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EFFECT OF DIFFERENT PRETREATMENT ON *IN VITRO* SEED GERMINATION AND SEEDLING DEVELOPMENT OF *SENNA ALATA* LINN

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Key words:

Senna alata, Seed culture, Seed germination, Murashigs and skoog medium, Pretreatment.

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ABSTRACT: *Senna alata* is an important medicinal and ornamental flowering tree, in the subfamily of Caesalpinioideae, which was found in diverse habitat in the tropics. Leaves or sap are used to treat several infections such as skin diseases, bronchitis, asthma and ringworm. The present study was design to investigate for conserve the important medicinal plant by establishing an efficient *in vitro* seed germination method. The seed germination was gradually decreased by increasing the age of the seeds. The seeds were pretreatment with different chemicals such as, sulphuric acid, hydrochloric acid and distilled water. After pretreatment the seeds were surface sterilized with various concentration of mercuric chloride. The objective of the present study the effect of different pretreatment as *in vitro* seed germination and seedling development of *S. alata* Linn. Seed propagation is still used as a specialized tool for breeding and propagation of pathogen-free plant.

INTRODUCTION: Seed germination is important to know the germination pattern of a plant, more particularly the medicinal ones that might need to bring under cultivation for the primary healthcare system. The significance of the seedling in plant population ecology has long been recognized [1]. The germination response pattern of seeds is also regarded as a key characteristic in plant life history [2, 3]. The variation in seed dormancy and the subsequent patterns of seedling emergence are controlled by environmental conditions.

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Important factors controlling the variation in seed dormancy within species include the environment of the mother plant during the time of seed maturation and environmental conditions after the seeds have been released [4]. Certain environmental conditions may be required to break dormancy, and other conditions are often required to permit germination after dormancy is broken [5]. Seeds of many species require days, weeks, or months at low temperatures to break dormancy [6]. However others require warm temperatures for after-ripening to germinate when permissive conditions arrive [7]. Many attempts have been made to investigate seed germination and seedling emergence of different annual and perennial species including medicinal plants [8, 9, 10, 11, 12].

Seed dormancy is the most limiting factor for plant propagation. However, the blocking of water access

into the seed is the most common cause of delay in seed germination[13]. *Cassia sp*. suffers from dormancy to the presence of water impermeable thick seed coat that prevents water and oxygen reaching and activating the embryo. The presence of germination-inhibitor chemical compounds and they require specific treatments for breaking dormancy [14, 15]. Acid scarification (H_2SO_2) for different periods was the most effective treatment is softening the seed coat of Cassia seeds [16].

S. alata Linn is an important medicinal plant as well as ornamental flowering plants in the subfamily Caesalpinioideae. It also known as a Candelabra Bush, Empress Candle plant, Ringworm Tree or "Candle tree". *S. alata* is native to Mexico, and can be found in diverse habitats. It is a large shrub with very thick finely downy branches. It is named for its flower buds which grow in a column and looks like fat yellow candles each complete with a flames. It is found commonly in Somalia, Saudi Arabia, some parts of Pakistan and Kutch area of Gujarat. It is largely cultivated in Madurai, Ramanathapuram, Salem and Tiruneleveli districts of Tamil Nadu for its medicinal purpose [17]. In the tropics it grows up to an altitude of 1,200 meters. The Shrub stands 3-4 m tall, with leaves 50-80 cm long. The inflorescence looks like a yellow candle.

The fruit shaped like a straight pod is up to 25 cm long. Its seed are distributed by water or animals. The seed pods are nearly straight, dark brown or nearly black, about 15 cm long and 15 mm wide. On both sides of the pods there is wing that length of the pod. Pod contains 50 – 60 flattened train angular seeds. The leaves close in the dark. In Sri Lanka this is use an ingredient of Sinhala traditional medicine. It the Indian system of medicine, namely Ayurveda, Siddha, and Unani, decoctions of the leaves, flowers, bark and wood are used in skin diseases such as eczema, pruritus, itching, and in constipation[18].

