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Effect of Different Pre-treatments on Seed Germination in *Adenanthera pavonina* L.

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Abstract

Adenanthera pavonina L. is an important medicinal plant with colorful seeds. The hard seed coat that covers the seeds causes them to go dormant. This study aimed to compare how various pretreatment techniques affected the germination percentage and mean germination time in *A. pavonina*. The pretreatments involve scraping seeds with sandpaper, immersing seeds in sulphuric acids for 10 minutes and boiling for ten minutes. The findings indicate that the pre-treatments have significant effect on rate of germination. The seeds that were scraped had the highest germination rate (78.33%) and the least mean germination time (6.91 days), followed by acid treatment (68.33%, 16.6 days). However, the seeds treated with boiling water showed the least germination (42.5%, 22.40 days). Environmental conditions like light and darkness did not show significant effect on rate of germination for large-scale seed germination, acid treatment like immersing in sulphuric acid can effectively increase the rate of germination and shorten the germination period because scraping takes a long time for an enormous quantity of seeds.

Keywords: Chemical treatment, Dormancy, Germination, Scrapping

Introduction

Adenanthera pavonina L. belongs to the family Fabaceae, subfamily Caesalpinioideae (The Legume Phylogeny Working Group [LPWG], 2017). The tree is known by a number of common names, including red-bead tree, red wood tree and is often used as an alternative timber of the Red Sandal wood. The tree has been planted extensively throughout the tropics as an ornamental and has become naturalized in many countries. In India, it has been cultivated in many parts of the southern region. Seeds are gaining more attention due to their attractive color and are used as beads in necklaces, jewelry, leis and rosaries and several decorative ornaments (Jaromin et al., 2011; Lim, 2012). Earlier, the seeds were used as weight measures for jewelry, apothecaries and gold smithing, since they have very little variation in their weight (Barwick, 2004; Lim, 2012). Besides this they are also used in curing gout, burning sensation. The plant possess analgesic, anti-inflammatory, anthelminthic, antihypertensive and antibacterial properties (Adeyemi et al., 2015; Hussain et al., 2011; Lim, 2012; Olajide et al., 2004). The plant's seeds are cooked and eaten by the locals (Burkill, 1966). However, studies have shown that raw seeds are toxic and may cause intoxication (Lim, 2012).

Adenanthera pavonina has nitrogen fixing capacity due to the presence of root nodules, which has a symbiotic relationship with certain soil bacteria and is often cultivated as a forage plant in agroforestry systems (Lim, 2012; Useful Tropical Plants, 2024). Decoction of leaves is often used in the treatment of rheumatism and gout (Barwick, 2004). Additionally, the leaves are rich in protein content and thus cooked as vegetables, used as fodder and are also used in making green manures (Lim, 2012; Useful Tropical Plants, 2024). The rapidly growing and spreading tree crown of light, feathery, bipinnate foliage makes it an attractive shade tree, suitable for avenue and roadside plantation (Lim, 2012). Considering its multipurpose uses as well as medicinal and economic value, it has been introduced into several countries including Nepal as well. The native range of this species is Tropical Asia to North Australia (Plants of the World Flora [POWO], 2023), but is cultivated in different parts of tropics for a wide range of uses including avenue tree as well as in agroforestry systems (Huxley et al., 1992), it has been naturalized throughout the tropics (World Flora Online [WFO], 2024). For plant germplasm to be conserved, seed germination and seedling growth are essential processes (Seng & Cheong, 2020). However, dormancy is the state in which certain seeds refuse to sprout even when placed in growth-promoting environments. Many tree seeds exhibit dormancy, which can serve as a useful survival strategy by postponing germination until the environmental circumstances favor active growth (Osborne, 1981). In many plants, the timing of seed germination is regulated by physical dormancy brought on by impermeable seed coverings (Baskin & Baskin, 2014). This is because during maturation drying, the palisade layer of Malphigian cells become lignified which makes this layer impermeable to gases and water (Baskin et al., 2000; Jaganathan, 2016; Rascio et al., 1998).

