

**BINDURA UNIVERSITY OF SCIENCE EDUCATION**  
**DEPARTMENT OF NATURAL RESOURCES**

**PROPAGATION AND NURSERY GROWTH PERFORMANCE OF *ERYTHRINA*  
*LYSISTEMON* (LUCKY BEAN TREE) IN BINDURA, ZIMBABWE**



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## **DEDICATION**

This work is dedicated to my dear parents who brought me to this world and gave me the gift of education, my loving grandmother who gave me words of encouragement, my caring brother and my lovely friends for their full support when i was doing my school work.

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## ABSTRACT

This study compared germination among different seed pre-treatment methods and early nursery growth (diameter and height) of vegetatively propagated cuttings and seedlings of *E. lysistemon* in Bindura, Zimbabwe. A completely randomised design was used for this study since it was carried out in a greenhouse at Astra campus. Pre-treatments that were applied to *E. lysistemon* seed included soaking in hot water at 100°C for 10 minutes, soaking in 98% concentrated sulphuric acid, soaking in cold water for 24 hours and the control with untreated seeds. Black polythene pots were used for sowing the pre-treated seeds whilst sand beds were used for planting the cuttings. Results obtained from this study showed that germination of *E. lysistemon* is increased with hot water and sulphuric acid treatments whilst cold water and control had very low germination. This study showed that there is significant difference in germination across pre-treatments ( $p < 0.05$ ). Results obtained from this study indicated that propagation of *E. lysistemon* through cuttings might produce good results when rooting hormones are added. Nursery growth performance was measured in terms of root collar diameter and height for a period of eight weeks. Seedlings from seeds subjected to hot water treatment recorded highest mean height and root collar diameter over the period of eight weeks. This study therefore recommends hot water treatment for improving *E. lysistemon* germination and sexual propagation for regeneration and domestication of this tree species.

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## **LIST OF ACRONYMS AND ABBREVIATIONS**

ANOVA	Analysis Of Variance
BUSE	Bindura University of Science Education
CM	Centimetres
FAO	Food and Agriculture Organisation of the United Nations
MM	Millimetres
SPSS	Statistical Package Software for Social Sciences
WHO	World Health Organisation



## CHAPTER 1: INTRODUCTION

### 1:1 BACKGROUND TO THE STUDY

The genus *Erythrina* from the Fabaceae family occurs virtually in every type of vegetation throughout the tropics, including moist and dry forests, savannah and semi-desert scrub. *Erythrina* occurs in some non-tropical regions such as South Africa, the southern United States, and the Himalaya Mountains. There are 31 species in Africa and 12 in Asia and Oceania. In Zimbabwe, the most customarily known species is *Erythrina lysistemon* well known for its multipurpose uses.

In the past years in forestry, *Erythrina* was mentioned as a “good for nothing” indigenous tree because of its soft perishable wood. Since it was regarded a woody weed by foresters it was eliminated for more valuable woody species. Later scientists from many disciplines recognised the multiple uses of *E. lysistemon* and changed from the limited wood focused view hence incorporating *E. lysistemon*. Man uses plants in different ways to meet their basic needs. *Erythrina* is increasingly noted as a producer of forage, green manure, medicine and wood for handicrafts, agroforestry, live fence; and a spectacular ornamental. Approximately, 80% of the population in the developing countries rely on plant-based medicines for primary care (WHO 1978). In Kenya, herbal products are recognized and scientific knowledge on the medicinal values of indigenous plants increases.

*E. lysistemon* is able to propagate from natural regeneration, cuttings and seed with a particularly hard seed coat, which under natural conditions would take a long time to germinate. In the past, in countries such as Kenya mostly natural regeneration was the common propagation method used. Wildings were protected from any destruction until a mature age then transplanted to desired sites. These seedlings were mostly found under mature trees or far from the trees due to dispersal. Sometimes, the area under a seeding tree was cleared of weeds and the soil loosened to help the seeds to germinate. When the rains start, the seeds will germinate and the wildings were collected soon thereafter. However, such wildings required good care.

Another method of propagation that was used was direct sowing at the desired site. The seeds were not cared for as those in nurseries and were prone to accidents like being washed away by rain, eaten by birds or even the seedlings being mistaken by weeds during weeding. The situation is exacerbated by the fact that seeds are the major means of propagation of *Erythrina* species, hence seed dormancy presents a serious problem for seed germination of *E. lysistemon* thereby affecting establishment of this multipurpose species. The few that manage to germinate are mostly affected by anthropogenic factors like frost, climate changes leading to rainfall and temperature irregularities, veld fires, mining and browsing by animals. *E. lysistemon* was also propagated through cuttings. The low-technology non-mist propagator currently is used in Kenya (Leakey *et al.*, 1990). In the indigenous woodlands of Zimbabwe, shoots of *E. abyssinica* are often destroyed during the first two years and only a very small part of sexual regeneration can survive.