In recent years, pharmaceutical companies have focused on developing drugs from natural products. Plants still remain the most effective and cheapest alternative sources of drugs [19]. In modern medicine, plants are used as sources of direct therapeutic agents, as model for new synthetic compounds and as a taxonomic marker for the elaboration of more complex semi-synthetic chemical compounds [20]. Wide variations in medicinal quality and content in phytopharmaceutical preparations have been observed. These are influenced mainly by cultivation period, season of collection, plant to plant variability in the medicinal content. Generally, herbal preparations are produced from field-growth plants and are susceptible to infestation by bacteria, fungi, and insects that can alter the medicinal content of the preparation [21]. However significant evidence to show that the supply of plants for traditional medicines is failing to satisfy the demand [22].

Seed germination is the easiest and cheapest method for propagation. Germination is the growth of an embryo genic plant continued within a seed which results in the formation of the seedling that emerges from a seed and begins the growth [23]. The present investigation is aimed at studying the standardization and seed culture of methods for the *in- vitro* seed germination of the species *S. alata*.

MATERIAL AND METHODS:

Senna alata seeds collection:

Matured pods were collected during 2012 on October to December from Tiruchirappalli. Tamil Nadu, India. Seeds were extracted manually and air dried at normal temperature. Complete dry seed were chosen for germination studies.

Seed Germination Medium:

Murashigs and Skoog (MS) basal medium was used for seed germination. The basal medium was added with (3%) sucrose and media were solidified with Agar (0.8%). The pH was adjusted between 5.6 to 5.8 before autoclaving at 121°C for 15 minutes. The inoculated bottles were maintained at 23±2°C under 18 h light and dark cycles. Healthy seedlings were selected and used as source of explants.

Pretreatment of seeds:

The collected seeds washed with tap water to remove the adhered soil particles and charcoal residues. Each 3g of seeds were soaked in 0.5% to 2% hydrochloric acid (HCl solution, sulfuric acid 0.5% to 1% and water for 12 h and finally rinsed three times with sterile water.

Seed surface sterilization:
The soaked seeds were placed in a detergent Teepol for 10minutes under mild shaking. They were rinsed with water to remove the detergent solution. Then the seeds were transferred into laminar air flow the 50 ml conical flash with 0.15 /v aqueous mercuric chloride ($HgCl_2$) for a period of 2 to 10 mins. Finally they were rinsed thrice with duration of 3 mins each in sterile distilled water to remove all the traces of $HgCl_2$. Sterilized seeds were aseptically inoculated in both MS medium supplemented with GA3 (1.0 mg/l) and cotton soaked with sterile distilled water. The inoculated seeds were incubated under optimal culture condition. The *in vitro* raised seedlings were transferred to earthen pots containing soil and sand (1:1) and maintained in the garden. The explants were collected from two months old *in vitro* derived seedling [24].

Statistical analysis:
All the experiments were conducted as a randomized complete design. For each experiment, a minimum of 25 replicates were taken and repeated thrice. Comparisons between treatments were made with Duncan's new multiple ranges test (DMRT) (Duncan, 1955). The results are expressed as P values < 0.05 (95% confidence limit) was considered statistically significant, using software Graph Pad Prism5.

RESULT AND DISCUSSION: The present study is to possibility of using MS medium to support the whole propagation system efficiently with various combinations of growth regulators and to standardize the *in vitro* regeneration methods for *S. alata.* The morphological traits of pod (pod length, width and no. of seeds per pod) and seed (seed length, width, thickness) were measure (**Table 1).**

TABLE 1: MORPHOLOGICAL CHARACTERISTICS OF *S. ALATA* PLANT

Plant parts	Observation
Leaves colour	Ever green
Seed colour	Dark-grey to black
Shrub stand	3-4m tall
Leaves	50-60 long
Straight pod	25 cm long
Pod colour	Dark brown and nearly black
Bloom colour	Bright yellow
Pod width	15mm
Pod	50-60 seeds
Seed shape	Triangular

Effect of pretreatment on seeds germination:
S. alata belongs to caesalpiniaceae family embryos with little proembryo embedded in endosperm. Some effective aids can induce maximum seed germination. *S. alata* seed were treated with various concentrations of H_2SO_4 (0.5% -1%) and HCL (1% - 2%) for the time period ranging from 6-24 hrs. Among these seeds soaked in 1% HCL for 12 hrs were found to be optimum with 95 % seed germination. Seeds treated with 0.5% H_2SO_4 for 12 hrs showed higher germination percentage (70%). Any increase or decrease in the concentration of H_2SO_4 and HCL or the time of soaking leads to decrease in the germination percentage (**Fig.1** and **2**).

