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Research article

INDUCTION OF SEED GERMINATION IN THE RET MEDICINAL PLANT, JYOTHISHMATHI (*CELASTRUS PANICULATUS* WILLD.)

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ABSTRACT: *Celastrus paniculatus*, one among the RET medicinal plants, shows poor germination under natural conditions. To induct germination, experiments were carried out by using different concentrations of ethyl alcohol at different time intervals (100%, 70%, 50% for 1 minute, 30 minutes, 60 minutes and 120 minutes), hot water with different temperatures (100, 90, 80, 70 and 50 degree Celsius for 1 minute, 2 minutes and 3 minutes), different concentrations of sulphuric acid (100%, 70%, and 50% for 10 seconds, 30 seconds and 1 minutes), rolled towel method and mechanical scarification. Maximum germination rate of 70% was obtained when the seeds were treated with 70% ethyl alcohol for 120 minutes. 50 % germination rate was obtained in the case of seeds treated with rolled towel technique. Treatment with hot water of 70°C for 3 minutes and 2 minutes also resulted in an increased rate of germination than the control. However, sulphuric acid treatment and mechanical scarification has not recorded a promising result in the present study.
Key words*: Celastrus paniculatus*, ethyl alcohol, germination, hot water, rolled towel.

INTRODUCTION

Celastrus paniculatus Willd. (Celastraceae) commonly known as Malkangani, Jyotishmati or Bitter sweet, is an important Indian medicinal plant which is a deciduous, woody forest climber growing mostly in the hilly regions of Northern India at an altitude of 1250 meters [1, 2] and in the lower elevation of (500-700m) of Western Ghats of South India. Oil obtained from the seeds of this plant is a source of herbal medicine, which is used in the treatment of gout, leprosy, skin diseases, fever, rheumatism, beriberi, sores and neurological disorders [3, 4]. Literature also suggests that the seed oil of *C. paniculatus* and its extract exhibit the following actions such as antiviral [5], antibacterial [6], insecticidal [7], analgesic, antinflammatory [8, 9], antifatigue [10], antispermatogenic [11], hypolipidaemic [12], sedative [13] and anti convulsant [14]. Celapagin, celapanigin, celapanin, celastrine and paniculatine are the alkaloids found in the seed oil which are responsible for making the plant medicinally highly potent [15]. Several studies have confirmed the memory- and grasping-power boosting properties of *C. paniculatus* [16, 17]. Apart from these therapeutic applications, its roots are also used in the treatment of cancerous tumours [18].

Researches are being conducted to find out the possibility of application that anticancer drugs like pristimerin, which is derived from the seeds of the *Celastrus* plant, as an effective means of treating or inhibiting the growth of specific types of cancer cells [19]. Yang et.al 2009 [20], conducted studies on pristimerin found it quite active against nine cancer cell lines. Many more such studies are still going on and the potential health benefit of *C. paniculatus* seems very promising.

Naturally, *C. paniculatus* is propagated through seeds. Indiscriminate collection of this plant from the wild has posed a serious threat to its existence in the wild, especially when the plants are harvested well before seed set [21]. Moreover, propagation either by seed or vegetative is rather difficult. However, the viability and germination (11.5 %) of the seeds are poor [22]. Dormancy in freshly harvested seeds of *Celastrus* spp. was primarily related to the inhibitory influence of hard seed coat [23]. Rekha et al., 2005 [22] reported that treatment with petroleum ether; ethyl alcohol and sulphuric acid improved the germination percentage. Alcohol for 3 hours and petroleum ether for 6 h almost doubled the germination percentage. Seed dormancy and germination are regulated by several plant hormones, such as abscisic acid, gibberellin, auxin (indole 3-acetic acid), ethylene, and brassinosteroid. Endogenous concentrations of a hormone are determined by the balance between biosynthesis and deactivation, and contribute to the regulation of physiological responses [24].

