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A study on the effect of physical and chemical treatments on breaking the dormancy of *Adenanthera pavonina* L. seeds

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ABSTRACT

Forests are store houses of biodiversity. Tropical forests are the most biologically diverse and ecologically complex of terrestrial ecosystems. Most of the trees have viable dormant seeds which lay inactive. Adenanthera pavonina L. is a deciduous tree which has ecological and medicinal importance. The present study on Adenanthera pavonina L. aims to investigate the fruit - seed characteristics and germination employing various physical and chemical treatments. The seeds were subjected to a host of physical treatments - mechanical scarification, dry heat, leaching, light and chemical treatments - acid scarification, inorganic compounds to break the seed dormancy. Physical and chemical treatments had a significant effect on breaking the physical dormancy which is due to the presence of hard impermeable seed coat.

Keywords: *Adenanthera pavonina* L., germination ecology, physical treatments, chemical treatments, seed dormancy.

INTRODUCTION

A forest is a complex ecosystem which is predominantly composed of the non-living (abiotic) and the living (biotic) component. Forests are store houses of a large variety of life forms such as plants, mammals, birds, insects, reptiles, fungi, microorganisms etc. Plants in the forest include the trees, shrubs, climbers, grasses and herbs. Upon the framework of the tree and within the microclimate of the canopy grow a range of other kinds of plants: climbers, epiphytes, strangling, plants parasites, and saprophytes [25]. The angiosperms and gymnosperms comprise the two groups in the seed plants. The trees frequently have viable seeds of low life expectancy or seeds of dormancy. The term dormancy means that seeds will not germinate under unfavourable conditions of temperature, moisture, and light, whereas non-dormant seeds will germinate over the widest range of conditions possible for the taxon or genotype. Five classes of dormancy like physiological, morphological, morpho-physiological, physical, and combinational dormancy was understood by Baskin & Baskin, (1998) [1] and Nikolaeva (1969) [19]. A seed is a small embryonic plant enclosed in a covering called the seed coat, usually with some stored food. It is the product of the ripened ovule of gymnosperm and angiosperm plants which occurs after fertilization and some growth within the mother plant. Plants have evolved many ways to disperse and spread the population through their seeds. *Adenanthera pavonina* L. is a medium-sized to large deciduous tree commonly known by the following names - Tamil: Ani kundumani, Hindi: Raktakambal, Bengali: Ranjana, English: Bead tree, Circassian bean, Circassian seed, Coral wood, Crab's eyes, False sandalwood, Jumbie bead, Red bead tree, Red sandalwood, Red wood. *Adenanthera pavonina* L. is endemic to India and South East China. *Adenanthera pavonina* L. are found scattered in primary and

secondary, evergreen to dry deciduous rainforests. Cultivated in forest clearings and village common areas, this useful tree provides quality fuel wood, wood for furniture, food, and shade for economic crops like coffee and spices. The scientific name is derived from a combination of the Greek aden, "a gland," and anthera, "anther"; alluding to the anthers being tipped with a deciduous gland. Synonyms: *Adenanthera gersenii* Scheffer, *Adenanthera polita* Miq. [17]. In India it is found in Sub – Himalayan tract, ascending up to an attitude of 1,200 meters in Sikkim, West Bengal, Assam, Meghalaya, Gujarat, Maharashtra, South India & in the Andamans [9]. Leaves bipinnate; 2-6 opposite pairs of pinnae, each with 8-21 leaflets on short stalks; alternate leaflets oval-oblong, with an asymmetric base and blunt apex, dull green on topside and blue-green underside; leaves turn yellow with age. The trees have been observed to be flowering and fruiting almost throughout the year, The inflorescence appears at the apex of the small fruit. Flowers borne in narrow spike like racemes at branch ends; flowers small, creamy yellow, fragrant; each flower star shaped with 5 petals, connate at the base, and having 10 prominent stamen bearing anthers tipped with minute glands. Pods long and narrow with slight constrictions between seeds, dark brown, turning black upon ripening, leathery, curve and dehisce to reveal 8-12 hard-coated, showy seeds lens shaped, vivid scarlet which adhere to pod. Ripened pods remain on the tree for long period. Traditionally it had been used to treat many diseases. Various parts of this plant have also been used in traditional medicine for the treatment of asthma, boil, diarrhea, gout, inflammations, rheumatism, tumor and ulcers, and as a tonic [24, 14, 7, 11]. The legume is generally considered to be nitrogen fixing. Sparse, fast-growing, brown nodules with *Rhizobium* have been observed, and Vesicular Arbuscular Mycorrhizae (VAM) have been found on the roots of nursery stock. The small leaves break down easily, making the species a good green manure to improve soil fertility. Raw seeds are poisonous. These seeds mostly remain inactive due to seed dormancy. The purpose of the study was to investigate and provide conclusions to the aspects of germination ecology such as fruit - seed characteristics and germination employing various physical and chemical treatments which were employed to break the seed dormancy.