Establishment of aseptic seedlings:
Contamination is the main stumbling block in the plant tissue culture, since plants are exposed to various microbial contaminants. The seeds of *S. alata* were directly used for *in vitro* germination contamination were a major problem. Among the various time exposures (2-8 mins) tested with different concentrations of $HgCl_2$ (0.1-0.2%), 6 min of exposure to 0.1% $HgCl_2$ was found to be optimum for seed germination (92%) (**Fig.3 A, B, C**).

In this study the seed exposed to 0.1% $HgCl_2$ for 6 mins shows good response with maximum survival percentage and higher percentage of contamination free plants. Decreasing the time exposure leads to lower percentage of contamination free plants. The results are in accordance with *Sterulia urens* where seeds surface sterilized with 0.1% HCl_2 produced aseptic seedling [25].

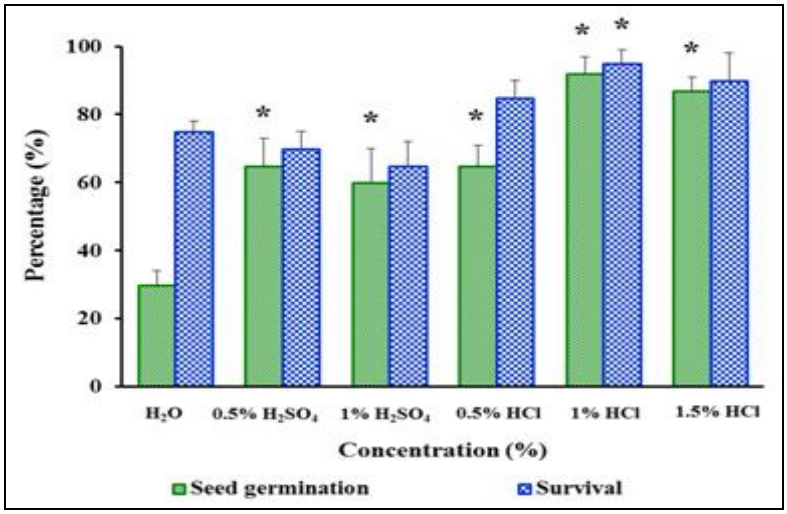

FIG.1: PRETREATMENT OF *S. ALATA* SEEDS (TIME EXPOSURE 12 Hrs). VALUES ARE PRESENTED AS MEAN ± SEM. COMPARED BETWEEN THE DIFFERENT TREATMENT EXPOSURE CONDITION GROUPS.
P value less than 0.05 was considered as significant. $^{*}p<0.05$ significant

