

RESTORATION OF *Santalum album* L. RESOURCE IN SRI LANKA: DISTRIBUTION, SEED STORAGE, GERMINATION AND ESTABLISHMENT

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ABSTRACT

Santalum album L., native to India, Indonesia and Sri Lanka, is the best quality oil yielding species among all members of the genus *Santalum*. Due to the high value of heartwood and oil, this species is illegally harvested in large numbers which can lead to possible extinction. Although it has been named as a protected species since 1964 in Sri Lanka, still the illegal harvesting is very high and the regeneration of this valuable resource is also hindered due to the slow growth rates and low germination rates. Moreover, all *Santalum* species are hemi-parasitic in nature so that they absorb certain nutrients from other plant species through the connections made *via* roots. Due to the decline at rapid phase, it is essential to take certain steps to restore *S. album* resource in Sri Lanka before it becomes almost extinct. Following this objective, the present paper describes the current distribution of *S. album* in Sri Lanka and attempts made for enhancing the germination rate and initial growth rate of the seedlings by means of seed treatments, seed viability loss and the effect of different host species at the seedling stage. According to the recent findings, *S. album* has a wider distribution in Sri Lanka despite of the belief that the best oil yielding trees grow in Badulla-Welimada region. According to the results of the experiments conducted, 0.05% Gibberellic acid was identified as the best seed treatment and the seed viability loses rapidly with the storage time. *Desmodium triflorum*, *Mimosa pudica* and *Clitoria ternatea* were identified as the best host species for the seedling stage of *S. album*.

Key words: Protected species, *Santalum album*, Seed treatments, Seedling hosts

INTRODUCTION

Sandalwood oil extracted from the heartwood of certain member of the genus *Santalum* (family: Santalaceae) has been used for perfumery, medicinal, religious and cultural purposes over centuries of years. In addition to oil, the wood and its powder are used for religious, cultural and medicinal purposes, especially in the Asian and Arab regions.

Although there are about 18 sandalwood species belonging to the genus *Santalum*, which are *S. freycinetianum*, *S. haleakalae*, *S. ellipticum*, *S. peniculatum*, *S. pyrularium*, *S. involutum*, *S. boninese*, *S. insulare*, *S. austrocaledonicum*, *S. yasi*, *S. macgregorii*, *S. accuminatum*, *S. murrayanum*, *S. obtusifolium*, *S. lanceolatum*, *S. fernandezianum*, *S. salicifolium* and *S. spicatum* (Fox, 2000), only a few species produce commercially valuable sandalwood oil. Among these few oil yielding species, *S. album* is renowned for its oil, which is highly rated for its sweet, fragrant, persistent aroma and the fixative property which is in high demand by the perfume industry (Joulain *et al*, 2012; Subasinghe, 2013; Xiaojin *et al*, 2011). All *Santalum* species are identified as obligate wood hemi-parasites, which means they absorb certain nutrients such as phosphates and nitrates from the host trees via root connections called haustoria (Brand *et al*, 1993; Fox, 2000).

The global distribution of *Santalum* species occur between 30 degrees N and 40 degrees S and these are mainly found in Australia, Fiji, Hawaii, India, Indonesia, Sri Lanka, Timor *etc.* Out of the 18 species mentioned above, about 6 species can be found in Hawaii Islands which shows the highest *Santalum* diversity (Senok, 2012; Septiani, 2012). *S. albumis* is found in the tropical mountainous regions of Southern India, Sri Lanka and several islands of the Indonesian archipelago. In Sri Lanka, *S. album* has been identified and harvested from the wild for traditional medicines since the known history (Subasinghe *et al*, 2013a).

Natural sandalwood resources are diminishing in all countries irrespective of the species and therefore, those states had to implement strict rules over harvesting and exporting sandalwood products. This, however, may have severely reduced the sandalwood product supply to the market causing rapid increase of price over a short period of time. This fact will remain until significant contributions from *Santalum* plantations occur, most probably by the private sector.

2 Sri Lanka context

S. album is commonly found in Badulla-Welimada region of the Uva province of Sri Lanka and the trees growing in this area are well-known for producing high quality oil. Badulla-Welimada region receives an annual rainfall of 1,000mm to 1,500mm which is a low amount when compared with the most of hilly areas of the country. The mean temperature varies from 22.5 to 25.0°C in this region which is about 2 to 5°C higher than the most mountainous areas. However, during the study conducted by Subasinghe *et al* (2013b), it was possible to identify that *S. album* has a wider distribution in the country as shown in Figure 1, covering all three climatic zones, *i.e.*, wet, intermediate and dry. However, it was not identified in high mountain areas where the elevation is more than 1,500m with temperature is less than 18-20°C and annual rainfall is higher than 2,000mm.

