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# Assessment of mutagenic sensitivity of *Urginea indica* Kunth (Synanthus variety) to ethyl methane sulphonate through the perceived degree of alterations in the pollen grains

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**Abstract**

Urginea indica Kunth is an important medicinal plant being used extensively to cure innumerable human ailments both by local people and by pharmaceutical companies. It shows great morphological variations and grows in the wild. The development of genetically improved varieties of Urginea indica Kunth which is having such a huge medicinal value would be beneficial in the field of pharmacy. But before the development of genetically improved varieties, the mutagenic screening (detection and verifications of mutagenic activity) of the mutagens on the plants should be done.Therefore, in this research work, the sensitivity of the Urginea indica KunthSynanthus variety to Ethyl Methane Sulphonate was assessed by estimating the frequency of aberrations in the pollen grains of Urginea indica Kunth after treatment for two generations i.e. $M_1$ and $M_2$ generations.The decreased pollen fertility and alteration in the shape and size of the pollen grains with the increasing concentrations of Ethyl Methane Sulphonate in both $M_1$ and $M_2$ generations showed the sensitivity of Urginea indica KunthSynanthus variety to Ethyl Methane Sulphonate.

**Keywords:** *Urginea indica* KunthSynanthus variety, Pollen fertility and sterility, Ethyl Methane Sulphonate, Genetic variability, mutagenic sensitivity

## Introduction

*Urginea indica* Kunth is a poisonous herb having great medicinal value used extensively for curing various human ailments since a long time and in modern medicine also, they find their important space. It is an economically important medicinal plant, having high therapeutic potential being used in human homeopathy, phytotherapy and in veterinary science. It is also demonstrated to have significant level of nutrients[1].

*Urginea indica* Kunth is a plant with considerable morphological variations. It shows distinct morphological alteration in shape, size and color of bulbs and leaves, inflorescence and flower color. Even the flowering seasons of *Urginea indica* Kunth shows great variations. Moreover, *Urginea indica* Kunth adapts to the seasonal peculiarities in unique manner. The bulbs are dormant during winter but with the advent of the spring, plants generally appear above the soil and complete their vegetative

and reproductive phases. They have a very effective method of vegetative propagation through the production of underground daughter bulbs in few populations. *Urginea indica* Kunth is divided into two forms on the basis of their vegetative and reproductive phases of life[2]:
(i) Hysteranthus type
(ii) Synanthus type

In Hysteranthus type of *Urginea indica* Kunth, bulbs produces inflorescence first and later the vegetative leaves appear, while in Synanthus type, leaves and inflorescence appear simultaneously. In the present research work the Synanthus variety of *Urginea indica* Kunth collected from Ranchi, Jharkhand was used. In this variety, bulbs enter a dormant stage in winter, but with the advent of the monsoon, leaves and flowersappear simultaneously. The flowers of this variety of *Urginea indica* Kunth are white in color and are comparatively smaller in size.
Since *Urginea indica* Kunth is having numerous medicinal values; therefore, to save and to produce genetically improved varieties of this species, effort is required to assess the possibilities of inducing genetic variabilities. The genetic variability leads to the increased yield and mutation breeding occupies an important space in inducing genetic variability in the plants both through chemical and physical mutagens. In this research work, an attempt has been made to find out the cytotoxic and genotoxic effects of Ethyl Methane Sulphonate (EMS) on *Urginea indica* Kunth. The cytological anomalies including aberrations in the root tip cells as well as pollen grains, induced by chemical mutagens are reported to be the dependable measure for estimating the effects of the mutagens. Any cytotoxic and genotoxic result give an indication of mutagenic effects. The pollen characters and pollen fertility of *Urginea indica* Kunth Synanthus variety after treatment with the different concentrations of Ethyl Methane Sulphonate was recorded for two generations i.e. $M_1$ and $M_2$ generations.