MATERIALS AND METHODS

Study area

Madras Christian College (MCC) campus supports a veritable piece of nature, in the form of Tropical Dry Evergreen vegetation. This forest ecosystem harbours both cultivated and wild species of angiosperms, a host of animals such as insects, reptiles, birds and mammals. Madras Christian College campus is located at 12°55' N latitude and 80°7' E Longitudes are tropical in distribution. The average elevation of the campus is about 30 m above sea level [18]. The climate is wet tropical moderate bio climate, where the mean temperature is > 20° C, annual precipitation is between 500 to 1000 mm and it covers 5 or 6 dry months in a year. However, four overlapping seasons may be recognized such as, South west monsoon (June-Sept), North- east monsoon (Oct-Nov), winter (Dec-Feb) and summer (Mar-May) [12]. During December and January, the maximum temperature is about 28°C there is a steady increase of temperature during summer that reaches maximum of 38 - 40° C. The relative humidity is about 85% during rainy season and it drops to about 55% during summer. The wind velocity is 4-6 Km/hr during winter and it goes up to 11 – 13 Km/hr by the end of summer [16].

A. Source of seeds and pods

Adenanthera pavonina L. seeds for the present study were collected in the Madras Christian College campus from a healthy tree near the Bell tower. The healthy viable uniform sized seeds were then surface sterilized in 1% HgCl2 for 5 minutes followed by treatment in 100% alcohol for 3 minutes. The surface sterilized seeds were then subjected to various physical and chemical treatments in the present study of seed dormancy.

B. Surface sterilization

The seeds were sterilized with two chemical disinfectants namely 1% $HgCl_2$ for 5 minutes, followed by 3 minutes exposure to 70% C_2H_5OH. 1% $HgCl_2$ is prepared by dissolving 1g of $HgCl_2$ in 100 ml of distilled water. The petri dishes used for the experimental purpose were first sterilized and lined with two Whattman No.2 filter paper. The treatment seeds were watered with 5ml distilled water once in two days throughout the germination period.

C. Moisture content of fruit

A batch of 20 leguminous pods was taken and fresh weight of the individual pods was analyzed using an electronic balance. The fruits were dried in a hot air oven at 80°C for about 12 hours and the dry weight of the fruits was also taken. Fruit moisture content was calculated as follows:

Percentage of moisture content (%) = $\frac{\text{FW – DW}}{\text{FW}}$ × 100

Where, FW – Fresh weight, DW – Dry weight

D. Moisture Content of seed

The seed moisture content is an important physiological attribute directly related to seed germination and seed viability [3].

A batch of 50 seeds of *Adenanthera pavonina* was chosen and fresh weight was taken using an electronic balance. The seeds were then dried using a hot air oven at 50°C for 12 hours. Later the dry weight was taken using a monobalance. The seed moisture content was calculated by using the formula:

Percentage of moisture content (%) = $\frac{\text{FW - DW}}{\text{FW}}$ × 100

Where, FW – Fresh weight, DW – Dry weight

E. Germination percentage

The germination percentage of the seeds of *Adenanthera pavonina* L. in each treatment in our present study was calculated by using the formula:

Germination percentage (%) = $\frac{\text{No of seeds germinated}}{\text{Total no of seeds}}$ ×100

F. Seed Viability

Test for seed viability of *Adenanthera pavonina* L. was carried out on a weekly basis. Seeds stored were soaked overnight in beaker containing water. Imbibed seeds were then cut into equal halves using a sharp blade and immersed in 0.1% (TTC) solution. Observations were made at hourly intervals and gradual changes in the colour of seed halves were noted. The seed halves which turned pink were viable / Tetrazolium positive and those that did not show any colour change were non-viable / Tetrazolium negative. The results were tabulated and the percentage of viability was calculated.