S. album grows at a slow rate and the average annual growth rates of diameter and height are about 0.3 to 1.0cm and 0.5 to 1.0m respectively at the young stage (Subasinghe *et al*, 2013b). According to the study conducted by Samarasekara (2013) on host-sandalwood relationship in three different areas, it was found that *Neolitsea cassia*, *Lantana camara*, *Tecoma stans*, *Ligiistrum robustiim* and *Psidium guajava* were the most common hosts of wild-grown *S. album* in Badulla-Welimada region. *Artocarpus heterophyllus*, *Persea americana*, *Erythrina variegata*, *Mangifera indica* and *Eucalyptus* species were also identified as hosts in the same region at lower frequencies. *Azadirachta indica*, *Phyllanthus polyphyllus*, *Tectona grandis* and *Zizyphus oenoplia* were the common hosts in Kurunegala district while *A. indica*, *Feronia limonia*, *Z. oenoplia* species were common hosts in Hambantota district.

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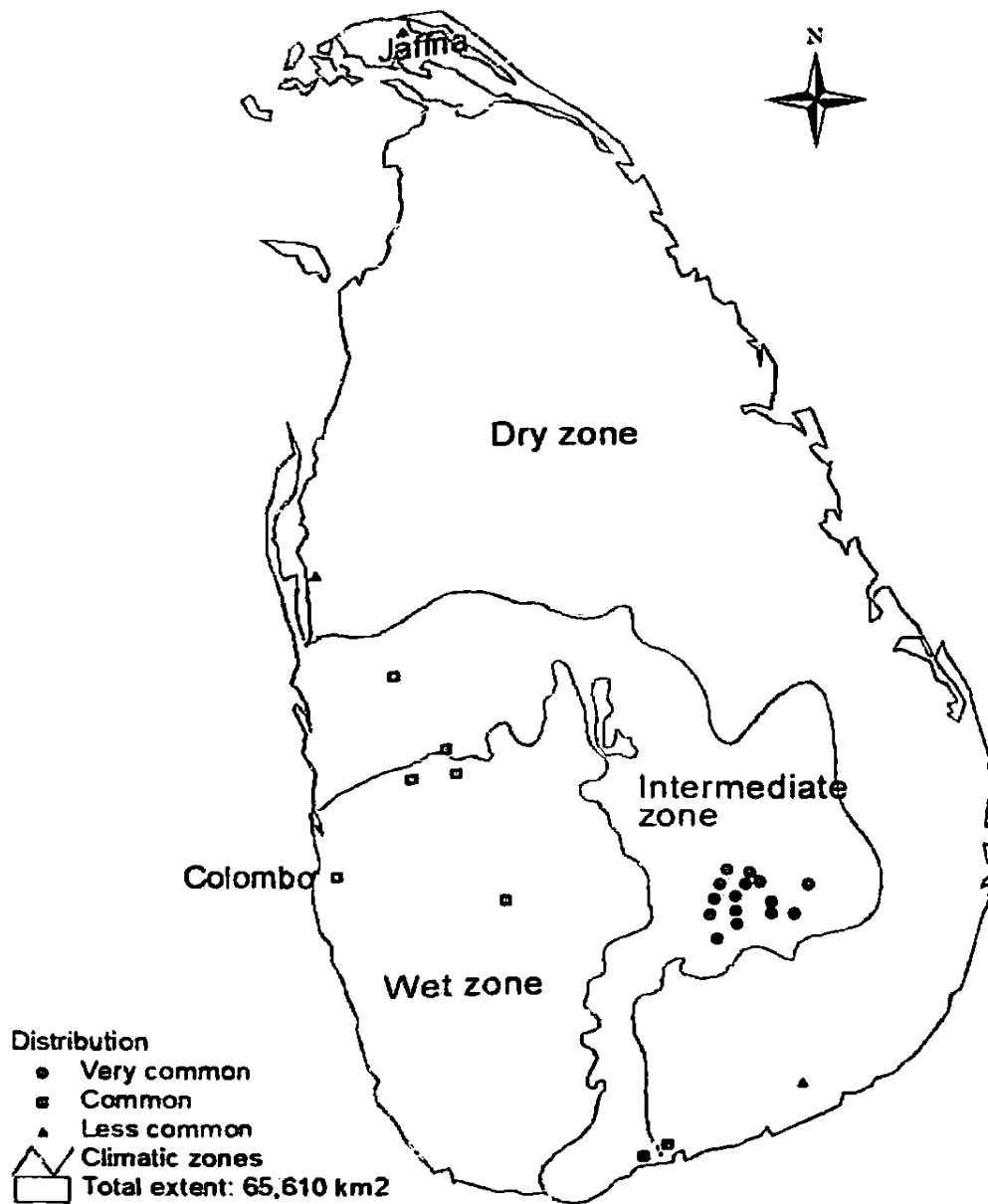


