations such as X-rays, gamma rays etc and concluded that naturally occurring variations in the plants are due to background radio activity of the environment.

Kumar et al (1979) conducted radiation studies with *Linun* sp and reported that in treated plants vegetative characters like plant height and number of frequency branches were not affected as much as the reproductive characters such as number of capsules per plant and number of seeds per capsule and the most affective character was pollen variability.

*Leucaena leucocephala* L. is favoured by some people as a useful plant with many beneficial properties, it is claimed to be a very fast growing well spread ever green species, the foliage and fruits of it are supposed to be excellent food for cattle

## 2. Material and Methods

### 2.1. Seed collection

Four hundred seeds of *L. Lucocephala* were secured from plants grown at Bannu KPK Pakistan. All the seed were mature, dry and normally healthy in appearance. Seeds thus collected were divided into four lots of 100 seeds and in turn each lot was divided into four groups of 25 seeds each (Table-1).

### 2.2. Treatment of the seeds

Seeds of the first three lots were exposed to X-rays at various stages of development. Each lot was divided in a group of 25 seeds each, which were allowed to germinate on a moist filter paper in a petridish. The 4th lot consisted on four groups of 25 seeds each, which were not exposed to X-rays and grown as control.

**Table-1 Showing Treatments**

| Treatments | Group | No. of Seeds |
|---|---|---|
| LOT A Germinating Seedlings. | I | 25 |
| | II | 25 |
| | III | 25 |
| | IV | 25 |
| LOT B Moist Seeds. | I | 25 |
| | II | 25 |
| | III | 25 |
| | IV | 25 |
| LOT C Dry Seeds | I | 25 |
| | II | 25 |
| | III | 25 |
| | IV | 25 |
| LOT D Control | I | 25 |
| | II | 25 |
| | III | 25 |
| | IV | 25 |

### 2.3. Exposure to Radiation

X-rays radiation is used of magnitude 4.5 Kv for 5 mints at 98.5 F.

Dates of showing and exposure:

a) In "LOT A" treatment was devised in seedling stage:
b) In "LOT B" treatment of the moist were devised:
c) In "LOT C" treatment of the dry seeds was devised:
d) In "LOT D" no treatment was devised as they were the controls.

## 3. Results

Categorically results of this investigation can be summarized as:

### 3.1. Germination / Survival Rates in Different Treatments:

In both "LOT A" and "LOT B" where treatment was given to the germinated seedlings and to the moist seeds respectively, the survival rate was zero. After the treatment seedlings "LOT A" died and the seeds of "LOT B" failed to germinate (Table-2). In "LOT C" where dry seeds were treated germination percentage was 61, whereas in "LOT D" (controls) percentage of germination was 98. Another observation of interest was that in "LOT C" not only the germination percentage was low, the pace of germination too was very much retarded, with an only nine percent final survival rate (Table-3).

### 3.2. Morphological and Anatomical Variation:

*As Leucaena leucocephala* is a perennial tree all the morphological and anatomical variations in the treated individuals will be noticeable in some year's time. However, some of the variations are quite evident even in the Juvenile plants which are more or less five months old and three variations are:

#### 3.2.1. Morphological:

**Table-2 Showing germination and Survival Rate**

| Treatment Lot | Group | No of Seeds | No of Seeds Germinated | %age | Final Survival Rate | Remarks |
|---|---|---|---|---|---|---|
| A | I | 25 | 25 | 100 | Zero | Overall survival rate is zero |
| | II | 25 | 25 | 100 | Zero | |
| | III | 25 | 25 | 100 | Zero | |
| | IV | 25 | 25 | 100 | Zero | |
| B | I | 25 | 0 | Zero | Zero | Overall survival rate is zero |
| | II | 25 | 0 | Zero | Zero | |
| | III | 25 | 0 | Zero | Zero | |
| | IV | 25 | 0 | Zero | Zero | |
| C | I | 25 | 16 | 64 | 12% | Germination rate is 61%, While Overall survival rate is 36% which is very slow |
| | II | 25 | 14 | 56 | 08% | |
| | III | 25 | 13 | 52 | 08% | |
| | IV | 25 | 18 | 72 | 08% | |
| D | I | 25 | 24 | 96 | 96% | Germination and survival rate is very high 98% |
| | II | 25 | 25 | 100 | 100% | |
| | III | 25 | 24 | 96 | 96% | |
| | IV | 25 | 25 | 100 | 100% | |