Physical Treatments

I. Mechanical Scarification

The seed batches of *Adenanthera pavonina* L. were subjected to mechanical scarification by slightly cracking the seeds by using the hammer but these seeds didn't germinate because their embryos were damaged by the blow from the hammer. So the seeds had to be rubbed against the floor for 10, 20, 30, 40, 50, 60 seconds duration to see the effect of mechanical scarification [22].

II. Dry Heat Scarification

The seeds of *Adenanthera pavonina* L. were exposed to dry heat in a hot air oven maintained at 30°, 40°, 50°, 60°,70° and 80° for 5 minutes along with a control maintained at room temperature [6].

III. Leaching

The seeds of *Adenanthera pavonina* were mechanically scarified by rubbing on the ground. Batches of 10 seeds of *Adenanthera pavonina* L. were pre-soaked in distilled water for 12, 24, 36 and 48 hours durations under lab conditions [10].

IV. Light Treatment

The seeds of *Adenanthera pavonina* L. were subjected to different radiation like white light, red light, far red light and complete darkness in the seed germination chamber. The respective lamps are used in the germination chambers for about 12, 24, 36 and 48 hours duration [23].

V. Hot water Scarification

10 seeds of *Adenanthera pavonina* L. were subjected to hot water scarification by heating the seeds over electric hot water heater. The different temperature is reached by turning the temperature knob and the required temperature is

maintained by adding cold water and hot water was displaced using a small beaker. The seeds are treated at varying temperatures of 60°C, 70°C, 80°C, 90°C and 100°C for about 30 minutes [26].

Chemical Treatments
I. Acid Scarification
A. Sulphuric Acid Treatment
Six batches of 10 seeds *Adenanthera pavonina* L. were pretreated with concentrated sulphuric acid for varying durations of time of 10, 20, 30, 40, 50 and 60 minutes respectively. After the acid treatment the treated seeds were subjected to leaching in running water. The seeds were then kept separately in petri plates for observation [4].

B. Nitric Acid
A sample of 10 seeds of *Adenanthera pavonina* L. were pretreated with concentrated nitric acid for varying durations of time of 10, 20, 30, 40, 50 and 60 minutes to test their ability to germinate. After the acid treatment the treated seeds were subjected to leaching in running water respectively.

C. Hydrochloric Acid
A sample of 10 seeds of *Adenanthera pavonina* L. were taken in six batches and pretreated with concentrated hydrochloric acid for varying durations of time of 10, 20, 30, 40, 50 and 60 minutes. After the acid treatment the treated seeds were subjected to leaching in running water and kept for germination in petri-plates respectively.

Inorganic compounds
I. Potassium Nitrate
The different concentrations of potassium nitrate such as 0.25M, 0.5M, 1.0M, 1.5M and 2.0 M respectively were prepared with distilled water. The seeds of *Adenanthera pavonina* L. seeds had to be rubbed against the concrete floor then the seeds were soaked in the respective concentrations of sodium nitrate for a day [21].

II. Ammonium Nitrate
The different concentrations of ammonium nitrate such as 0.1%, 0.2%, 0.3%, 0.4% and 0.5% were prepared by adding 0.1g, 0.2g, 0.3g, 0.4g and 0.5g respectively to 100 ml of distilled water. The seeds of *Adenanthera pavonina* L. seeds had to be rubbed against the concrete floor then the seeds were soaked in the respective concentrations of ammonium nitrate for a day and a control [15].

RESULTS AND DISCUSSION

I. Seed Viability, Fresh weight, Dry weight and Percentage of moisture content
Test for seed viability of *Adenanthera pavonina* L. was carried. Seeds stored were soaked overnight in beaker containing water. Imbibed seeds were then cut into equal halves using a sharp blade and immersed in 0.1% (TTC) solution. Observations were made at hourly intervals and gradual changes in the colour of seed halves were noted. The percentage of viability was 75 %.The fresh weights and dry weights of the pods and seeds of *Adenanthera pavonina* L. were noted using the electronic balance. The average fresh weight of the pod is 4.97g while the average dry weight is 4.81g. The average moisture content percentage of the pod is 3.8% (Table: I). The average fresh weight and dry weight of the seed are 0.48g and 0.44g respectively. The average moisture content percentage of the seed is 8.02%. (Table: II)

Table I: Fresh weight, Dry weight and Percentage of moisture content of *Adenanthera pavonina* L. pods