Increasing human and livestock populations have affected the status of wild plants, particularly those used in medicines [25]. Owing to its pharmaceutical importance, over-exploitation and poor natural regeneration either by seed or other methods have resulted into depleting population of *Celastrus paniculatus* in natural habitats and therefore, it is currently listed as threatened species [4, 21, 26 -27]. Thus, there is an urgent need to conserve the natural stock and multiply the plants on a larger scale to reduce the dependence on the forests for the supply of raw drugs. Realizing the threat of extinction there is a need to develop propagation protocols, conservation strategies, and commercial cultivation of the plant. Moreover, its cultivation and multiplication will meet the increasing demand of the seed oil in pharmaceutical industry. Hence the present work is aimed at developing the seed germination techniques of *Celastrus paniculatus* Willd.

MATERIALS AND METHODS

The plant material used is the seeds of *Celastrus paniculatus* Willd. Collected from Malabar Botanical Garden, Calicut. *Celastrus paniculatus* flowers in December and produces mature fruits and seeds by March every year. The fruit is a loculicidal capsule each bearing six seeds. Seeds are with reddish brown testa but provided with soft juicy reddish brown aril (Fig. 1D). The function of aril is probably propagation through birds.

The germination experiments were done with ethyl alcohol, sulphuric acid, hot water, rolled towel method and mechanical scarification. The percentage of germination was noted in a time interval of 20, 30, 40 and 50 days in all the experiments.

Treatment with Ethyl alcohol

A total of 10 seeds were taken for each experiment. The seeds were treated with 50%, 70% and 100% of Ethyl alcohol for a time interval of 1, 30, 60 and 120 minutes. After treatment, all the seeds were allowed to germinate by placing them on moist filter paper in a Petridish. The same set of seeds treated with distilled water was taken as control. The percentage of germination was noted in a time interval of 20, 30, 40 and 50 days.

Treatment with Sulphuric acid

The same experiment was repeated here for a time interval of 10 seconds, 30 seconds and 1 minute for the treatment of seeds with Conc. sulphuric acid (H_2SO_4).

Treatment with Hot water

A total of ten seeds were treated with hot water (100°C, 90°C and 70°C) for a time interval of 10 seconds, 30 seconds and 1 minute and seeds treated with distilled water at room temperature were taken as control.

Rolled towel method

The seeds were placed inside rolled towels and were dipped in hot water of 50°C, 70°C and 80°C separately. These wet rolled towels with seeds were kept for 2 days. The seeds were then taken out and placed on separate petridishes (10 seeds in each petridish) and labeled. 10 seeds were placed on wet filter paper without any treatment in another petridish as control.

Mechanical scarification

Seeds of *Celastrus paniculatus* were mechanically scarified, washed with distilled water and placed in a wet petridish. Control was maintained in another petridish on wet filter paper without scarification. The percentage of germination was noted in a time interval of 20, 30, 40 and 50 days.

RESULTS

Ethyl alcohol treatment

Among the seeds treated with various concentrations of Ethyl alcohol at different time periods, seeds treated with 50% ethyl alcohol for 30 minutes showed 20% increase in germination rate when compared to the seeds kept as control. 30% of the seeds germinated on 20^{th} day, 40% on 30^{th} day, 50% on 40^{th} day and 60% within 50 days whereas the control showed 40% germination rate within 50 days. The seeds treated with 70% Ethyl alcohol for 120 minutes showed maximum rate of germination. 20% seeds germinated in the petridish on 20^{th} day of treatment; on 30^{th} day the percentage of germination was found to be 50 and 70% on 40^{th} day. Whereas the control showed a germination rate of 10% on 20^{th} day, 30% within 30 days and 40% within 40 days. i.e., the rate of germination was found to increase by 30% through treatment with 70% ethyl alcohol for 120 minutes. Seeds treated with 100% ethyl alcohol for 1 minute showed 10% germination rate on 20^{th} day and 30% on 30^{th} day. The seeds treated with 70% ethyl alcohol for 1 minute and those treated for 60 minutes and the ones treated with 50% ethyl alcohol for 1 minute showed 10% germination rate in 30 days which is less than that of the control (Fig. 2 & 5).

Sulphuric acid treatment

Zero percentage of germination was observed with conc. H_2SO_4, showing this method is injurious to seeds/embryos.

