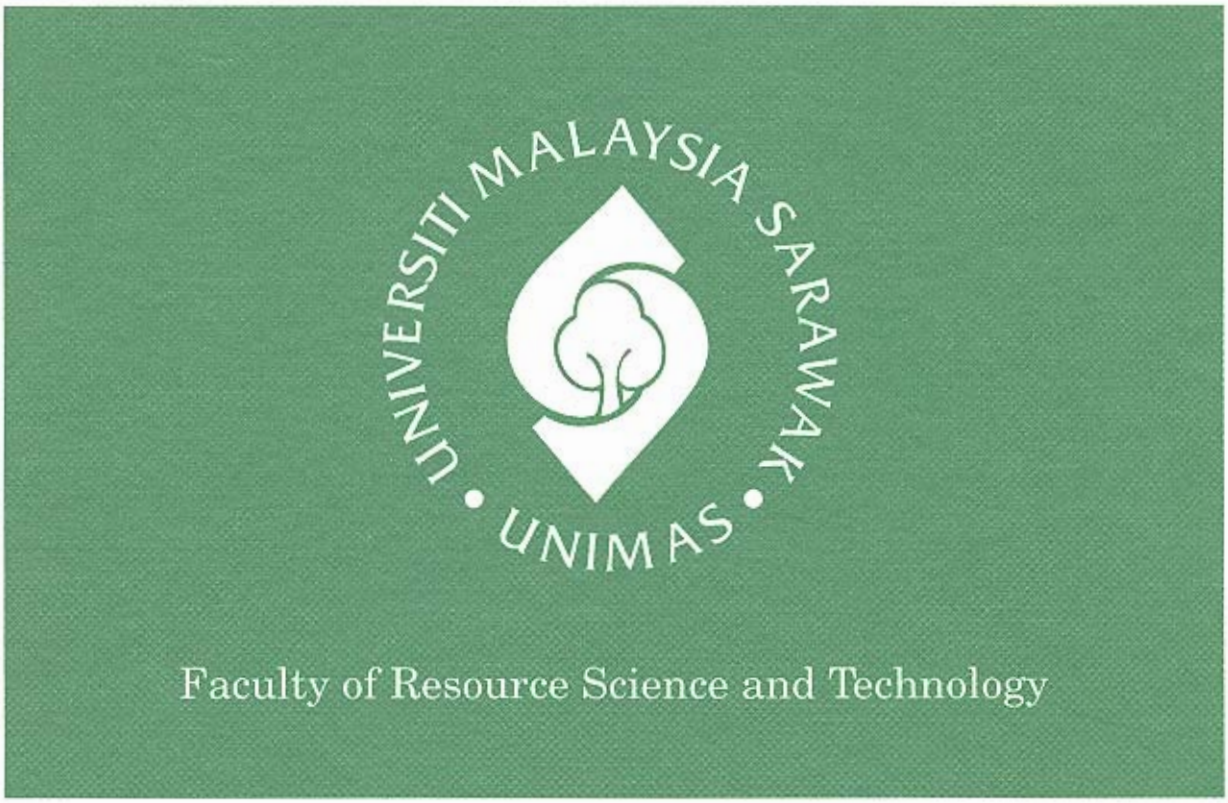

VIABILITY OF *SHORA RESINOSA* FOXW. SEEDS FOLLOWING DEHYDRATION TECHNIQUE

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Bachelor of Science with Honours
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ABSTRACT

A study was conducted to evaluate the suitable dehydration techniques to preserve *Shorea resinosa* Foxw. seeds. The dehydration techniques used and compared were silica gel, laminar flow and sucrose solution. Initial germination and moisture content of the seeds used were 85% and 67.38% respectively. Dehydration using silica gel for 48 hours gave the highest germination, which was 92.5% with moisture content of 47.71%. The highest germination obtained was dehydrated using laminar flow for 6 hours and sucrose solution at 0.6M for 120 minutes were 87.5% and 40% with the moisture content of 46.53% and 52.36% respectively.

Keyword: *Shorea resinosa* Foxw seeds., dehydration, germination and moisture content.