Figure 1. Sandalwood distribution in Sri Lanka (Source: Subasinghe *et al*, 2013b)

Illegal felling is the main threat to the *S. album* resource in Sri Lanka. Due to the high value of oil, 1 kg of heartwood is priced at USD 31.25 to 58.60 (Subasinghe *et al*, 2013b). However, these prices are highly depended on the quality. Due this high value, poachers fell not only the mature trees, but also young and immature ones which contain very low amounts of heartwood. Those immature trees do not produce considerable oil amounts and therefore the leftovers, after seizing of the stem parts by the poachers, do not bear the commercial value. Despite of the attempts made by the villagers to protect the trees (Figure 2), mostly the trees growing in the home gardens are illegally felled at mid night and very early morning. After felling the tree, a 1 to 2m log is taken away leaving the top and butt regions at the site. Due to the difficulty of protecting and due to the lack of an easy procedure of selling the trees, the attempts by the villagers in cultivating this valuable species in large scale are very low.



Figure 2: Attempt in protecting a homestead tree by wrapping a tin sheet around the lower stem

Due to the low natural germination and high illegal harvest, *S. album*, has been identified as a species under threat for many years. The protected fauna and flora list in Sri Lanka was declared in Fauna and Flora Protection Ordinance, Chapter 469. *S. album* was included into this list in the 2009 amendment (Fauna and Flora Protection Amendment Act No. 22, 2009). It has also been mentioned in the same Act that this law can be overruled if another regulation becomes more effective. However, due to the above mentioned documented protection status, felling and harvesting of *S. album* have become a lengthy procedure. Further, export of *S. album* oil is prohibited without the permission of the Department of Wildlife Conservation.

METERIALS AND METHODS

3.1 Effect of different seed treatments on germination

Since *S. album* seeds bear a low germination rate, different seed treatments were recommended in the past (Annupurna *et al*, 2006, Nagaveni and Shrimathi, 1985). In order to identify the best seed treatment method, seeds were collected in October 2012 from about 15 year old healthy *S. album* trees growing in Ella Arawa of Welimada, Badulla and they were de-pulped by soaking in water. De-pulped seeds were then thoroughly washed with water to remove the seed coat and were air-dried to remove the moisture. Those seeds were treated with different media listed in

Table 1 after one week from the collection. After treating, those were separately sown in sand beds and the number of seeds germinated after two months was counted.

Table 1: Seed treatments used and duration of soaking

Treatment	Duration of soaking
0.05% GA ₃	12 hours
Water at R.T.	12 hours
0.1% BAP	24 hours
50 mM KNO ₃	24 hours
150 mM KNO ₃	24 hours
Conc H ₂ SO ₄	10 minutes
Conc H ₂ SO ₄	20 minutes
Conc H ₂ SO ₄	30 minutes
Scarification	-

3.2 Effect of seed storage on germination

Seed storage has an influence on the success of the most of the regeneration attempts of *S. album*. Due to the high dormancy of sandalwood seeds (Doran, *et al*, 2005), an experiment was conducted to identify the relationship between the seed storage time and the germination. Seeds were collected for this purpose from the same trees mentioned in the above section. De-pulped and air-dried seeds were loosely packed in cotton bags and stored under dark conditions at room temperature. Based on the results of the experiment conducted on the seed treatments, it was decided to use 0.05% GA₃ for this trial.

Starting from a storage period of 3 weeks, 100 seeds from the seeds stored were treated with 0.05% GA₃ for 12 hours and then the seeds were sown in sand beds. This process was continued at one week intervals up to the 12th week and then it was continued at two week intervals up to 32 weeks. Water was used as a control for soaking at four week intervals.