Sno	Fresh Weight (g)	Dry Weight (g)	Moisture Percentage (%)
1	5.04	4.87	3
2	4.41	4.2	5
3	5.58	5.47	2
4	4.12	3.87	6
5	3.9	3.79	3
6	3.07	2.86	7
7	4.36	4.16	5
8	7.67	7.56	1
9	7.21	7.09	2
10	4.37	4.2	4

Table II: Fresh weight, Dry weight and Percentage of moisture content of *Adenanthera pavonina* L. seeds

Sno	Fresh Weight (g)	Dry Weight (g)	Moisture Percentage (%)
1	0.49	0.45	8.16
2	0.48	0.42	12.5
3	0.54	0.51	5.55
4	0.49	0.43	12.24
5	0.52	0.5	3.84
6	0.48	0.45	6.25
7	0.42	0.38	9.52
8	0.56	0.53	5.35
9	0.4	0.37	7.5
10	0.43	0.39	9.3

A. Physical Treatment:

I. Mechanical scarification:

The mechanically scarified seeds had a significant impact on breaking the dormancy. The seeds that were mechanically scarified for 10 and 30 seconds showed 100 % germination percentage whereas the germination percentage of seeds that were mechanically scarified for about 20, 40 and 50 seconds showed the germination percentage of 80% , 80% and 60 % respectively (Figure: I; Table: III). In our present study mechanically scarified seeds showed very high germination percentage when compared to any other treatment. Mechanical scarification was very effective in overcoming dormancy in seeds of *Adenanthera pavonina* L. The study of Okunlola *et al*., (2011) revealed that seeds mechanically scarified improved seed germination and seedling growth. It is therefore recorded that seeds mechanically scarified with sandpaper had germination of 83.3% in *P. biglobosa* [20]. This shows that mechanical scarification may be effective for breaking dormancy and improving the seedling vigour.

Figure I: Mechanical scarification

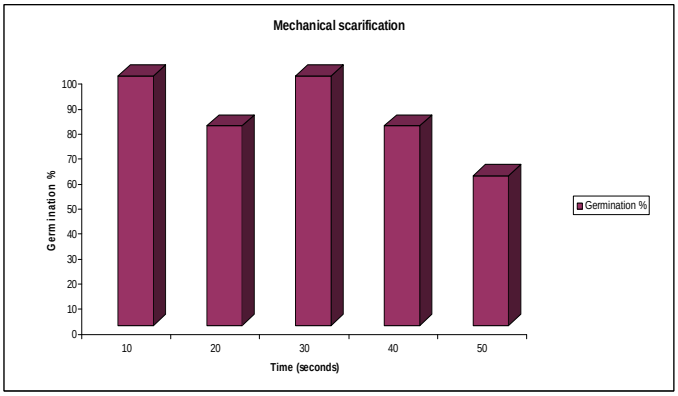

Table III: Seed germination in response to mechanical scarification in *Adenanthera pavonina* L.

S.No	Time(in seconds)	No of seeds germinated
1	10	10.33±0.46 (100)
2	20	9.33± 0.24 (80)
3	30	10.33±0.46 (100)
4	40	8.33± 1.24 (80)
5	50	6.33± 0.46 (60)

Germination percentage is written within brackets.

II. Dry Heat Treatment:

The seeds treated at different temperatures in hot air oven were not found to be very effective on breaking the dormancy. The highest germination percentage was observed at 50°C (40%) and 40°C (30%) (Table: IV). The seeds treated at 30°C, 60°C, 70°C and 80°C didn't show any significant change in breaking the seed dormancy. However, incubating seeds at 30/15°C, 40/25°C and 80°C – 100°C were ineffective in breaking the dormancy in *Senna marilandica* were similar results obtained [2].

Table IV: Seed germination in response to Dry Heat Treatment in *Adenanthera pavonina* L.

S.No	Temperature (C°)	No of seeds germinated
1	30	*
2	40	3.66± 0.46 (30)
3	50	4.66± 0.46 (40)
4	60	1.2± 0.46 (10)
5	70	*
6	80	*

*Germination percentage is written within brackets and * represents zero.*

III. Light treatment:

At different light treatments (white light, red light, far red light and darkness) , the seeds of *Adenanthera pavonina* L. showed highest of 70% germination at 36 hours in darkness and least germination of 10% was observed in white light and far red light at the same time interval. Germination percentage was observed to be very low at 48 hours of treatment in all light treatments. Red light shows better results in all time duration next to darkness treatment (Figure: II; Table: V). Seeds of *Eucalyptus regnans* require light for germination, but those of *E.pauciflora* do not [8]. Among the lights that were used red light favours the germination of seeds next to darkness was observed in our present study.