**Table-3 Showing Anatomical Variation**

| Organ | Tissue | Lot A | Lot B | Lot C |
|---|---|---|---|---|
| Root | Epiblema | - | - | Cells weaker and thin |
| | Cortex | - | - | No noticeable difference |
| | Stele | - | - | Cells slightly thicker. |
| Stem | Epidermis | - | - | Cells slightly thicker and smaller. |
| | Cortex | - | - | Cortex proportionately thicker |
| | Endodermis | - | - | Not clear |
| | Stele | - | - | Proportionately smaller |
| Leaf | Epidermis | - | - | Like normal |
| | Mesophyll | - | - | Like normal |
| | Guard cells | - | - | Like normal |

| | |
|---|---|
| Flower | Not yet produced |
| Fruit | Not yet produced |

Treated individuals are noticeably smaller in size with their stems and branches quite thinner than that of controls. However, variations appeared in the leaves, size of the leaves in the treated and control remained the same at the average. The size of epidermal cells and the guard cells also remained unchanged.

**3.2.2. Anatomical:**

The anatomical variations in the stems and root tissue of the treated individuals will become more prominent by the time; the plants will mature in the years to come. However, the noticeable variations even at this stage are: (i) Epiblema of the root and epidermis of the stem appeared to be made of weaker and smaller cells as compared to the controls (Table-3). (ii) Treated plants showed more thicker cortical tissue than that of control ones (Table-3) (iii) Proportionately the amount of cortical tissue appeared to be much different from the untreated (control) in the stem (Table-3). Some of these recorded variations may become prominent as the plants will advance towards maturity, but at the same time some of these variations now traceable may assume normal form and may not be noticeable at the maturity of the plants.

Another interesting point to note is the normal shape and form of the leaves and the various leaf cells and tissue. Shape of the compound leaf and its leaflets was normal, and also no variation of any nature was traceable neither in the epithermal cells and nor in the cells of mesophyll tissues (Table-3).

**3.3. Physiological Variations:**

Some of the physiological variations are also quite evident right from the beginning and they are: delayed germination of the treated seeds, sharp fall in percentage of seed germination, slow pace of seed germination, extremely low final survival rate of the treated individuals and slow rate of growth. Variation regarding flowering habit, viability of male and female gametes, fruit selling and seed formation etc are yet to appear. Moreover, a few more physiological variation such as rate of respiration, photosynthesis and transpiration were not studied because the plants were tender and weak and also very few in number. The prime object was providing them a chance to grow and set in; as such studies of this nature were not conduced to this stage.

**4. Discussions**

The finding of this investigation brings to light some facts, regarding the influence of X–rays on the *Leucaena leucocephala* seeds and on the individuals which originated from them, which can be discussed as:

Influence of the X-rays on the germinated seedlings showed very young seedlings varying in age from 8 to 2 days when subjected to the treatment of X–rays died in a short time, this is a clear indication of the lethal effect of X–rays on the seedlings. Probably the young protoplasm could not bear the ionizing effects of the X-rays or the chromosomal aberrations caused by the X–rays were so deleterious, that is the individuals fail to survive. However, 100 percent morality in (Table-2) the X–rays treated seedlings can attribute to the following reasons:

(i) In the young seedlings, the cells are full of protoplasm in the young protoplasm. The amount of cytoplasm and is always very high with very few vascular bodies and perhaps at this stage, the ionizing effects of X-rays disturbed the metabolic activities thus causing highly damaging effects.

(ii) In the very young seedling the rate of cell division is very high and the chromosomes of actively dividing cells are more liable to become the targets of any sort of radiation such as X- rays.