3.3 Impacts of the seedling host on the initial growth

Since *S. album* is a hemi-parasitic tree, it needs the support of another species for the growth even from the seedling stage. Therefore, different hosts were tested with *S. album* seedlings using 18×30cm poly pots filled with sand:soil:copost at 1:1:1 ratio. The seedlings of the selected hosts listed in Table 2 were transplanted into pots a week prior to transplanting the *S. album* seedlings. Two host seedlings of same species were planted in one pot opposite to each other, closer to the pot edge. *S. album* seedlings of 10.0-12.0cm in height, which were raised from seeds, were transplanted in the middle of the pot. Foliar fertiliser was used once in two weeks as a separate treatment for the *S. album* pots which did not contain any host. Hosts were not planted

in the control treatment and neither was the fertiliser added. 10 replicates were used for each treatment.

Table 2. Selected host species

Scientific name	Vernacular name
<i>Alternanthera species</i>	Malmukunuwenna
<i>Cassia tora</i>	Pethi Thora
<i>Clitoria ternatea</i>	Katarolu
<i>Desmodium triflorum</i>	Undupiyaliya
<i>Mimosa pudica</i>	Nidikumba
<i>Tagetes erecta</i>	Daspethi

Seedlings were kept under 50% shade for four months from the transplanting date. Watering was done once in two days, about 200 ml per pot. Chlorpyrifos, an insecticide, was sprayed once a month to avoid insect attacks on all seedlings. Weeding was manually carried out whenever required. The height growth of the *S. album* seedlings were measured weekly from the date of sowing.

RESULTS AND DISCUSSION

4.1 Effect of different seed treatments on germination

Table 3 illustrates the results of the seed treatment experiment. Seeds treated with 0.05% gibberellic acid (GA₃) showed 15% germination rate and 0.1% benzyl amino purine (BAP) showed 10% germination rate after one week of sowing. Seeds treated with 0.05% GA₃, 1% BAP and scarification, showed a germination percentage of 60%, 10% and 5% respectively after two weeks. According to the results, 0.05% GA₃ was identified as the best treatment for *S. album*.

Table 3. *S. album* seed germination % with different seed treatments

Treatment	Germination %	Germination %
	After 1 week	After 2 weeks
0.05% GA ₃	15	60
Water	0	2
0.1% BAP	10	10
50 mM KNO ₃	0	0
150 mM KNO ₃	0	0

Conc H ₂ SO ₄	0	0
Conc H ₂ SO ₄	0	0
Conc H ₂ SO ₄	0	0
Scarification	0	5

4.2 Effect of seed storage on germination

The change of germination percentages with the seed storage time are given in Figure 3. The germination was over 80% at the 3rd week of storage when soaked with 0.05% GA₃ and it was less than 40% when soaked with water. However, from the 12th week, both treatments produced approximately similar results (Figure 3). Germination of 50% when soaked with 0.05% GA₃ was achieved at the 7th week of storage and the germination was 0% after 28 weeks of storage under the same treatment.