In *Adenanthera pavonina*, seeds are bright red, dormant and seed coats are impermeable due to the presence of palisade layer throughout the seed coats (Jaganathan et al., 2018). But water gaps called 'lens' or 'pseudolens' are present in seeds through which the water first enters inside the seeds through imbibition (Gama-Arachchige et al., 2013; Jaganathan et al., 2018). When seeds are dormant, they are less likely to germinate under circumstances that would otherwise be favorable (such as high moisture, light and warmth) (Egley & Chandler, 1983; Morrison et al., 1998). Physical dormancy is interrupted when specific 'water gaps' in the seed coat break up, allowing the embryo to imbibate (Baskin & Baskin, 2014). Pretreating seeds before germination has been proven to increase germination rates in a variety of mimetic plant species (Bhandari & Chalise, 2019; Raja et al., 2019) by dislocating the palisade layer and opening the water gaps (Jaganathan et al., 2018).

Adenanthera pavonina is a potential species being widely introduced outside its native range due to its multipurpose uses. Hitherto, very little studies have been done regarding seed germination potential, physical dormancy and pre-sowing treatments of seeds in this species (Bahar, 2015; Joshi et al., 2022; Thilakar & Rathi, 2013; Vineeta et al., 2018). However, studies reflect that physical dormancy due to impermeable seed coat is an adaptive strategy in *A. pavonina* to synchronize germination with its favorable growing season (Jaganathan et al., 2018). Therefore, we aimed to assess the seed germination percentage and mean germination period in *A. pavonina*, subjecting to different pretreatment methods under ambient laboratory conditions.

Materials and Methods

Study species

Adenanthera pavonina is a multipurpose, erect, and medium to large sized, deciduous tree belonging to family Fabaceae. This tree is characterized by the presence of bipinnate leaves, spreading crown, axillary and terminal raceme inflorescence, minute yellow flowers, linear to slightly curved pods and bright red seeds. The flowering starts in March and lasts till May (eFlora, 2024). Pods are green, flattened, with slight constrictions between seeds, glabrous, dehiscing from top to bottom by twisting valves (eFlora, 2024; WFO, 2024). This species was first reported from India and described in Linnaeus Species Plantarum (Linnaeus, 1753). The generic name *Adenanthera* comes after two Greek words 'aden' meaning 'glands' and 'anthera' meaning 'anther' in English.

It grows on a variety of soils from deep, well-drained to shallow and rocky, this tree prefers neutral to slightly acidic soils (Lim, 2012). It can germinate very fast in tropical areas where the temperatures are between 25 °C and 40 °C (Zpevak et al., 2012). It is well recognized for its timber and seeds. Its timber is used as a substitute of red sandalwood and the bright red polished seeds are used as weights and for making beads and jewelries (eFlora, 2024).

Seed collection

Seeds of *A. pavonina* were collected from different places of Udayapur District, Koshi Province during November, 2022. Small sized, injured, and discolored seeds were discarded to maintain uniformity and to control the quality of seeds used in experiment. The collected seeds were subjected to the viability test by immersing the seeds into a beaker containing water. The seeds that floated were regarded as non-viable (Usman et al., 2010). Viable seeds were then air dried and used in the experiment.

Pretreatments and seed germination

Four different pretreatments were used to assess the germination of seeds in two different environmental conditions; light and dark. The four different pretreatments; control, boiling in water for 10 minutes, immersion in concentrated sulphuric acid (acid treatment) for 10 minutes, and physical scarification, were applied to the seeds. Physical scarification was carried out by scrapping the seeds with sandpaper to remove some parts of the seed coat ensuring the embryo is not damaged and seeds can easily absorb water. The acid treated seeds were properly rinsed with tap water, then distilled water, and allowed to air dry for 24 hours before use.

In each treatment, six petri dishes (three in dark condition and three in light) were kept with 20 seeds in each. For dark condition, the petri dishes containing seeds were covered properly with aluminum foil and kept in completely dark cabinets. For the light condition, the experimental setup was performed in normal daylight in glasshouse with light intensity ranging from 535 to 9500 lux in different time period of the day. Petri dishes were lined with filter paper and kept moist with distilled water during the germination study (Zpevak et al., 2012).