**Materials and methods**

A Chemical Mutagen, Ethyl Methane Sulphonate (EMS) was used in this research work to treat *Urginea indica* KunthSynanthus variety. Their fresh and healthy bulbs of uniform size were treated with different concentrations of Ethyl Methane Sulphonate (EMS) for six hours. Then the treated bulbs were thoroughly washed in running tap water to remove the residual effect of the chemical used. The control and the treated bulbs were grown in the experimental plots in the randomized block design to rise $M_1$ generation.
Pollen studies were performed from flowers of *Urginea indica* KunthSynanthus variety raised from the bulbs treated with Ethyl Methane Sulphonate (EMS). Pollens were dusted on the glass slides for acetocarmine staining. They were suspended in a drop of acetocarmine, warmed over a spirit flame, squashed and examined after five to ten minutes. Stained pollen grains were considered to be fertile and unstained one as sterile. The percentage pollen fertility and sterility was determined by the formulae:

$$\text{Percentage Pollen Fertility} = \frac{\textit{Total Number of fertile pollens}}{\textit{Total number of pollens studied}} \times 100$$

$$\text{Percentage Pollen Sterility} = \frac{\textit{Total Number of sterile pollens}}{\textit{Total number of pollens studied}} \times 100$$

Shapes of the pollen grains of treated flowers were determined by using Erdtman (1952)[3] table based on the relation between polar axis and equational axis (P/E) of the pollen grains of *Urginea indica* KunthSynanthus variety.

**Results**

In the present study, the pollen morphology, percentage pollen fertility and percentage pollen sterility of the *Urginea indica* Kunth Synanthus variety after treatment with five different concentrations of Ethyl Methane Sulphonate (EMS) were studied for two generations i.e. $M_1$ and $M_2$. The results are depicted in table 1-2 and fig. 1-5.

Dose dependent increase in the percentage pollen sterility was observed in $M_1$ generation, which continued to decrease in $M_2$ generation. The variations in shape and size of pollen grains were also observed after treatment with different concentrations of Ethyl Methane Sulphonate (EMS). Polar diameter and equatorial diameter of pollen grains were observed to determine their shape on the basis of polar and equatorial diameter ratio (P/E).

In *U. indica* Kunth Synanthus variety, pollen grains were reported to show variations in shape at different concentrations in $M_1$ generation, however, it was reported to be same in shape as in control, at all the concentrations for $M_2$ generation. Similarly, percentage pollen fertility was reported 83.007% in control which showed dose dependent decrease for both the generations. It decreased considerably in $M_1$ generation, which showed slight increase in $M_2$ generation (table 2). At lower to higher concentrations it was reported to decrease from 71.047% to 53.826% in $M_1$ generation, while 78.947% to 58.017% in $M_2$ generation. On the other hand percentage pollen sterility increased with increased concentrations in $M_1$ generation which was found decreased in $M_2$ generation. It was reported 16.993% in control which increased to 46.174% at higher concentration (0.5%) in $M_1$ generation and slightly decreased to 41.983% in $M_2$ generation at the same concentration (table 2; fig. 1-2, 5).

**Fig. 1-4: Photomicrographs: Figures showing abnormalities in the pollen grains of *Urginea indica* Kunth Synanthus variety after treatment with different concentrations of Ethyl Methane Sulphonate (EMS).**