Lack of adequate knowledge, especially in propagation techniques for woody species, inhibits farmers in Africa, including Zimbabwe, from successfully growing this tree (Schreckenber *et al.*, 2002) since most of the work to date has been skewed towards exotic tree species. However, dependence on seed although cheaper may not be the best solution as there is delayed harvest of useful products.

## **1:2 PROBLEM STATEMENT**

Indigenous plants are being harvested in an unregulated manner from natural stands thus putting pressure on natural populations of *E. lysistemon* severely compromising their contribution to health care. The rise in market and public demand for indigenous medicinal plants exhibits the important risks such as extinction to *E. lysistemon* as medicinal plants. Seedlings of *E. lysistemon* in the forest survive only if they can overcome a wide range of anthropogenic obstacles to their growth including rainfall irregularity, bush fires, pathogens and livestock browsing, thus posing threats which include loss of genetic diversity of the certain species. Practical knowledge in rural communities especially farmers when it comes to propagating *E. lysistemon*, is generally inadequate hence cultivation of important woody species with the goal of conserving them is hampered by little, or no, information on how such species can be propagated or their seedlings established

### **1:3 JUSTIFICATION**

Documentation of information about *E. lysistemom* is needed so that the results could contribute to the conservation and domestication of this important woody species. In fact, when it comes to propagating, managing and using *Erythrina*, the practical knowledge of farmers is generally far ahead of scientific research. With the increasing worldwide demand for medicinal plants as an alternative to prescription drugs, ex situ, in situ conservation programs and true to type mass propagation of *E. lysistemom* could benefit from the findings of this study. There is need to develop mass propagation protocols which produces true to type plants irrespective of season. Cultivation has several advantages, for instance, postharvest handling can be controlled and a steady source of uniform quality raw material can be secured. Considering the important values that the species have, it is important that they are conserved to minimise the suffering of the marginalised who cannot afford expensive allopathic medicines, and to reduce poverty levels of practitioners and other people who depend on medicinal plants for income generation. The study will provide useful information for sustainable forest resources management to various stakeholders in Zimbabwe including the Forestry Commission, Environmental Management Agency and silviculturists.

### **1:4 AIM**

To carry out an assessment into propagation and nursery performance of *E. lysistemom*.

### **1.5 OBJECTIVES**

1.5.1 To determine the most effective pre- treatment methods in the germination of *E. lysistemom* seeds.

1.5.2 To compare nursery growth performance of seedlings across pre- treatment methods

1.5.3 To assess nursery growth performance across different selected propagation methods

### **1.6 HYPOTHESES**

There is no significant difference in germination of *E. lysistemom* seeds across different pre-treatment methods.

There is no significant difference in growth across selected pre-treatment methods.

There is no significant difference in nursery growth performance of seedlings and cuttings.

## **CHAPTER 2: LITERATURE REVIEW**

### **2.0 INTRODUCTION**

Africa is one of the continents with mostly degraded forests in the world with high population. Most of the rural populations depends on forest products for their survival through harvesting of non –timber forest products. Non timber forest products provides an easy accessible and affordable source of medication contributing over 57% revenue in Madagascar (Walter, 2001). Apart from medicinal values wood species also have other values such as provision of firewood ,timber , and ornamental values.

### **2.1 *Erythrina abyssinica***

#### **2.1.1 Origin and Description**

*Erythrina* has about 115 species where *E. abyssinica* is one of the species. The place of origin of *Erythrina* is not known but South America tends to be since a large sum of the species are found there .Africa is also likely to be the origin since some of the endemic species are still found .Orwa *et al.*, (2009) describes *E .abyssinica* as a deciduous tree with a short trunk, thick spreading branches and a rounded crown, 6-12 m with deeply grooved, brown, thick and corky, with or without woody spines. Compound with 3 leaflets, largest leaflet rounded to 15 cm with branch lets and underside of leaves covered with grey-brown hairs, veins and stalks sometimes prickly. This tree species produces orange-red heads that appears on the bare tree. Both narrow calyx lobes and petals are coloured, each flower about 5 cm long. *E. abyssinica* bears woody pods, 4-16 cm long, hairy, strongly narrowed between seeds, opening to set free 1-10 shiny red seeds with a grey-black patch.