ABSTRAK

Kajian ini dijalankan untuk mengkaji kesuaian teknik dehidrasi untuk tujuan penyimpanan jangkamas panjang bagi biji benih Shorea resinosa Foxw. Teknik dehidrasi yang digunakan adalah dehidrasi 'silika gel', 'laminar flow' dan larutan sukrosa. Peratus percambahan dan kandungan kelembapan awal bagi biji benih masing-masing adalah 85% dan 67.38%. Teknik dehidrasi menggunakan silika gel dengan tempoh pendedahan selama 48 jam memberikan peratusan percambahan yang terbaik iaitu 92.5% manakala peratus kelembapannya adalah 47.71%. Bagi teknik dehidrasi menggunakan laminar flow yang didedahkan selama 6 jam dan larutan sukrosa (0.6M) yang didedahkan selama 120 minit masing-masing mempunyai peratus percambahan sebanyak 87.5% dan 40%. Manakala peratus kelembapan pula ialah 46.53% dan 52.36% bagi kedua-duanya.

Kata kunci: Biji benih Shorea resinosa Foxw., dehidrasi, percambahan dan kandungan kelembapan.

CHAPTER 1

1.0 INTRODUCTION

Shorea resinosa Foxw. is a member of the Dipterocarpaceae family. *S. resinosa* also known as remesa, similar to meranti and meranti belang. The plant is widely distributed throughout Sri Lanka, India, Myanmar through Indochina and Malaysia.

Shorea is the largest and economically most important genus in the family Dipterocarpaceae (Symington, 1943). However, taxonomically, it is a problematic genus and can be confused with other genera, especially *Hopea*. The genus shorea consists of 188 species of mainly rainforest trees and this genus has been divided into 10 groups based on the appendages to the connectives, the form of the anthers and the number of pollen sacs (Ashton, 1982).

Dipterocarpaceae is a family of 17 genera and approximately 580-680 species of mainly tropical lowland rainforest trees with two-winged fruits. The largest genera are *Shorea* (360 species), *Hopea* (105 species), *Dipterocarpus* (70 species), and *Vatica* (60 species). Many are large forest emergent species, typically reaching heights of 40-70 m tall. The species of this family are of major importance in the timber trade. Some species are now endangered as a result of over cutting and extensive illegal logging. They provide valuable woods, aromatic essential oils, balsam, and resins.

S. resinosa is small to large deciduous or evergreen tree. Bark is shallowly fissured. Buds minute, usually ovoid. Stipules are small, caducous or persistent. Leaves not plicate; more or less symmetrical at base; secondary nerves pinnate, curved or rather straight, arched near to margin; intermediate nerves usually absent; tertiary nerves scalariform. Domatia is frequently present. Calyx lobes imbricate, deltoid, 3 outer lobes ovate to ovate-oblong, slightly narrower than the inner ones. Panicles are axillary or terminal. Flowers buds are almost sessile. Petals usually partially joined at base or free, usually pubescent outside, white to creamy, yellow or red. Fruiting calyx lobes developing into wings, 3 lobes slightly or much longer than the other 2, calyx bases saccate thickened. Nut is subglobose or ovoid, free from calyx, apiculate. Cotyledons are fleshy, emergent or not, bilobed, unequal.

Dipterocarpaceae is also economically important and its timber as well as non-timber products have long been recognized in Southeast Asian and other regions. In Malaysia, the main export timbers are mainly produced by *Shorea sp.* The dipterocarpaceae are very valuable source of hardwood timber production in South and Southeast Asia and thus have been heavily logged. Natural regeneration of some species depleted and species diversity is being reduced (Abdulhadi *et al.*, 1981)

There is always some flowering of this shorea every year, however, the amount of flowering and consequent seed production is very small (Ng, 1977). The dipterocarp seeds are produced sporadically and exhibit loss of viability after four days of maturity due to the reduction of moisture content below 37% and become nonviable on the 8 day.

Thus, seeds cannot be preserved or stored for long periods as they lack dormancy. Beside that, the recalcitrant seeds also intolerant to desiccation (Appanah and Cossalter, 1994; Krishnapilly, 1994; Roberts, 1973).