Figure II: Light treatment

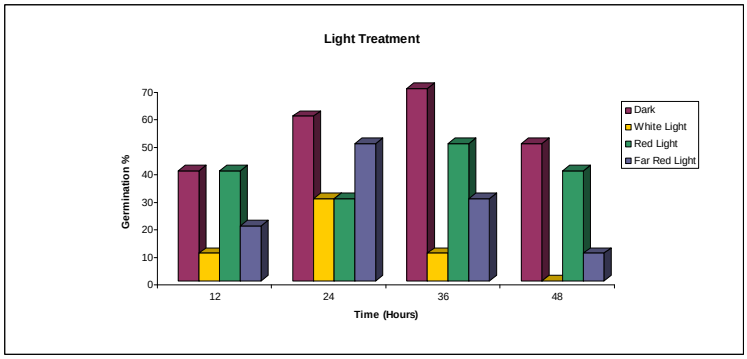

Table V: Seed germination in response to light treatment in *Adenanthera pavonina* L.

Sno.	Time (hours)	No of seeds germinated			
		Dark	White light	Red light	Far Red Light
1	12	4±0.81 (40)	1.5±0.5 (10)	3.66±0.93 (40)	2±0.81 (20)
2	24	6.33± 0.46 (60)	3± 1.29 (30)	4±0.81 (30)	5± 0.81 (50)
3	36	7.33± 0.88 (70)	1.33 ± 0.89 (10)	5±0.81 (50)	3± 1.29 (30)
4	48	5±0.81 (50)	*	4±0.81 (40)	1.33±0.46(10)

*Germination percentage is written within brackets and * represents zero*

IV. Hot water Treatment and Leaching Treatment:

Adenanthera pavonina L. seeds in hot water treatment did not respond to any range of temperature. More or less similar results were obtained in leaching treatment with water at room temperature. Only 10% results were obtained in both 24 and 48 hours treatments. Similar results were observed by Bralewski *et al.*, (2004) in carrot seeds soaked for 24 and 48 hours respectively [5] (Table: VI).

Table VI: Seed germination in response to Leaching in *Adenanthera pavonina* L.

Sno.	Time (hours)	No of seeds germinated
1	24	1.5±0.5 (10)
2	48	1.33±0.89 (10)

Germination percentage is written within brackets.

B. Chemical treatment:

I. Acid scarification:

Adenanthera pavonina L. seeds soaked in concentrated sulphuric acid at different time intervals showed significant difference in seed germination. The treatment of concentrated sulphuric acid promoted seed dormancy breaking in *Adenanthera pavonina* L. in all the time intervals. The germination percentage of the seeds in different time intervals such as 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes and 60 minutes are 10%, 50%, 30%, 100%, 100% and 20% respectively. The germination percentage is highest in seeds soaked for 40 and 50 minutes (100%) and the lowest germination percentage for seeds treated for 10 minutes (10%) (Figure: III; Table: VII) Analysis indicated that H2SO4 concentrations had significant effects on seed germination and final germination percentage. The results showed that the seeds of *Asparagus cyclophyllon* had 55% germination by breaking the seeds dormancy [13]. Among these acids used for scarification treatment concentrated sulphuric acid promoted seed dormancy breaking more significantly than concentrated nitric acid and concentrated hydrochloric acid. The seeds treated with concentrated hydrochloric acid showed very poor germination. Germination percentage analysis of seed germination effect with HNO_3 showed similar results in lower time intervals when compared to concentrated H_2 SO_4. Higher percentage of 100% result was significantly seen in seeds soaked for 50 minutes in concentrated H_2SO_4.

Figure III: Acid scarification

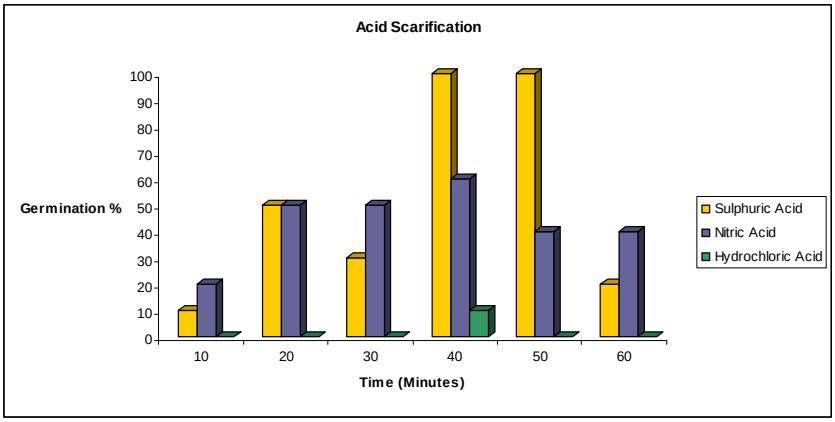

Table VII: Seed germination in response to Acid scarification in *Adenanthera pavonina* L.