Hot water treatment

Among the seeds treated with hot water of different temperature, those treated with hot water of 70°C for 3 minutes showed 30% germination rate in 20 days and 50% within 30 days whereas the control showed only 10% in 20 days and 20% in 30 days. So treatment with hot water of 70°C for 3 minutes has enhanced the rate of germination by 20%. 20% of the seeds treated with hot water of 70°C for 2minutes germinated in 20 days and within 30 days 40% of the seeds were germinated. So the rate of germination is increased by 10% when compared with the control which showed a germination rate of 30% within 30 days. 20% of the seeds treated with 70°C hot water for 1 minute germinated on 30th day, which is 10% less than that of the control. Seeds treated with 100°C and 90°C hot water for a period of 1 minute, 2 minutes and 3 minutes each failed to germinate within 50 days (Fig. 3 & 5).

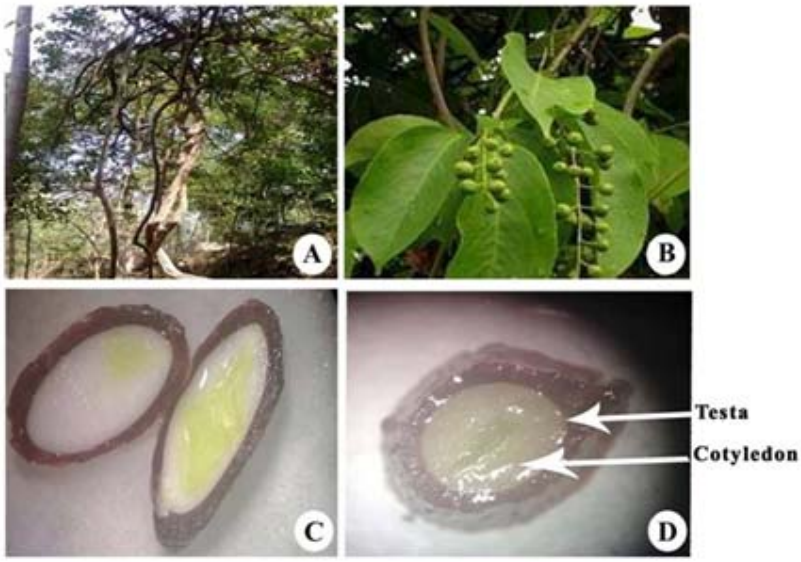

Figure 1: A - Habit of *C. paniclatus*, B - Inflorescence bearing flower buds, C- L.S. of seed and D- T. S. of seed.