Increasing demands for forest products have risen sharply over the decades and shortage have been forecasted by the end of this century (Keays, 1974). Thus, immediate actions must be taken to conserve this species from extinction. So, the cyropreservation is important and is one of the techniques that can be used to preserve this species for the long term conservation.

The main objectives of this study were to analyze the viability of *S. resinosa* seeds using dehydration technique and to determine the suitable cryopreservation technique use to conserve the seeds as a germplasm for long term storage.

CHAPTER 2

2.0 LITERATURE REVIEW

S. resinosa is a woody plant species of tropical rain forest in South and Southeast Asia. This species is widespread throughout Peninsular Thailand, Peninsular Malaysia (type locality), Borneo, and Sumatra lowlands (sea level) to upper hills (800 m altitude). Flowering of *S. resinosa* usually occurs in September to October (Appanah and Chan, 1981) and the fruit ripen in the following January and February. The yield may continue to late March and April in very favorable years.

2.1 Seed Viability

To most technologists, viability is defined as a seed is capable of germinating and producing a normal seedling. In this context, a seed is either viable or nonviable is depending on its ability to germinate and produce a normal seedling, thus only seed lots representing populations of seeds may exhibit levels of viability. In other words, viability donates the degree to which a seed is alive; metabolically active possesses enzymes that capable to catalyze metabolic reaction needed for germination and seedling growth. That mean, the seed viability is probably highest at the time of physiological maturity, though environmental conditions on the parent plant may not permit germination. And then, after physiological maturity, the viability of seeds gradually declines. The tetrazolium test is widely used for testing the seed viability. This method was developed in Germany in

early 1940s by Professor Georg Lakon (1928). The tetrazolium test can be distinguishes between live and dead tissues of the embryo on the basis of their relative respiration rate in the hydrated state. This test utilizes the activity of the dehydrogenase enzymes that are respiration rate and seed viability. Dehydrogenase enzymes react with substrates and release hydrogen ions to the oxidized, colourless, tetrazolium salt solution. It is change into red *formazan* as it is reduced by hydrogen ions (Copeland and McDonald, 1995).

2.2 Moisture Content

The critical moisture content of the seeds and cell is playing the main role to determine the viability of the seeds and cell. Seed likes soybean and peanut with 11 to 12 percent moisture content and kept at 20^0C will lose its viability over a year of storage (Copeland and McDonald, 1995).

Storage temperature and moisture content of the seed play the important part in the seed viability while in the storage. This is because, its will be influence the metabolism of the seeds and cells. High moisture content will increase the biochemistry activity of the seeds and cells. The increase in biochemistry activity also influences the hydrolytic enzyme activity and enhances the respiration and increase in free fatty acids production. The high temperature enhances the rate of the enzymatic activities and metabolism reaction which will cause rapid rate of deterioration. Thus, high moisture content accelerates deterioration of stored seeds as compared with high temperature. The seeds with low

moisture content kept in liquid nitrogen will store better (Copeland and McDonald, 1995).

Beside that, the storage of seed also dependent on the type of seed, whether the seed is an orthodox or a recalcitrant seed. Recalcitrant seed depends on the critical moisture content for it to be successfully store in a seed bank or in cryostorage. The recalcitrant seed is a short-lived seeds which are usually conserved in field gene bank as the seeds die after relatively brief period of storage (Roberts, 1973).

2.3 Dehydration

There are four types of cryopreservation techniques that are commonly used and these are: dehydration, encapsulation, vitrification and slow freezing. The main purpose of dehydration methods is to induce drought tolerance of the seeds and it have been studied extensively to preserve much kind of seeds in the three ways that were used laminar air flow, cryoprotectant (sucrose solution) and silica gel dehydrations. The dehydration using laminar air flow is successfully to preserve the seeds and embryos. This is proven by the demonstrated the almond seeds which has been dehydrated to 5 – 10 percent moisture content can be cryoperserved without using cryoprotectant (Chaudhury and Chandel, 1995). For dehydration using silica gel is widely used to prevent the seed from contamination by the non-sterile environment. The example is the seeds of tea (*Camellia sinensis* (L.) O. Kuntze) have been dehydrated to 24 percent of moisture content and successfully cryopreserved in liquid nitrogen (Chaudhury *et al.,* 1990). Sucrose is

examples of cryoprotectant that used widely in cryopreservation but others cryopectant that are still available to use such as mannitol, amino acids and dimethyl sulphoxide (DMSO).