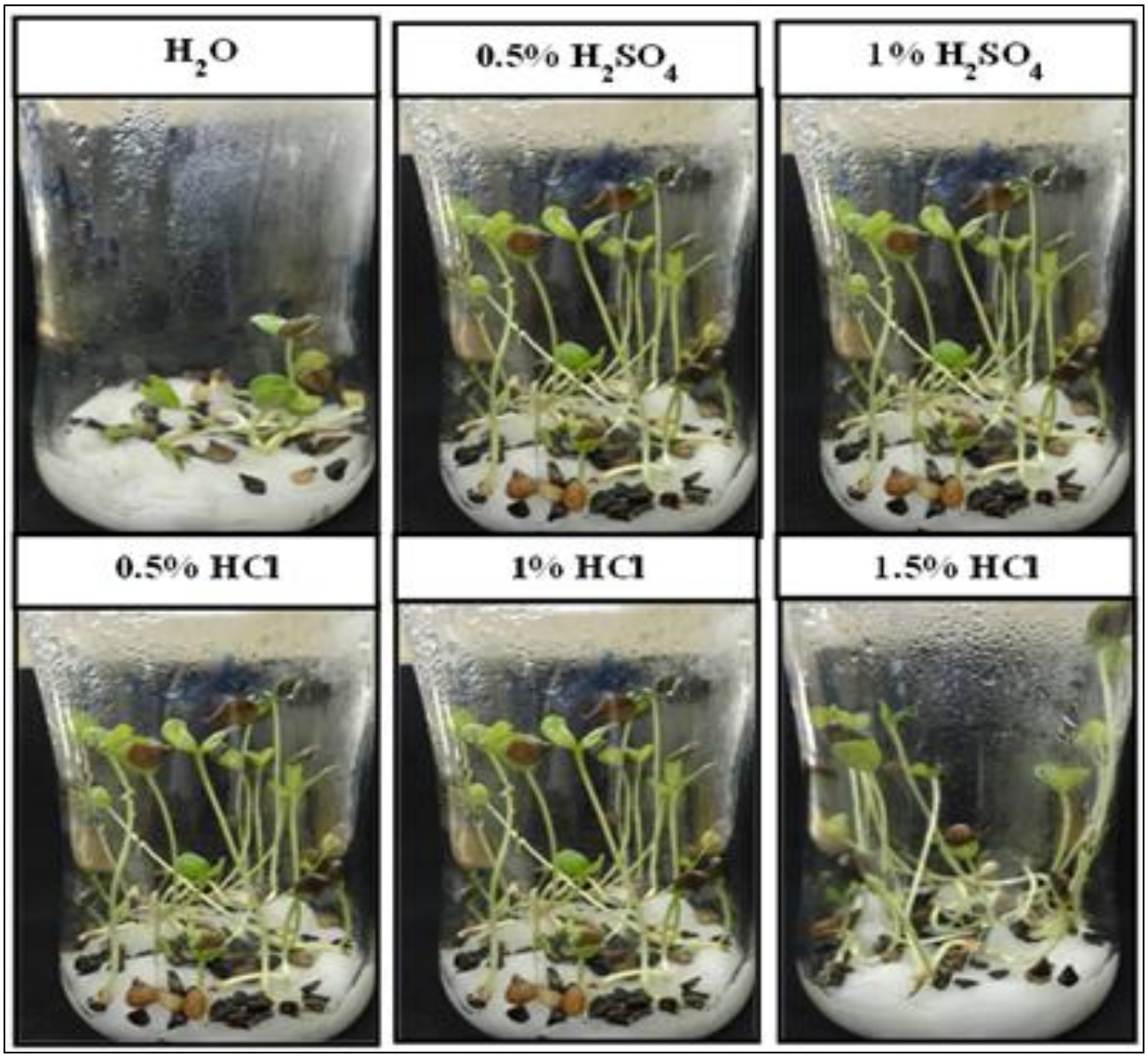

FIG. 2: VARIOUS FROM OF *IN VITRO* SEED GERMINATION OF *SENNA ALATA* (TIME EXPOSURE 12 Hrs).