When the roots were ≥ 2 mm long, seeds were considered to have germinated. Each day, germination was noted at the same time (11:00 a.m.). Some photographs of the experimental setup are provided in Appendix 1. The germinated seeds were removed and water was added to maintain the saturation of the filter paper. The germination percentage and mean germination time (MGT) were calculated. Germination percentage was calculated based on the total number of germinated seeds divided by the total number of seeds used. MGT is a measurement of the germination rate and time-spread (Bewley et al., 2013). It is equivalent to $\sum(n,t)/\sum n$, where 'n' is the number of newly germinated seeds at Time 't' and 't' is the time in days since the beginning of the experiment (Zhang et al. 2014).

Data were tested for normality (Shapiro–Wilk test, $p > 0.05$). The mean values were compared using one way ANOVA and Tukey's HSD test was used for multiple comparisons between the treatment types. To test the significant difference of means between two populations, unpaired Student's *t* test was performed. All the analyses were done using Microsoft Excel 2016 and IBM SPSS (Version 27).

Results and Discussion

Germination data clearly indicates that pre-sowing treatments had significant influence on the germination of seeds of *A. pavonina* ($F= 110.27$, $p<0.001$; Figure 1). The highest germination was seen in seeds that were scrapped (78.33%) and the lowest germination was seen in control seeds (36.67%). When compared to scraping, acid treatment results in less germination, although it is still higher than control. The reduced germination compared to scraping could be the result of sulphuric acid damaging the seeds (Vineeta et al., 2018). Similarly, mean germination time was found to be less in seeds scrapped with sandpaper and seeds treated with acid in comparison to seeds treated with boiling water and control (Figure 2). Dissolving the tough, impermeable seed coat, chemical treatment significantly contributed to the breaking of physical dormancy of seeds (Egley, 1989; Thilakar & Rathi, 2013).

The hard seed coat serves as a water barrier, causing the seeds to go dormant. The application of chemicals and scraping causes the seed coat to break, increasing its permeability for water absorption (Franke & Baseggio, 1998), thus resulting in increased germination percentage and less germination time. Use of acids gave better results in germination than the control, this might be due to the softening of the seeds' hard coat (Joshi et al., 2022). Seeds treated with boiling water showed slightly higher germination than the control but the difference was not significant (Figure 1). Boiling water also helps to break seed dormancy (Jaganathan et al., 2018). Lower germination in seeds treated with boiling water might be due to the time of treatment, as the seeds were boiled for 10 minutes. Boiling the seeds might have been enough to kill the embryo in the seeds or not enough to break their dormancy (Alves et al., 2004), this might have also resulted in higher germination time. Compared to the control, the use of acids produced improved germination results; this could be because the acids softened the hard shell of the seeds (Joshi et al., 2022).

Pre-sowing treatments has influence on the time taken for germination. Early germination (on 6th day with about 45% germination, MGT=6.91 days) was seen in seeds treated with mechanical scarification i.e. scrapping, followed by acid treatment (Figure 2 and 3). Late germination was seen in seeds without any treatment i.e. control (MGT=23.93 days). Effect of light on the germination of seeds was not significant (t=0.164, p=0.871) (Figure 4). The percentage of seeds that germinated in the light and dark was almost equal.

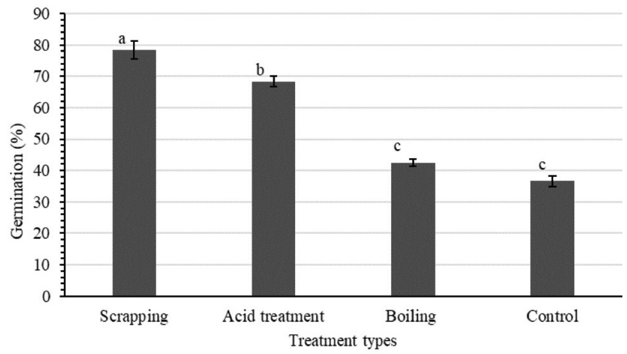

Figure 1: Germination percent (mean±S.E.) in different treatments. Different letters in the graph denote significant differences ($p < 0.05$)