CHAPTER 3

3.0 MATERIAL AND METHODS

3.1 Material

Fresh and matured seeds of *S. resinosa* were used in this study, collected from the Forest Reserve in Damai, Kuching. Separation of seeds was carried out to remove damaged, broken seeds and other inert matter to acquire quality seed lot. The seeds were than sterilized by soaking in 70% w/v of alcohol for 4 – 5 minutes. Then, seeds were rinsed three times with distilled water. Afterward, the seeds were placed in laminar flow hood for two hours to remove excess water. The seeds were treated with Captan 50 WP fungicide to protect them from fungi attack, before these seeds were kept in an air-tight bottle and placed in the store.

3.2 Methods

3.2.1 Moisture Content Test

Four replicates of 10 seeds per replicate each were used for evaluation of the moisture content. The seeds were arranged in a single layer in an aluminium plate and weighed to determine their wet-weight. Then, the seeds were put in an oven at 60^0C for 48 hours. After this drying period, the seeds again were weighed and the percentage of moisture content was calculated base on AOSA, 1985.

Formula:

$$\text{Moisture content (\%)} = \frac{b - c}{b - a} \times 100\%$$

Where; a = weight of aluminium plate container

b = weight of aluminium plate container + weight of the seeds before placing in the oven.

c = weight of aluminium plate container + weight of seeds after drying in the oven.

3.2.2 Viability Test

The viability test (tetrazolium test) was conducted using tetrazolium chloride solution to assess the viability of seeds. Respiration from dehydrogenase enzymes in living cells can be estimated by their capacity to reduce 2, 3, 5-triphenyl tetrazolium chloride (TTC) (Towill and Manzur, 1975).

Four replications of 25 seeds in each replication were used immersed in the TTC solutions of 0.1%, 0.5%, and 1.0%. The pH of the solution was in range of 6 to 8 for optimum staining result. After that the seeds were incubated in an oven at 35^0C for durations of 0, 30, 60, 120, 150 and 180 minutes.

After each incubation periods, seeds were withdrawn from the oven and TTC solution rinsed at least for 4 to 5 times with distilled water. The red colour (formazan) indicated

reaction of the solution of TTC with dehydrogenase enzymes in the living cells of the seeds. In the other word, the percentage of viability was calculated as below.

Formula:

$$\text{Viability (\%)} = \frac{a}{b} \times 100\%$$

Where; a = total of red stained seeds

b = total of seeds used

3.2.3 Germination Test

The Standard Germination Test was conducted based on the Rules for Testing Seed (AOSA, 1985). Four replications of 25 seeds each were used and seeds were planted in a uniform layer of moist sand and then, covered to a depth of about one cm in the sand. The trays were placed in Plant Growth Chamber (29^0C) and checked daily. The seeds were assumed to germinate when the radicles were ≥ 5 mm length emerged from the seeds. Total germinated seeds were recorded between 4 – 10 days after planting.

Formula:

$$\text{Germination (\%)} = \frac{a}{b} \times 100\%$$

Where; a = total of germinated seeds

b = total of seeds used

3.2.4 Dehydration

Dehydration of the seeds was conducted in three ways, these were dehydration using laminar air flow, using silica gel in desiccator and using sucrose. A set of four replicates of 25 seeds each were used for each treatment using dehydration technique.

3.2.4.1 Dehydration using laminar air flow

This technique generated in laminar air flow is a modification technique from Chaudhury (1995). Seeds were placed on trays in a single layer to enhance the effectiveness of the drying process. Dehydration of seeds was set at interval of 0, 2, 4, 6 and 8 hours. Every each period of dehydration of seeds were be evaluated for their viability. Prior to evaluation of germination and moisture content, seeds were stored in liquid nitrogen for an hour, following by thawing process for about 15 minutes in the water bath at 35^0C.