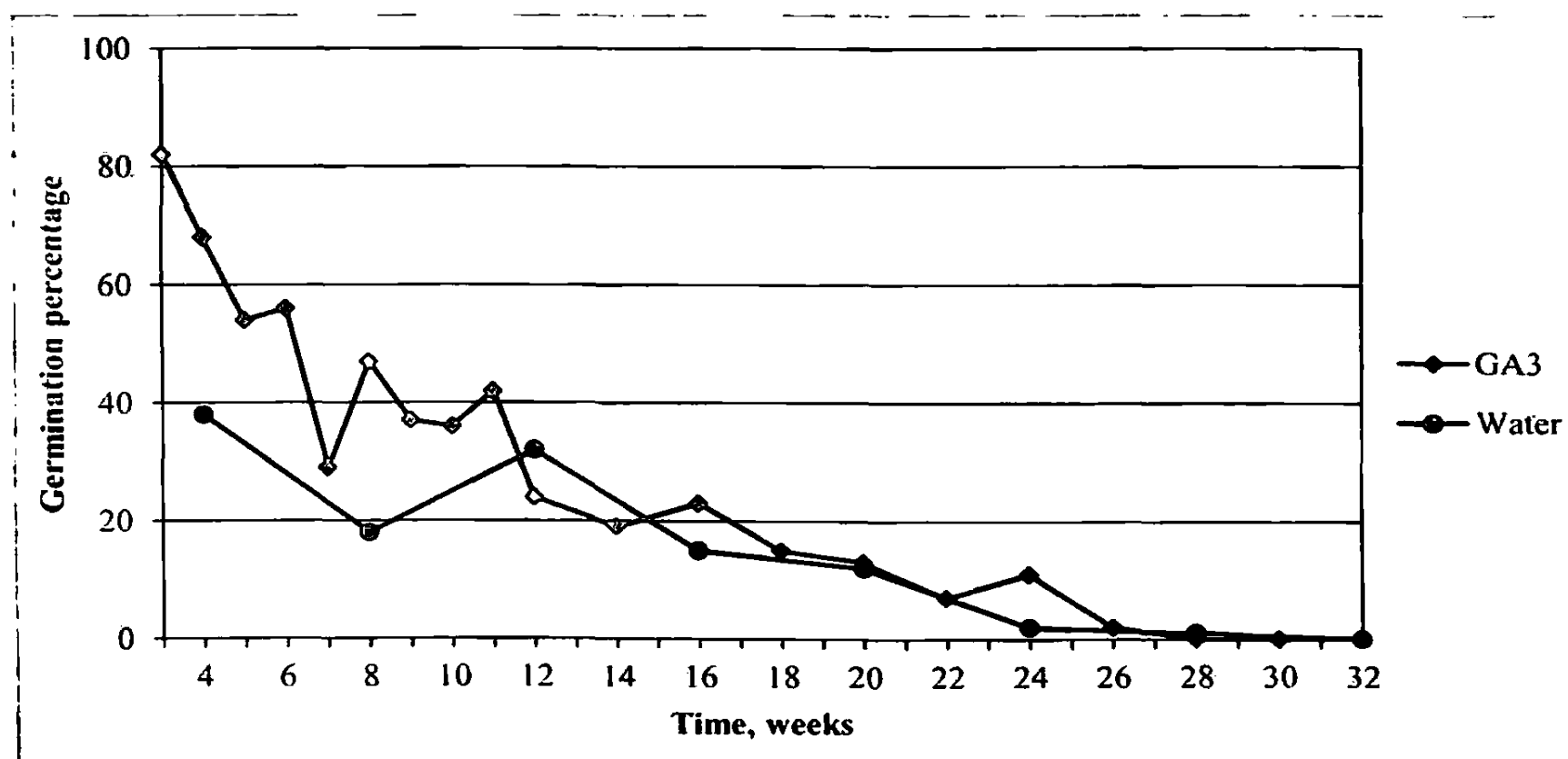


Figure 3. Germination percentage of sandalwood seeds with storage time

4.3 Effect of the seedling host on the initial growth of *S. album*

Figure 3 illustrates the height growth of *S. album* seedlings grown with different hosts. According to that figure, the maximum height growth of *S. album* seedlings was produced by *D. triflorum* followed by *M. pudica* together with *C. ternatea*. At the 24th week, the average height of the *S. album* seedlings grown with *D. triflorum* was 18.0cm was taller than the control. *M. pudica* and *C. ternatea* were identified as the next best hosts among the tested ones. Height growth of the seedlings planted with *T. erecta*, *C. tora* was the lowest, showing no difference to the control and the fertiliser applied *S. album* seedlings.

Significant results were shown for the *S. album* seedling height growth by the Kruskal-Wallis test. Growth of the *S. album* seedlings grown with the *D. triflorum* was significantly taller than the rest of the treatments. The next significant results were found for the *M. pudica* and *C. ternatea*.