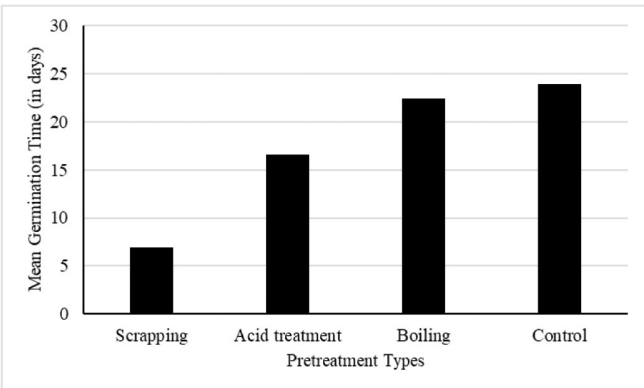

Figure 2: Mean germination time (MGT) in different treatments

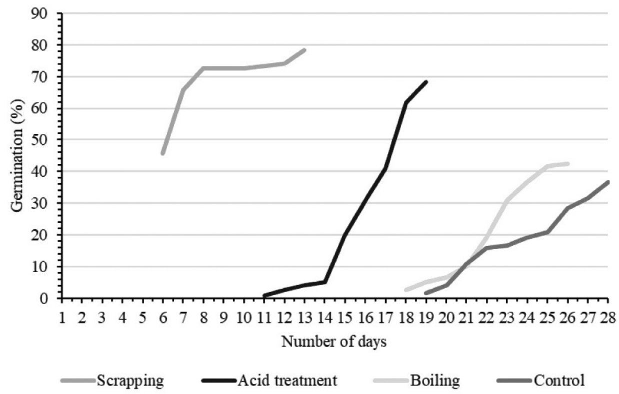

Figure 3: Diagram showing germination percent vs time taken for different treatments

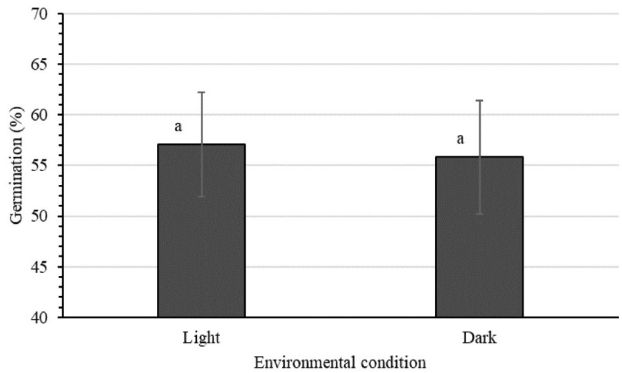

Figure 4: Germination percent (mean±S.E.) in light and dark condition. Same letters in the graph denote not significant differences ($p > 0.05$)

Conclusion

Adenanthera pavonina is an important medicinal plant having seeds with hard seed coat. The presence of hard seed coat prevents seeds from germination resulting in dormancy. To break the physical dormancy produced by the hard seed coat, pre-treatment is required. Pre-treatments have been found to be significantly effective in increasing germination percentage and reduce the mean germination time. Among different pre-treatments applied, scrapping was found to be the most effective, followed by acid treatment. Boiling the seeds did not show significant contribution in increasing seed germination. Seeds without pre-treatments take longer to germinate. Scrapping the seeds is the most effective way to break through the dormancy and shorten the germination period as well as increasing the germination percentage. However, this approach is not practical for mass germination because it requires a lot of labor and time to scrape. If there is a large amount of seeds for germination, chemical treatment may be preferable, but caution should be taken to ensure that acid does not damage the seeds.

Author Contributions

Y R Paneru and P Chalise designed and performed the experiments, analyzed the data and wrote the manuscript. A K.C. assisted during experiment and data collection.