3.2.4.2 Dehydration using silica gel

Silica gel was dried at 60^0C in an oven for two days before used for dehydrating materials. About 250 gram of silica gel was placed at the bottom of each desiccator. Then, seeds were put on the petri dish and placed in desiccators to dehydrate for 0, 24, 48, 72 and 96 hours. After each treatment period, moisture content and germination test were conducted. A set of seeds that were obtained from the storage bottle were used as a

control and put in liquid nitrogen for an hour followed by thawing process for 15 minutes, similar as in the treatments.

3.2.4.3 Dehydration using sucrose

This technique used different concentrations of sucrose conducted using 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 M solution of sucrose at intervals of 0, 60, 120 and 180 minutes of dehydration. After each period of dehydration, moisture content and germination test were carried out. A set of seeds were used as a control obtained from the seeds in the bottle and put in liquid nitrogen for an hour followed by thawing for 15 minutes.

3.2.5 Seed Storage

Seeds were stored in four different environments: ambient room ($28^0 - 30^0$C), refrigerator ($3^0 - 5^0$C), cold room (-12^0C) and in oven ($38^0 - 40^0$C). The seeds were stored for two weeks. Moisture content, viability and germination tests were carried out every two days to determine the percentages of moisture content, viability and germination.

Experimental design used for this research is Completely Randomized Design (CRD) and the data were analyzed using the Analysis of Variance (ANOVA). The data were discriminated using Least Significant Different (LSD) when significant.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Initial Seed Quality

The initial moisture content and germination of *S. resinosa* used was 67.38% and 85% respectively. *S. resinosa* seeds showed high initial germination. This is because the fresh seeds began to germinate quickly beginning on second day after planting whereas the seeds that were treated showed early germination, beginning on the first day of planting.

4.1.1 Tetrazolium (TZ) Staining

Figure 1, showed that the viability of seeds was generally higher when stained for a period between 90 to 120 minutes for the all three concentration of tetrazolium solution.

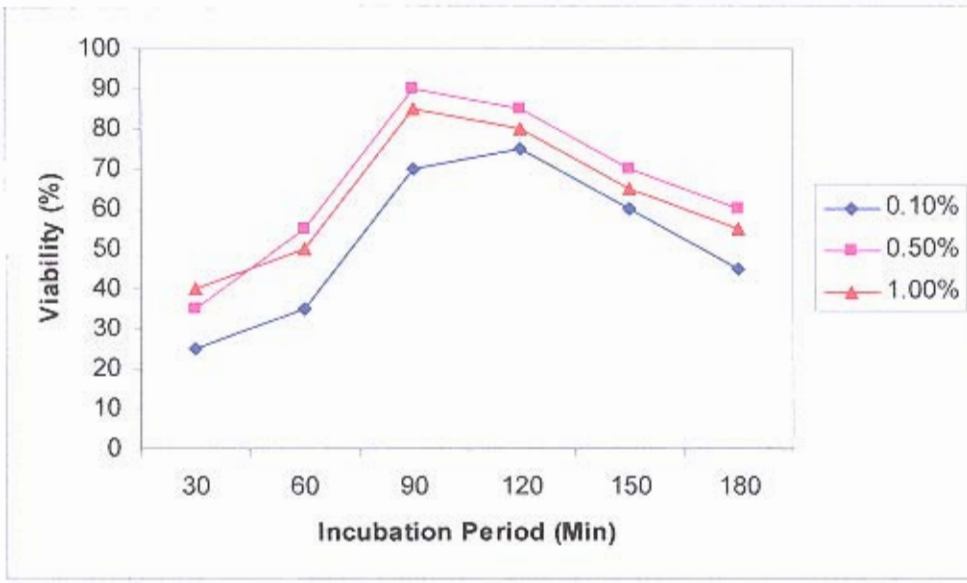

Figure 1: Viability of *S. resinosa* seeds in different concentrations of tetrazolium solution at 35^0C.