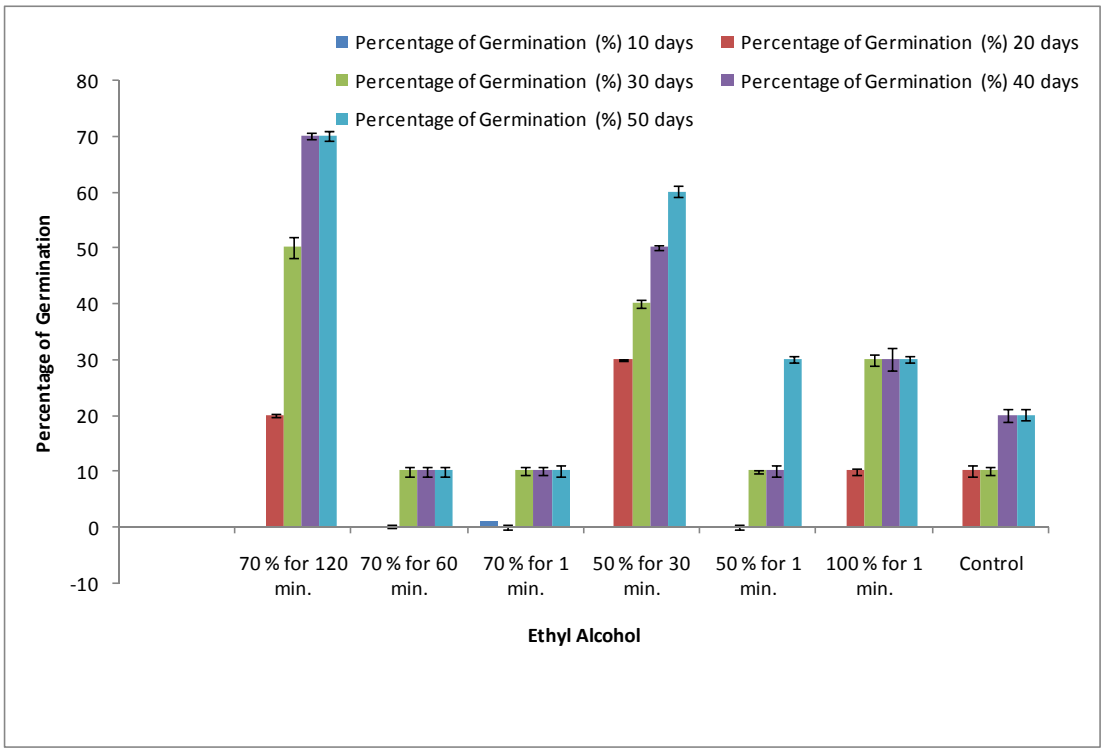

Figure 2: Figure showing the rate of germination at various intervals in the *C. paniculatus* seeds treated with Ethyl alcohol for different concentrations. Here the percentage of germination for 10 days is one in 70% of alcohol for 120 minutes, while the otheres showed zero gerrmination.

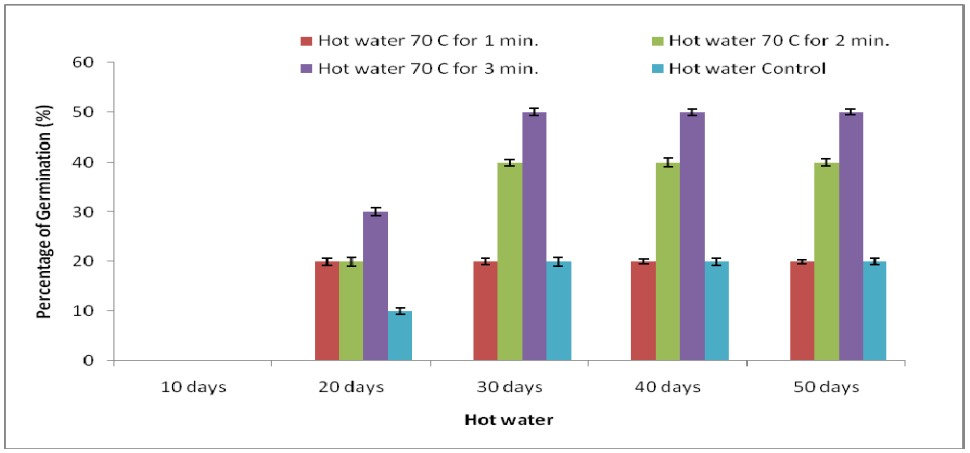

Figure 3: Figure showing the rate of germination at various intervals in the *C. paniculatus* seeds treated with Hot water of different temperature for different durations. The seeds showed zero percentage of germination in 10 days for all the treatments.

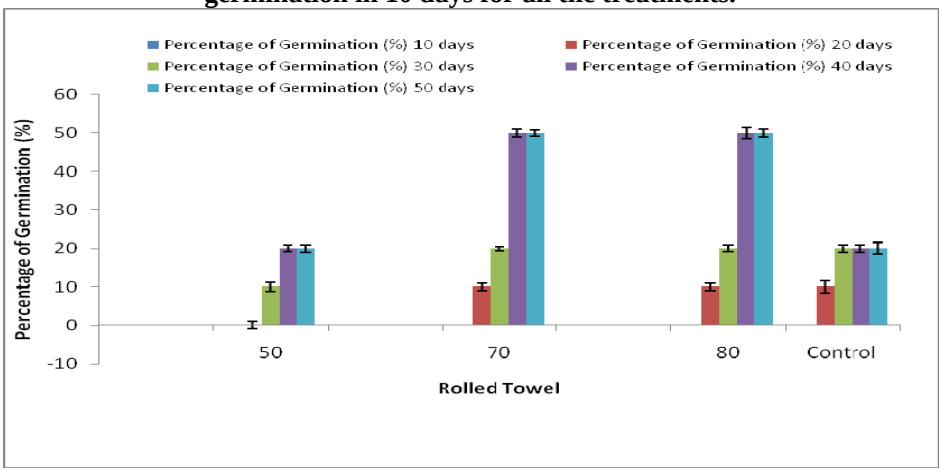

Figure 4: Figure showing the rate of germination at various intervals in the *C. paniculatus* seeds treated with Rolled towel method in different temperature for different durations. The seeds showed zero percentage of germination in 10 days for all the treatments.