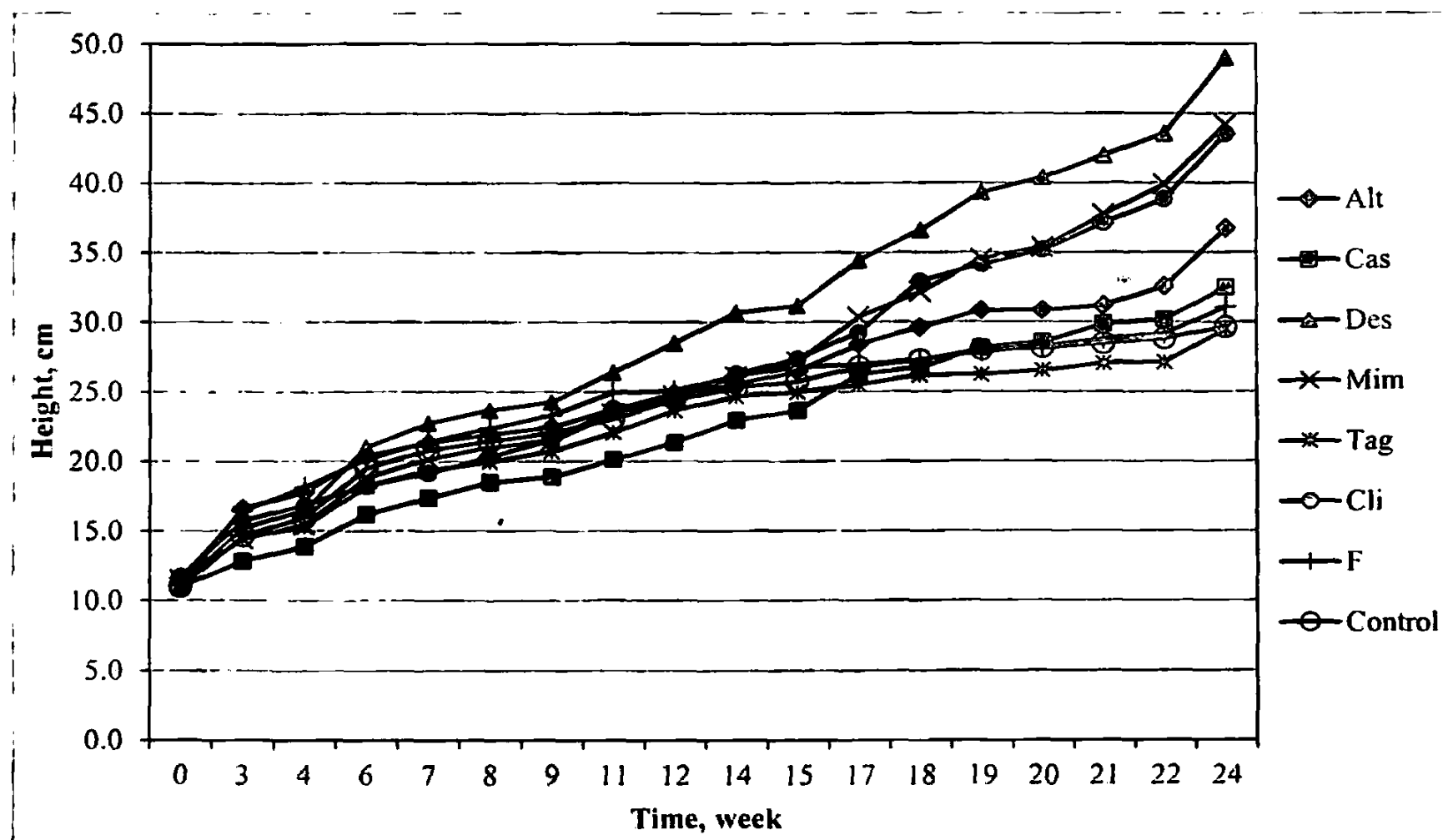


Figure 3. Height growth of *S. album* seedlings grown with different hosts -*Alternanthera* species, Cas-*Cassia tora*, Des-*Desmodium triflorum*, Mim-*Mimosa pudica*, Tag-*Tagetes erecta*, Cli-*Clitoria ternatea*, F-Fertiliser only, Control-No host used

Various pre-treatments have been tested in the past to accelerate the germination of sandalwood seeds. Scarification and soaking in gibberellic acid have been identified as the most successful methods by various researchers (Nagaveni & Srimathi, 1985; Rai, 1990; Tennakoon *et al*, 2000). Moreover, there can be other treatments which could enhance the seed germination in order to provide fast and uniform germination. However, though scarification has been mentioned as a good method by some researchers (*e.g.* Doran *et al*, 2005; Rai, 1990), it was not effective in this experiment.

A seed collection is economically valued if at least 50% or more of the seeds are viable. The viability of sandalwood seeds is decreased with the length of time in storage (Scharpf, 1970). Similarly, the results of the seed storage trial indicated a negative relationship between the

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storage time and the germination. Tennekoon *et al* (2000) identified the same pattern when *S. album* seeds were stored in cotton bags at room temperature. However, the germination percentages observed by them were slightly higher than the results of the present study, which could be due to the genetic variations of the mother trees used to collect the seeds.

Some researchers recommended *Alternanthera* as a good host for the growth of *S. album* in the seedling stage. However, in the present study it was not identified as the best host. *Desmodium triflorum*, *Mimosa pudica*, *Clitoria ternatea* and *Cassia tora* are the leguminous plants tested in the present study as seedling hosts while *Alternanthera* and *Tagetes erecta* are non-legumes. The results of the present study confirmed that other than *C. tora*, the rest of the leguminous plants used can significantly enhance the growth of *S. album* seedlings. In addition, the growth results of the foliar application on sandalwood seedlings did not show a favourable effect on growth compared to the seedling hosts.

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