The 0.10% concentration of tetrazolium solution exposed for 30 minutes gave the lowest viability only 25%. The maximum viability of 90% was obtained at 0.50% concentration of tetrazolium solution exposed for 90 minutes. This concentration of tetrazolium solution and period of exposed was used for viability evaluation in the subsequent experiments. In a similar study, Peter (2003) observed that *S. lasiocarpum* seeds had the highest viability of 87% which was obtained at 0.5% concentration of tetrazolium solution when exposed for 120 minutes. The TZ staining to assess viability in seeds is universally accepted in the seed industry.

4.2 Dehydration of Seeds

4.2.1 Silica Gel

Figure 2, exhibited the viability of *S. resinosa* seeds after treated using the silica gel. Maximum viability of 90% that was obtained at 48 hours when treated in silica gel. That mean the *S. resinosa* seeds have the most suitable moisture content for germination at this period. The lowest viability of *S. resinosa* seeds was 70% after exposed in silica gel for 96 hours. This might indicated that extending the dehydration of the seeds longer decreased the moisture content of the seeds critically low and reduced the viability of the seeds.

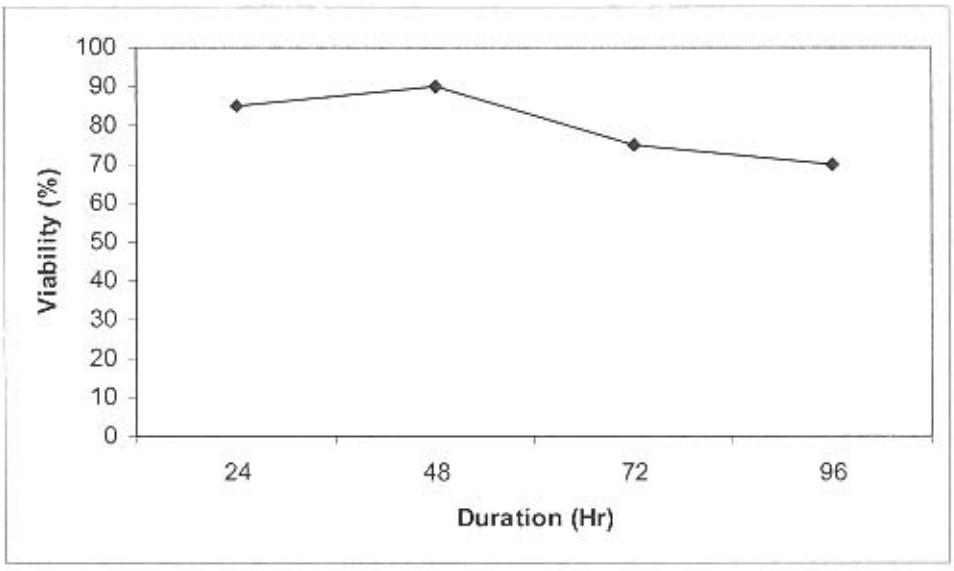

Figure 2: Viability of *S. resinosa* seeds after dessicated using the silica gel.

Figure 3, showed the moisture content and germination of *S. resinosa* seeds following dehydration using silica gel for 0, 24, 48, 72, and 96 hours. For 0 hours, germination percentage was low as compared to other periods which were 85%, with moisture content of 67.38%.

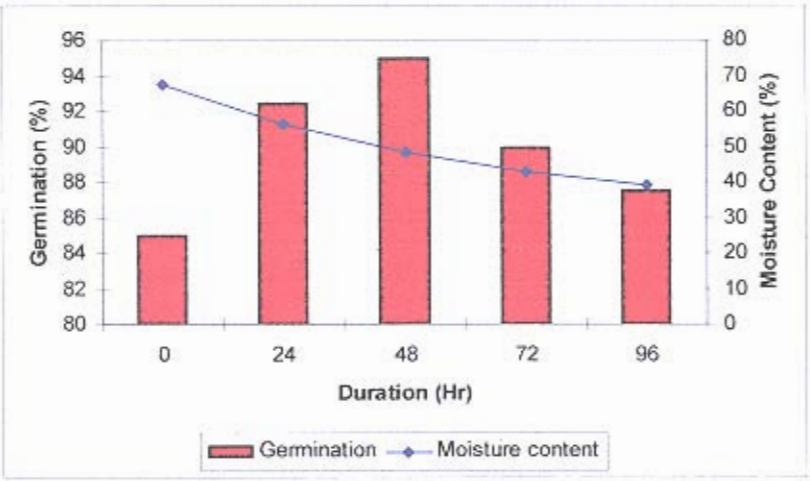

Figure 3: Moisture content and germination of *S. resinosa* seeds following dehydration using silica gel.