S.No	Time (in minutes)	No of seeds germinated		
		Sulphuric Acid (H_2SO_4)	Nitric Acid (H_2SO_4)	Hydrochloric Acid (HCl)
1	10	2 .0± 0.81 (10)	2.10± 0.81 (20)	*
2	20	5 .0± 0.81 (50)	5.40 ± 0.81 (50)	*
3	30	6.66±0.46 (30)	5.5± 0.81 (50)	*
4	40	9.33± 0.66 (100)	6.33±0.46 (60)	2.70± 0. 81 (10)
5	50	9.33±0.66 (100)	4.66±0.46 (40)	*
6	60	1.33 ± 0.89 (20)	4.66±0.46 (40)	*

*Germination percentage is written within brackets and * represents zero.*

II. Inorganic compounds:

The treatment with potassium nitrate did not have much significance on the germination of *Adenanthera pavonina* L. seeds in all concentrations. The highest germination percentage was observed in 0.5M concentration (40%). Whereas, other concentrations with potassium nitrate were noted to be 0% respectively. Like potassium nitrate, ammonium

nitrate also responded only in low concentration (1M) of 10%. The rest of the concentrations (0.25M, 0.5M, 1.5M and 2M) did not show any sign of germination. When comparing both the chemical treatments, no more significant effect on breaking the seed dormancy of *Adenanthera pavonina* L. was observed. (Figure: IV; Table: VIII).

Figure IV: Chemical treatment using inorganic compounds

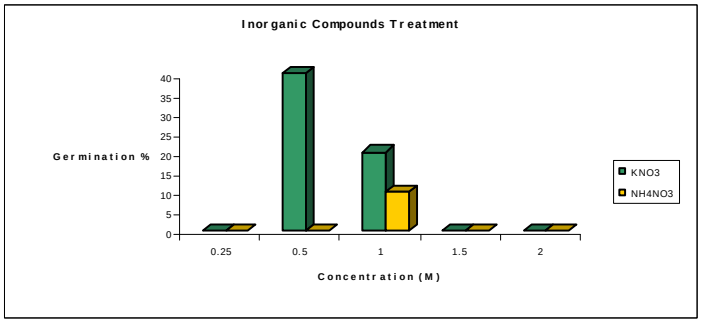

Table VIII: Seed germination in response to Chemical treatment using inorganic compounds in *Adenanthera pavonina* L.

Sno.	Concentration of inorganic compound (M)	Germination response to KNO_3	Germination response to $NH4NO_3$
1	**0.25**	*	*
2	**0.5**	**4.66± 0.46 (40)**	*
3	**1**	**2.33± 0.88 (20)**	**1.5± 0.5 (10)**
4	**1.5**	*	*
5	**2**	*	*

*Germination percentage is written within brackets and * represents zero.*

CONCLUSION

The seeds of the deciduous tree *Adenanthera pavonina* L. are dispersed by wind when the mature pods dry up. The twisted pods dehisce to release the bright red shiny hard seeds. The seeds are dormant and do not germinate readily. The seed germination is prevented by the hard seed coat. The seeds of *Adenanthera pavonina* L. were subjected to a host of physical and chemical treatments to break the seed dormancy. The seeds were subjected to mechanical scarification, dry heat, leaching, light, acid scarification and inorganic compounds. The physical and chemical treatments had a significant effect on breaking the seed dormancy. The seeds have physical dormancy due to the hard impermeable seed coat. It prefers shade and direct sun light reduces germination percentage. This study provides data about the germination of *Adenanthera pavonina* seeds which will be useful in regeneration of this species. This tree is not only ecologically important but it also serves as a source of medicine and livelihood. So it is important to conserve this species. More intense investigation on the reproductive biology of *Adenanthera pavonina* L. is the need of the hour to conserve and regenerate this species.

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