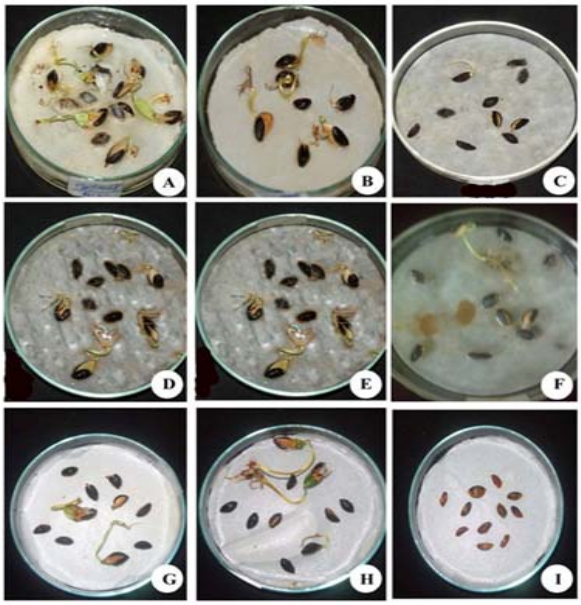

Figure 5: Induction of germination in C. paniculatus seeds. A- in 70 % Ethanol for 120 minutes, B - 50% Ethanol for 120 minutes, C - in 100% Ethanol for 120 minutes, D-Hot water treatment at 70 0 C for 3 minutes, E - Hot water at 70 0 Cfor 2 minutes, F - Hot water at 70 0 Cfor 2 minutes,G - Rolled towel at 80 0 C, H - Rolled towel at 70 0 C and I – Control.

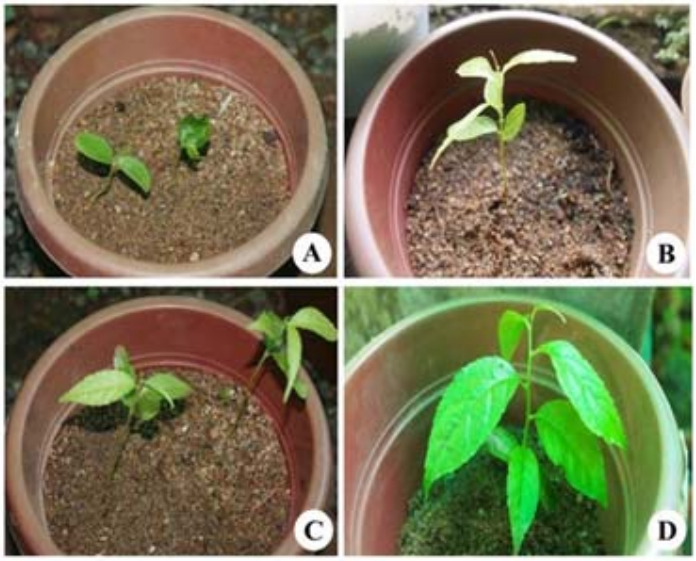

Figure 6: Seedling hardening in *C. paniculatus*. A - Cotyledonary stage, B & C- Different stages of growth in 30 & 40 days and D- seedling ready to transplant on land.

Rolled towel test

Seeds rolled in towel and treated with hot water of 80°C when kept in rolled wet towel for 2 days and changed to a petridish, 10% of the seeds germinated on 20th day. On 30th day 20% of the seeds were germinated, on 40th day 50% of the seeds were germinated whereas the control showed only 20% of germination. Seeds kept in rolled towel and treated with hot water of 70°C also showed 50% of germination whereas the control showed only 30% of germination. Among the seeds kept in rolled towel and treated with hot water of 50°C showed only 10% of germination on 30th day and 20% on 40th day, which is less than that of the control. The results are shown in (Fig. 4 & 5).