Desiccation for 24 hours exhibited germination of 92.5% with moisture content of 56.23%. However, as the seeds were dehydrated for a period of 72 hours, germination and moisture content decreased, where germination percentage was 90% and moisture content was 42.76%. The seed that were dried for 96 hours gave the lowest germination which was 87.5% and moisture content was 38.97%.

Seed selected for stored in liquid nitrogen were dehydrated in silica gel for 48 hours. This was because at this period dehydration seeds gave the highest percentage of germination. The percentage obtained for germination was 95% with moisture content of 48.52%. But, it was different result with other species likes *R. sativus* in which the best dehydration

using silica gel that gave the highest germination was for 24 hours and the germination was 100% with moisture content was 2.82% (Hamuddin, 2003). One way analysis of variance (Table 12 in appendix) showed that there is a significant different in germination after cryopreserved in liquid nitrogen for seeds dehydrated to different moisture content for different periods using silica gel.

For dehydration using silica gel was widely used to prevent the seed from contamination by the non-sterile environment. The example was the seeds of tea (*Camellia sinensis* (L.) O. Kuntze) that was dehydrated to 24 percent of moisture content and successfully cyropreserved in liquid nitrogen (Chaudhury *et al.*, 1990)

4.2.2 Laminar Flow

Figure 4, showed the viability of *S. resinosa* after treated using the laminar flow. Maximum viability of 95% was obtained when exposed to laminar flow for 6 hours. *S. resinosa* seeds at this period probability had a good germination. The lowest viability of *S. resinosa* seeds was 75% after exposed in laminar flow for 8 hours.

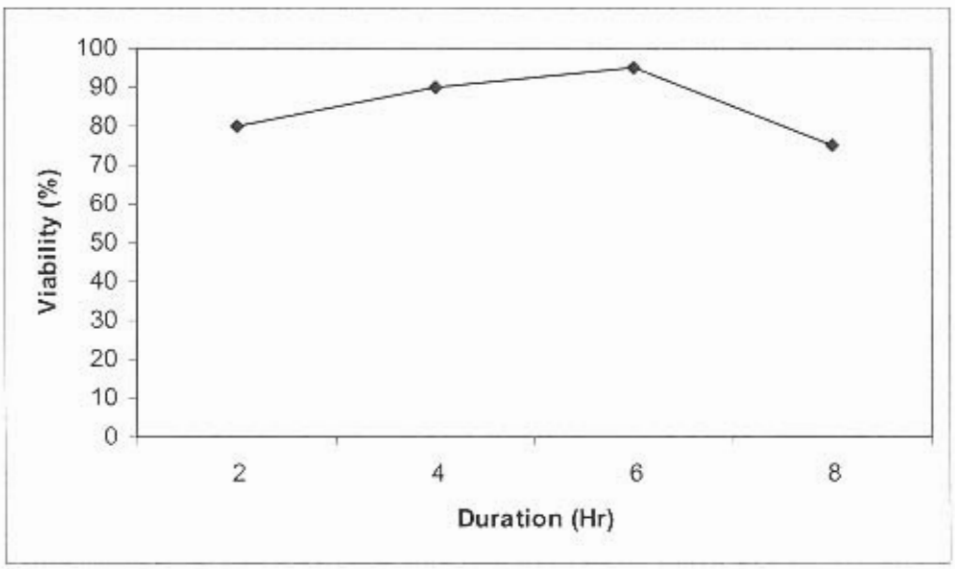

Figure 4: Viability of *S. resinosa* after desiccated in the laminar flow.