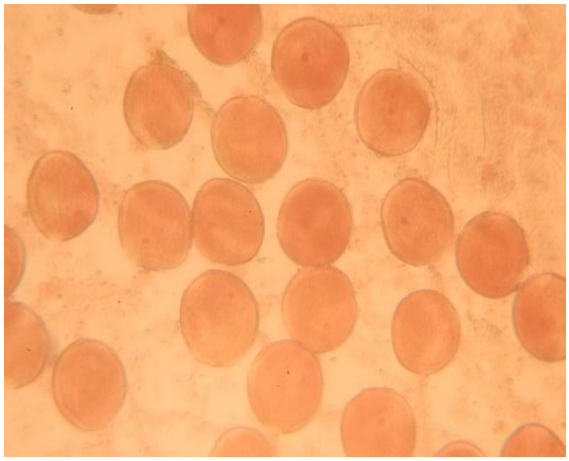

**Figure 1: Fertile Pollen grains.**

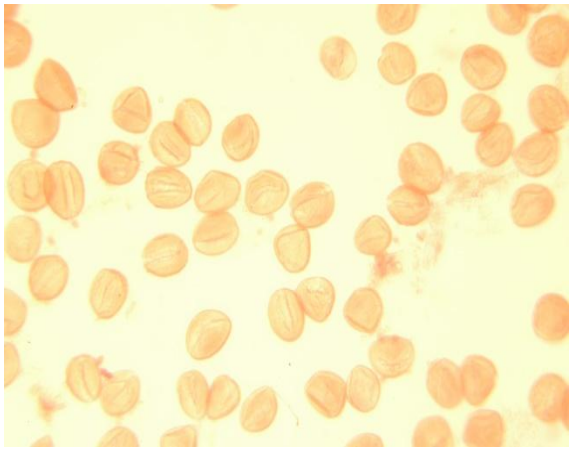

**Figure 2: Sterile Pollen grains with changed shape and size.**

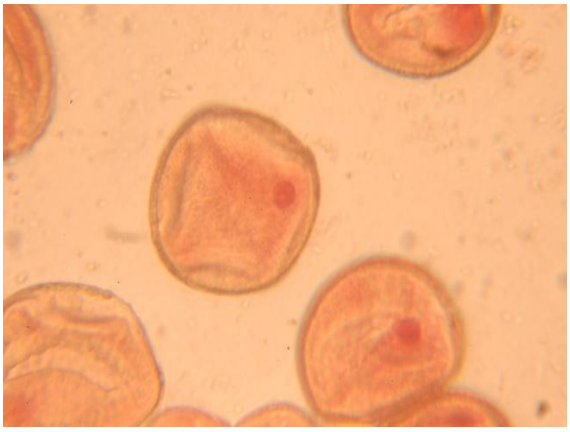

**Figure 3: Distorted pollen grains.**

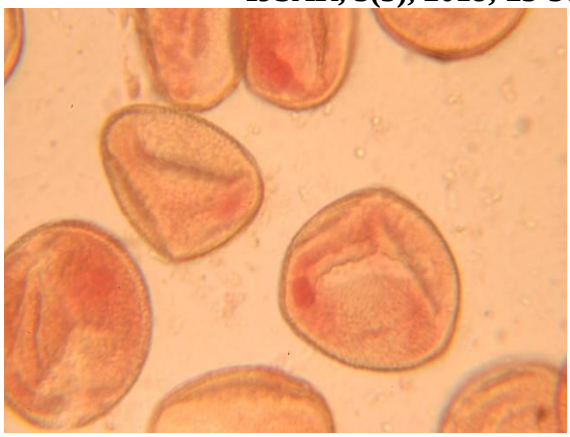

**Figure 4: Triangular shaped pollen grains.**

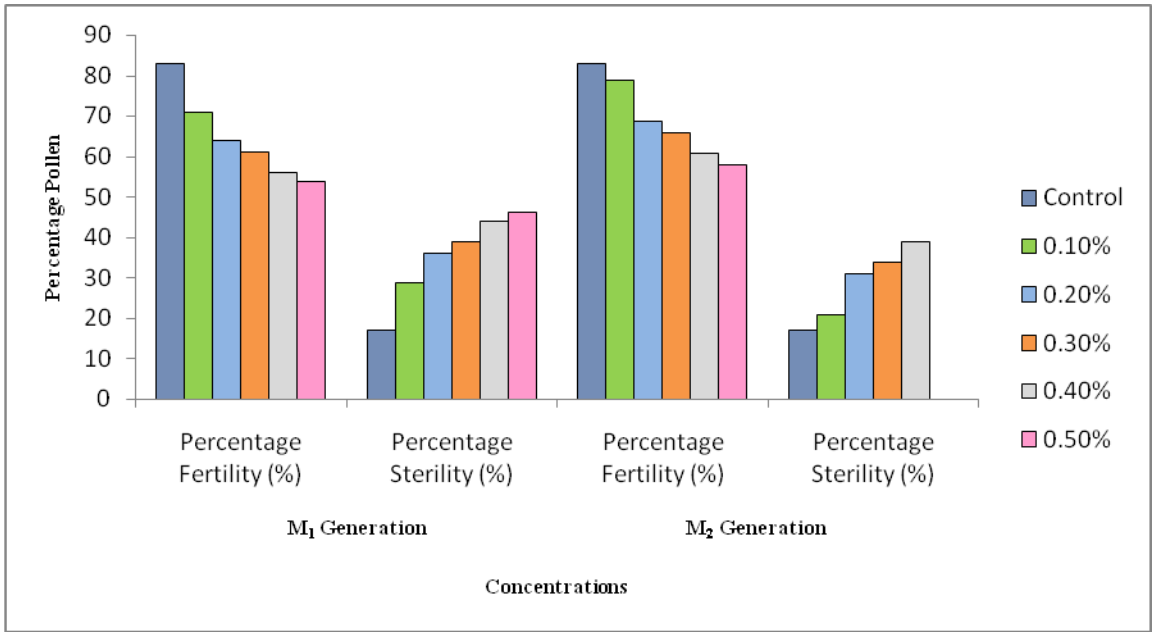


**Figure 5: Column graph showing Percentage Pollen Ferfility and Percentage Pollen Sterility in *Urginea indica* Kunth Cytotype II after treatment with different concentrations of Ethyl Methane Sulphonate in $M_1$and $M_2$generations.**

**Table 1: Pollen morphology in *Urginea indica* Kunth Cytotype II after treatment with different concentrations of Ethyl Methane Sulphonate (EMS) in M1 and M2 generations.**

| $M_1$ GENERATION | | | | | $M_2$ GENERATION | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | Pollen Grains | | | | Concentration | Pollen Grains | | | |
| | Polar Diameter (P) µ | Equatorial Diameter (E) µ | P/E | Shape | | Polar Diameter (P) µ | Equatorial Diameter (E) µ | P/E | Shape |