Mechanical scarification

20% of the mechanically scarified seeds germinated within 30 days whereas the control showed only 30% of germination within the same time.

Hardening

The germinated seeds were allowed to grow in plastic pots. Different stages of growth were observed for a period of 80 days. The seed germination is of epigeal type in which the cotyledons emerge above the ground. After the root emerges out, each day an increase in length of the root took place by 0.2 cm. As the root increases in length to 0.8 cm the narrow rootlets of 0.2 to 0.4 cm length start emerging, 4 to 5 days after initiation of germination. After 1 week as the root attains a length of 1 cm, the shoot begins elongation. Every day an increase in length of 0.2 cm was observed. On the 14th day, as the shoot attains a length of 2.5cm and the root 1.9 cm the leaf emerges above. The seedlings were planted in soil taken in small plastic pots and kept in mist chamber. The growth of the seedlings was observed and the seedlings obtained through different treatments as well as the control showed normal growth (Fig. 6). The potted plant is now conserved in the RET section of Malabar Botanical Garden.

DISCUSSION

Celastrus paniculatus Willd. (Celastraceae) is an important medicinal plant of the RET category (vulnerable) frequently used in ayurvedic and indigenous medicinal practices. It is a strong woody climber (Liana) naturally found in the Western Ghats but the number of population is very few. Field observation has revealed that the natural seed germination is very poor and less than 1%. Though thousands of seeds are produced by a plant no seedlings are found growing in and around the mother plant. Though the seed remains 1-2 years in the soil, germination does not take place showing that the reason is not only dormancy but also some problems with the viability and germination. This has necessitated artificial methods for augmentation of seed germination using the standard methods. However, conventional germination method has failed in this case and hence artificial methods were employed for the germination of *C. paniculatus* seeds. So the present study envisaged developing a congenial methodology for maximum germination of *C. paniculatus* seeds. Vegetative propagation through stem cuttings was also tried but the success was only 5% even in a mist chamber and hence this programme was discontinued. The various mechanisms usually employed in breaking seed dormancy are treatment with hot water, acid scarification, involving acids (like sulphuric acid, nitric acid, etc.), alcohol treatment, physical and mechanical scarifications, gibberellic acid treatment, etc.

The time required for dormancy break at high temperatures ranges from several weeks to many months, depending on the species [28].

In the present study the control showed a germination percentage of 10- 20%, but only after 30 days in wet petriplates and some of the seed treatments resulted in higher percentage of germination than the control. Maximum percentage of germination (70%) was recorded in seeds pretreated with 70% ethyl alcohol for 120 minutes (Fig. 5A). Pre treatment of seeds with 50% ethyl alcohol for 30 minutes has resulted in a germination percentage of 60% (Fig. 2). No promising results were observed in treatments with 70% ethyl alcohol for 1 minute and 60 minutes where the germination percentage obtained was less than that of the control (Fig. 5 I). Pretreatment of seeds with 50% ethyl alcohol and 100% ethyl alcohol for 1 minute does not give a promising result (Fig. 2). The effect of alcohol on seed germination results from the softening of waxy or other water insoluble compounds in the seed coat.

The improvement in rate of germination in the seeds treated with 70% ethyl alcohol for 120 minutes when compared to those treated with 50% ethyl alcohol for 30 minutes showed that increase in the concentration of alcohol as well as increase in the period of treatment has an enhancing effect on germination. Further increase in concentration of alcohol resulted in a decline in germination rate i.e., when the seeds were treated with 100% ethyl alcohol for the same periods as that of 70% ethyl alcohol, the germination percentage declined indicating the lethal effects of absolute alcohol. Even in the case of 70% ethyl alcohol, when the soaking time of seeds was reduced below 120 minutes the germination percentage of the seeds decreased (Fig. 2). So, the optimum concentration and duration of treatment of *Celastrus* seeds with ethyl alcohol to improve its germination rate could be inferred as 70% ethyl alcohol for 120 minutes (Fig. 5 A).