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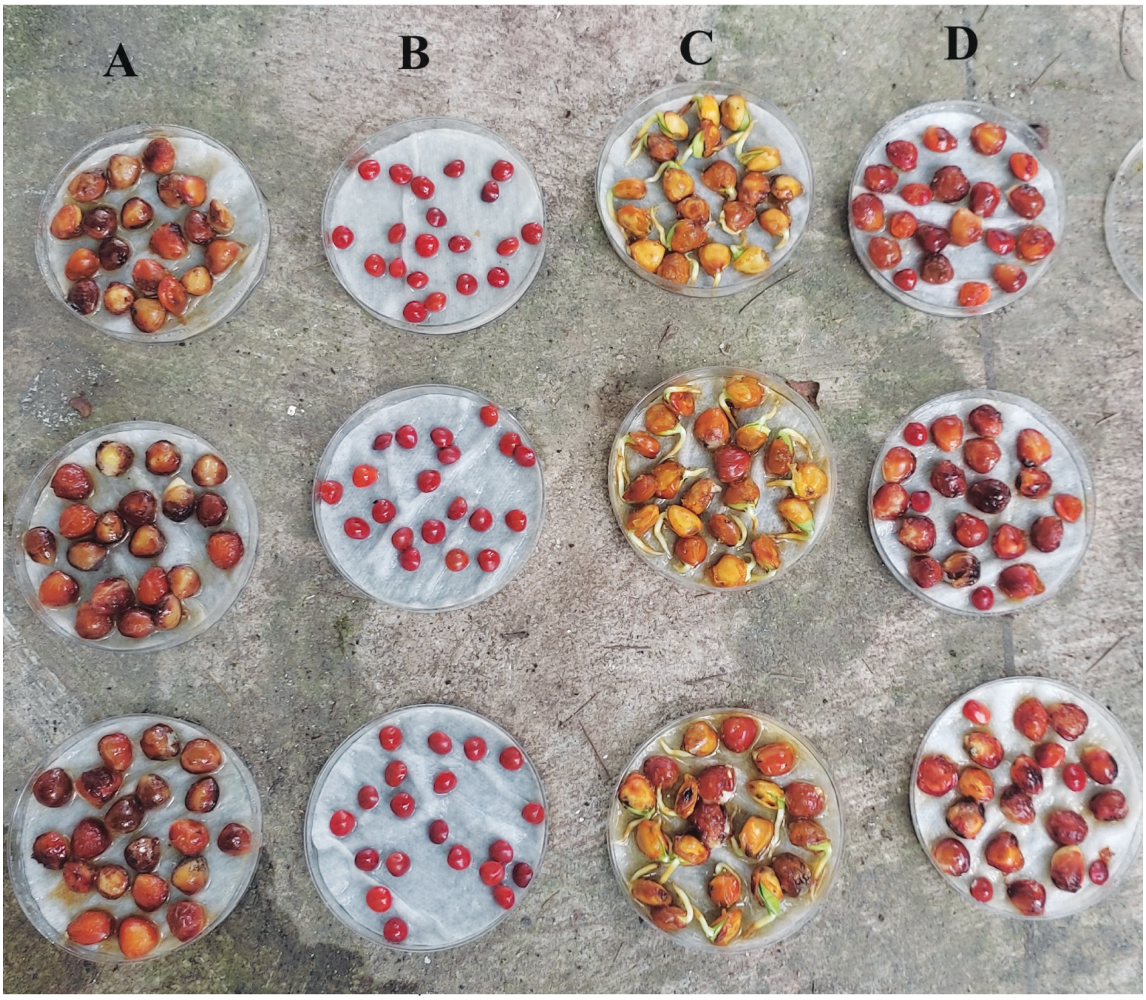
Appendix 1: Some photographs taken during germination test of *A. pavonina*



Seeds in different pre-treatments on 8th day of experiment setup, **(A)** Acid treatment, **(B)** Control, **(C)** Scrapping, **(D)** Boiling

Seedlings on 22th day of experiment setup

Seedlings on 28th day of experiment setup