| **Control** | 37.5 ± 0.688 | 37.5 ± 0.831 | 1.0 ± 0.801 | Oblate spheroidal | **Control** | 37.5 ± 0.688 | 37.5 ± 0.831 | 1.0 ± 0.801 | Oblate spheroidal |
| **0.1%** | 35.8 ± 0.630 | 34.9 ± 0.513 | 1.0 ± 0.109 | Prolate spheroidal | **0.1%** | 30.8 ± 0.519 | 29.4 ± 0.286 | 1.1 ± 0.876 | Oblate spheroidal |
| **0.2%** | 28.6 ± 0.580 | 24.7 ± 1.074 | 1.2 ± 0.789 | Sub prolate | **0.2%** | 36.1 ± 0.758 | 35.9 ± 0.808 | 1.0 ± 1.054 | Oblate spheroidal |
| **0.3%** | 28.7 ± 0.740 | 26.1 ± 0.932 | 1.1 ± 0.453 | Prolate spheroidal | **0.3%** | 37.5 ± 0.688 | 36.5 ± 0.432 | 1.0 ± 0.213 | Oblate spheroidal |
| **0.4%** | 29.1 ± 0.549 | 26.2 ± 0.50 | 1.1 ± 0.234 | Prolate spheroidal | **0.4%** | 37.6 ± 1.029 | 37.4 ± 1.093 | 1.0 ± 0.345 | Oblate spheroidal |
| **0.5%** | 29.3 ± 0.443 | 24.8 ± 1.424 | 1.2 ± 0.056 | Sub prolate | **0.5%** | 38.3 ± 1.348 | 37.0 ± 0.827 | 1.0 ± 0.510 | Oblate spheroidal |

**Table 2: Percentage Pollen Fertility and Percentage Pollen Sterility in *Urginea indica* Kunth Cytotype II after treatment with different concentrations of Ethyl Methane Sulphonate (EMS) in M1 and M2 generations.**