Pretreatment of seeds with hot water of 70°C for 2 minutes and 3 minutes has recorded a germination percentage of 40% and 50% respectively against the control which has resulted in 30% germination percentage (Fig. 5 D & E). However, the treatment with hot water of 70°C for 1 minute has not given a promising result (Fig. 5F). The seeds treated with hot water of 90°C and 100°C for the same periods as that of 70°C has not germinated (Fig. 3). This shows that the seeds have failed to withstand temperatures around and above 90° C. The increase in germination percentage in seeds treated with hot water of 70°C can be inferred to have occurred as a result of the removal of the cuticle and the palisade layers of the seed coat due to the effect of hot water. According to Corner [29], the seeds showing impermeable seed coat have a palisade layer of lignified cells in the seed coat. Impermeable palisade layers are composed of sclereid cells that have thick lignified secondary walls. The most common type of sclereid cell in palisade layers of seeds is the macrosclereid or malphigian cells [30]. Macrosclereids are impermeable to water because they are impregnated with water repellant substances, including cutin, lignin, quinones, pectic insoluble materials, suberin and wax [31, 32]. In addition to the development of impermeable layers in the seed coat, all the natural seed openings, including the micropyle, hilum and chalazal area, also became impermeable to water [28].

According to Baskin et al., 1999 [33], the endocarp of a mature seed may also contain brachysclereids and osteosclereids in addition to macrosclereids. When treated with alcohol, hot water, etc. these get eroded. Treatment with hot water produces a crack in the seed coat which can act as the site of water entry. But very high temperatures of 90°C and 100°C were found to have injurious effects on the embryo. High temperature could damage the delicate radicle which is immediately below the micropyle as reported by Brown and Buoysen 1969 [34]. So the seeds treated with hot water of these temperatures failed to germinate. Also, the seeds treated with hot water of 70°C for 1 minute has shown only 20% germination rate, but those seeds soaked for 2 minutes and 3 minutes showed a germination rate of 40% and 50% respectively (Fig. 3 & 5).This shows that 70°C can be considered to be the optimum temperature of hot water required for improving germination in *Celastrus* seeds.

Rolled towel method has also resulted in a germination percentage of 50% when the hot water used for dipping the seeds was of 70°C and 80°C (Fig. 5 G & H), against the control (Fig. 5 I) which showed a germination rate of 30%. But hot water of 50°C has not recorded a promising result (Fig. 4). This shows that the enhanced rate of germination occurred due to the softening effect of high temperature of 70°C and 80°C on the seed coat. Germination proceeds once the seed coat is made permeable. In the present study mechanical scarification has resulted in a germination rate of 20%. Scarification aims to abrade the seed coat so as to permit water absorption.

Piercing, chipping, filing the testa of individual seeds with abrasive paper is a technique which is usually considered to be the most reliable method of pretreatment. But in the present study the percentage of germination obtained was less than that of the control. The seeds treated with sulphuric acid fail to germinate. Sulphuric acid of 100%, 70% and 50% concentrations were used in treating the seeds each for a period of 10 seconds, 30 seconds and 1 minute which was found to be detrimental. So the highest germination percentage obtained among all the treatments and the control was 70% obtained in the treatment with ethyl alcohol (70%) for 120 minutes (Fig. 2 & 5A).

The present study scope with the seed germination techniques reported by Rekha et al., [22] in *C. paniculatus.* They found that gibberellic acid showed an increased rate of germination of 74.75%, ethyl alcohol treatment for 1 hour resulted in 26.20%, treatment of petroleum ether showed 22% germination rate, acid scarification 20.50%, and mechanical scarification resulted in a germination percentage of 22.75% against the control which showed a germination percentage of 11.5%.

The non germination of seeds in the near vicinity of the mother plant indicates that the seeds of *Celastrus paniculatus* are naturally dormant. However, the germination of seeds subsequent to hot water treatment and alcohol treatment indicates that these seeds possess 'seed coat imposed' or 'mild type of seed coat imposed dormancy'. This is generally because of the suberin and tannin materials present on glazing materials over the seed coat. Upon the application of hot water and alcohol, these materials dissolve and enable the seed for germination. In fact the delay in seed germination is an ecological mechanism for not germinating in unfavorable condition where the seeds are immediately disturbed. However, these seeds will germinate when they are transported to such conditions where the seeds can germinate and develop into a mature plant in future. The germination of seeds in control after 30 days is due to the decay of the outer layer of the seed coat.

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