In the moist seeds too, the X–rays caused very deleterious influence, leading to 100% mortality (Table-2) of the embryos and not a single such seed germinated to produce the seedlings. Perhaps in this too the damage is caused by the (i) ionization of the moist and turgid protoplasm of the cells (ii) the death of embryo, when the seeds are soaked in water usually the resting embryo enter into active phase and the growth of its tip commences perhaps in this case also the tips of embryos which were just awakened from dormancy and in which the chromosomes of the cells were spreading were damaged. Cause of this damage may be high degree of ionization of the nuclear material couple with chromosomes aberrations (iii) when a dormant embryo enters into active phase, the food stored in the endosperm or cotyledons becomes soluble because of enzyme action, and this food is made available to embryo, and so this embryo resume growth. These growths of embryo ultimately lead to death. X-rays appear to bring such metabolic disturbances.

X-rays can be attributed to the disturbance in enzymatic activity due to (i) High degree of ionizations, either totally blocks the enzymatic activities by disturbing the bio-electric balance of the cells or it may retard or weaken the enzymatic action. (ii) They gene become direct target of the X-rays, which at least amend their structure to the extent that they fail to produce the required enzymes which tells upon metabolic activities of the individual.

Amount of cortex was proportionately more in the stem of treated plants as compared to the controls.

Though the stem as a whole in the treated plants was less thick, and so the cortex, but the ratio between the stele and cortex was different in the treated ones with proportionately greater amount of cortex. (Table-3)

Moreover, the cells of epiblema in the roots and epidermis in the stem appear weaker and smaller in treated individuals as compared to the controls. Likewise the cells of cortex in stem appeared smaller in the stem whereas, in the root they appeared like normal (control). The cells of steler tissue of the stem appeared proportionately smaller. There appeared no differences in the cells of the epidermis mesophyll and guard cell tissue between the treated and control individuals (Table-3).

The physiological variations appear mostly due to the changes in the enzyme actions, either because of a change in the structure of enzyme in the system. The genes are ultimately responsible for the production of enzymes, when a gene is destroyed on altered the enzymes associated with it is also affected. Thus, most of the physiological variations are the manifestations of gene mutations but some of the physiological variations may be due to non-genetic reasons as well. Usually such are not permanent, such as nutritional deficiency symptoms. The physiological variation recorded during this investigation such as: Fall in the germination percentage and very low survival rate of germinated seedlings appear to be due to a change in the gene structure, because they are of permanent nature. The seeds in which the genetic material was totally damaged by X-rays amounted to 39% (Table R-I). In other words in 61% seeds. The damage was not that severe to bring about immediate death of the embryo, rather they were capable of germination with some difficulty. Out of the 61% of the germinated seeds only 9 survived. Thus giving a final survival rate of 9% (only up to this date).This is a clear indication that in 39% of the treated seeds, the genetic material was damaged so adversely that their embryos died immediately. In 52% of the seeds, the genetic material was so altered that it was some low to produce the enzymes needed for growth of the embryo during germination but not the enzymes needed in the advanced stage thus causing their death after germination.

Upon treatment with X-rays mortality rate was 100% in case of the young seedlings and moist seeds. Whereas, in dry seeds at least there was a survival rate of 9%.This is an indication that in a comparatively drier medium the deleterious effects of X-rays are less intense. The amount of moisture in the moist seeds and in the germinated young seedlings was decidedly higher than what it was in the normal dry seeds. Mortality in the first two cases was complete with a zero percent survival rate. In case of dry seeds mortality rate was 91% with a survival rate of 9 percent. X-rays appeared to have induced no morphological variations upon the leaves and leaflets of mutants (Table-3). But physiological variation like amino acid metabolism etc not yet determined. If any such change leading to the synthesis of greater amount of amino acids or any other important substance of nutritional importance appeared in any of these mutants or in the off spring these usefulness as fodder for cattle can be considered. Further investigations with the object to get some insight into the biochemistry and cytology along with morphological and physiological variations of these mutants will provide more information about the influence of X-rays radiation on *Leucaena leucocephala*.

**4. Conclusion**

X-rays causes mutations in *Leucaena leucocephala*. Influence of X-rays on the young seedling is highly lethal and brings about the death of seedling. X-rays also exert lethal effect on the moist seeds. The lethal effect of X-rays on dry seed is not as drastic as upon young seedling and moist seeds. Further plant breeder can search for the breeding material among this mutant or their off-spring. The cytology of these mutants is not studied as yet but there is need of cytological study of these mutants.

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