| Concentrations | $M_1$ GENERATION | | | | | Concentrations | $M_2$ GENERATION | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total No. of Pollen grains studied | No. of fertile pollen grains | Percentage Fertility (%) | No. of sterile pollen grains | Percentage Sterility (%) | | Total No. of Pollen grains studied | No. of fertile pollen grains | Percentage Fertility (%) | No. of sterile pollen grains | Percentage Sterility (%) |
| **Control** | 818 | 679 | 83.007 | 139 | 16.993 | **Control** | 818 | 679 | 83.007 | 139 | 16.993 |
| **0.1%** | 860 | 611 | 71.047 | 249 | 28.953 | **0.1%** | 817 | 645 | 78.947 | 172 | 21.053 |
| **0.2%** | 875 | 560 | 64.00 | 315 | 36.000 | **0.2%** | 804 | 554 | 68.905 | 250 | 31.095 |
| **0.3%** | 854 | 521 | 61.007 | 333 | 38.993 | **0.3%** | 890 | 587 | 65.955 | 303 | 34.045 |
| **0.4%** | 818 | 458 | 55.990 | 360 | 44.010 | **0.4%** | 886 | 540 | 60.48 | 346 | 39.052 |
| **0.5%** | 872 | 471 | 53.826 | 458 | 46.174 | **0.5%** | 817 | 474 | 58.017 | 343 | 41.983 |

**Discussion**

Induction of mutation in medicinal plants or crops can provide new genetic variability and allow them to adapt to the new environments. Therefore, to improve and increase the yield of crops or medicinal plants, mutation breeding is applied extensively. Plant system is reported to display numerous genetic and chromosome changes to determine the effects of mutagens. Moreover, plants also play an important role in various aspects of mutagenesis research. It is used extensively for mutagen screening (detection and verification of mutagenic activity), mutagenic monitoring and determining mutagen effects and mechanisms of mutagen action of certain mutagen[4]**.**The effect of induced mutagens in plants can be estimated by the observation of frequency of chromosomal abnormalities, increased pollen sterility and alteration in the shape and size of pollen grains induced in them by the mutagens. Therefore, in this investigation mutagenic effect of Ethyl Methane Sulphonate on the pollen grains of *Urginea indica* Kunth Synanthus variety was examined. The mutagenic effects of EMS on the pollen grains were observed for $M_1$ and $M_2$ generations. It was noticed that with the increasing concentrations of Ethyl Methane Sulphonate (EMS), size as well as shape of the pollen grains changed. In addition to that percentage pollen fertility was also found to be reduced in both the generations.

It has been reported that the changes induced in the shape, size and other properties of pollen grains is caused by the irregular or abnormal meiosis. This happens due to the fact that the structure and physiology of pollen grains is under genetic control[5, 6].

In *Urginea indica* Kunth, the polar diameter and equatorial diameter showed significant decline as the concentrations of Ethyl Methane Sulphonate (EMS) increased in $M_1$ generation. In control the shape of pollen grains was found oblate spheroidal but as the concentrations increased it was reported to show variations in shape. Thus, the changes observed in the shape and size of pollen grains of *Urginea indica* Kunth showed its sensitivity to Ethyl Methane Sulphonate (EMS).

The percentage pollen fertility and percentage pollen sterility of *Urginea indica* Kunth was also observed after treatment with Ethyl Methane Sulphonate (EMS). It was reported to show significant decrease in percentage pollen fertility, while increase in percentage pollen sterility in $M_1$ and $M_2$ generations. Percentage pollen fertility was however, reported to show some increase in $M_2$ generation. It was suggested[7, 8] that sterility following mutagenic treatments might be attributed to detectable chromosomal aberrations and cryptic deficiencies. The increase in the percentage sterility of pollen grains after treatment with Ethyl Methane Sulphonate (EMS) might be the result of gene mutation or invisible deficiencies[9]. It has also been reported that pollen fertility is directly correlated with meiotic anomalies. Fertility depends on the efficiency of the meiotic process[10, 11]. The relationship between aberrations and sterility suggests that mutagen induced sterility is mainly the result of chromosomal aberrations which might have passed from somatic to germ cells.

Decreased pollen fertility and increased pollen sterility in *U. indica*Kunth after treatment with Ethyl Methane Sulphonate was observed. Moreover, since the changes in shape and size of pollen grains and reduction in percentage pollen fertility was significantly high, it may be considered to show greater sensitivity to the different concentrations of Ethyl Methane Sulphonate.

## Conclusion

The result showed the sensitivity of *Urginea indica* KunthSynanthus variety to Ethyl Methane Sulphonate, which means that this chemical mutagen can be used to induce genetic variability in *Urginea indica* Kunth Synanthus variety to produce genetically improved varieties.

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