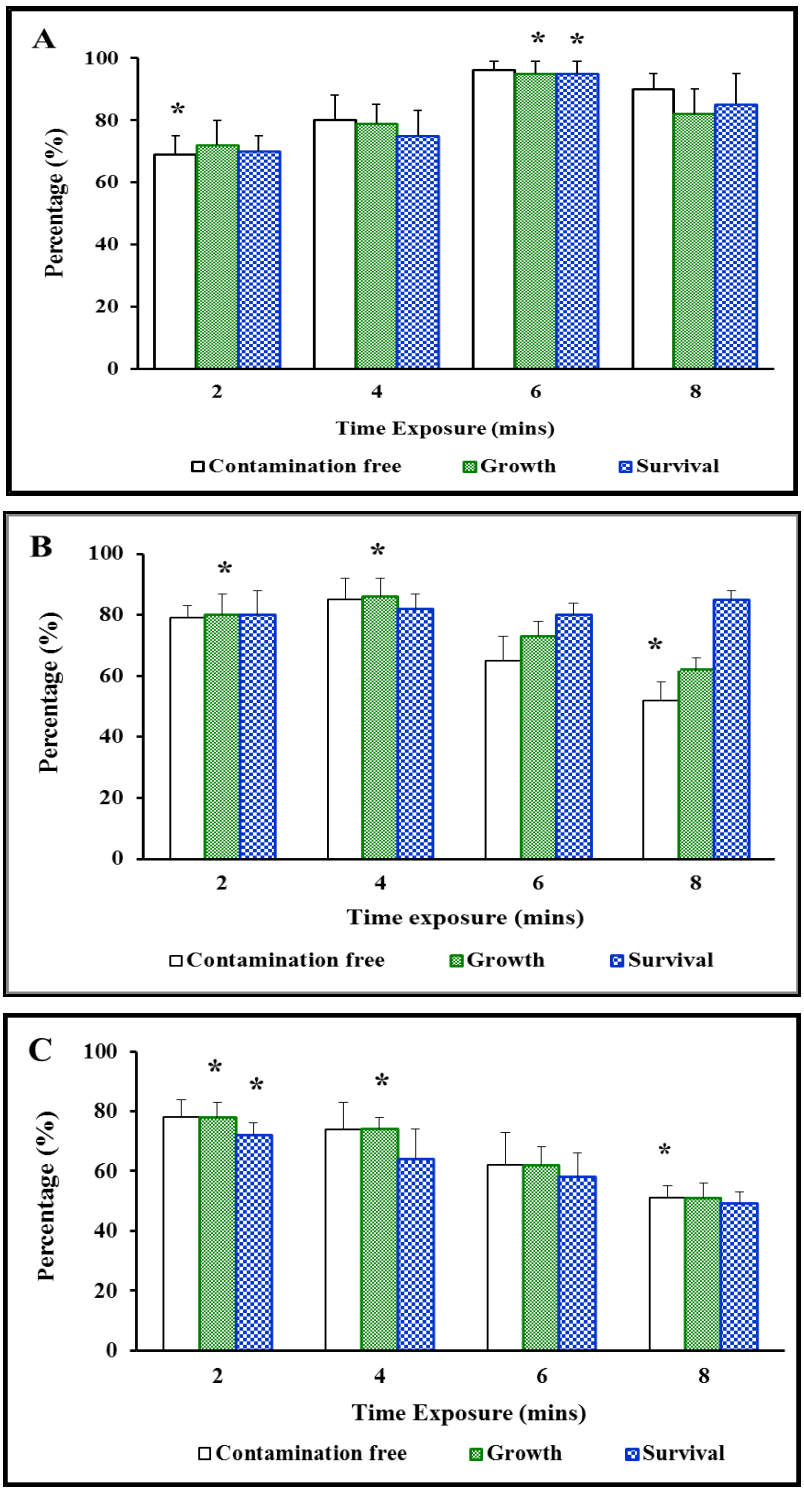

FIG.3: EFFECT OF MERCURIC CHLORIDE ($HgCl_2$) ON SURFACE STERILIZATION OF *S. ALATA* SEEDS.

A. 0.1% $HgCl_2$, **B**. 0.15% $HgCl_2$, **C.** 0.2% $HgCl_2$. Values are presented as mean ± SEM. Compare between the different treatment exposure condition groups. P value less than 0.05 was considered as significant. $^{*}P<0.05$ significant.

CONCLUSION: *S. alata* is an important medicinal plant, having rudimentary embryos require some effective aids for germination. The plants raised through seeds are highly heterozytes and shows a great variation in growth. However the yield may have to be discarded because of poor quality of products for their commercial release.

The results clearly demonstrated that the culture condition was the most effective for seedling production of *in vitro* propagation of the plant *S. alata*. This method will be useful to commercial grower for mass propagation of this plant.

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