#### **2.1.2 Taxonomy and Distribution**

*Erythrina* is included in the subfamily of Papilionoideae which is of the Legumionaise family commonly or mostly known as the Fabaceae family. The thorny tree is found all over Africa in warm temperate and tropical areas, as well as in Central America, Australia, southern Asia and Hawaii and many parts of Kenya in open woodland or grassland, but not in very dry or high-altitude areas. It is distributed in the coastal hills, 300-450 m and in inland areas; 900-2,250 m. *E. abyssinica* is native to the tropical Africa found in the savannah throughout the eastern and southern Africa. Therefore it is found in countries like, Ethiopia, Uganda, Kenya, Tanzania



and Zimbabwe. In northern and western Ethiopia, it is found at elevations between 1600 and 2100 m as a deciduous grassland legume. It grows in open woodland and grassland. This tree occurs on a variety of soils from loams to clay loams. The tree prefers deep well-drained soils on plateaus and slopes (Egli and Kalinganire 1988). *E. abyssinica* grows well in areas with rainfall maximum of 1500mm and minimum rainfall of 500 mm with temperatures ranging from a minimum of 15 and a maximum of 25 degrees Celsius.

### **2.1.3 Uses**

*E. abyssinica* has a number of uses differing with tropics and subtropics where they exist. In India the leaves and bark are mixed with honey and fresh juice as a cure for roundworms. Leaf juice mixed with castor oil is used to treat chronic dysentery. The bark is used to cure trachoma and can be roasted and applied to burns and swellings. Powdered roots are also used for treating syphilis, anthrax and snakebites (Rulangaranga, 1989). It is also roasted and applied to burns and swellings. Powdered root is used for syphilis, anthrax, and snakebites (Rulangaranga, 1989). Seeds, flowers and wood of *E. abyssinica* are used for a number of handcrafts such as earrings, necklaces and beads. In Mexico the wood is the most common material used for figurine carvings for religious ceremonies Russo et al., *E. abyssinica* has some ornamental uses. In Los Angeles, California and *E. crista galii* it is regarded as the Argentina's national flower. It is grown in sideways of the roads in streets and towns. The bark of young stems is used to treat trachoma. It is also roasted and applied to burns and swellings. Powdered root is used for syphilis, anthrax, and snakebites (Rulangaranga, 1989). *E. abyssinica* is useful in the agriculture sector. It can be used as a shade tree in coffee plantations in Tanzania. In Ethiopia after a test it was concluded that *E. abyssinica* can effectively act as a cheap source of protein supplement in the dry season to farmers who rear goats and sheep.

## **2.2 PROPAGATION**

Since the beginning of civilisation, plant propagation played an important role. Plant propagation is the technique of creating new plants from existing plants and this can be done in a number of ways. Most of the common forms of plant propagation were discovered by 2400 B.C. As civilization progressed, growing of different plants that provided fibres, medicines, ornamentals and beauty in addition to food crops. Throughout time man discovered the ability of plants to regenerate and started to use different vegetative propagation techniques to

propagate a variety of plants (Hartmann & Kester, 1983). Among these propagation techniques, cutting is reported to be an extensively practiced and economical means of vegetative propagation for a wide range of woody plants cultivated for use as ornamentals. Regeneration of different woody species can take place in a number of sexual and asexual ways which are not completely understood. Coates-Palgraves (1998) were convinced that, in the natural woodlands of Zimbabwe, the number of trees that grow from seeds is very low. The conventional methods commonly used in the propagation of *E. abyssinica* produce a high degree of genetic variability and consequently decrease the medicinal value of the plant. Propagation of a wide range of tree species have been successfully achieved (Pankaj and Toshiyuki, 2001).

### **2.3 SEXUAL PROPAGATION**

Propagation of plants can be done through seeds (sexual propagation) the most common method used. This is the multiplication of plants using seeds. Germinability of the seeds is the most important factor in this propagation method. This ability of the seed to germinate is mainly affected by a number of environmental factors such as availability of moisture, photoperiod (light and dark), temperature and air (oxygen and carbon dioxide) and the requirement of these factors by seeds vary with plant species (Hartmann & Kester, 1983; Copeland & McDonald, 1994 and Hartmann *et al.*, 1997). As reported by Copeland & McDonald (1994) there are different definitions on seed germination and understanding their difference is important. Physiologists' defines seed germination as the emergence of the radical through the seed coat by seed. Seed analyst defines it as the emergence and development from the seed embryo of those essential structures, which for the kind of seed in question are indicative of the ability to produce a normal plant under favourable conditions. In general, seed germination can be defined as the active growth of the embryo, which results in the rupture of the seed coat and the emergence of young plant. Time taken for seed germination can shorten by taking different pre –treatment method.

### **2.4 SEED DORMANCY**

This state of not germinating unless the required conditions are met is called dormancy. Missanjo *et al.*, (2014 ) defined seed dormancy as the inability of the seed to germinate under normal conditions due to hard coat which is water impermeable. Dormancy can take place even if there are optimal environmental conditions. It usually operates through a chemical or

physical inhibitor to delay germination tough seed coat. The fleshy pulp around some seeds may also contain inhibitors that prevent germination. This challenged of seed dormancy has been noticed on *Acacia* species such as *Acacia Auriculformis* Nigeria (Olatunji *et al.*, 2013).

## **2.5 PRE-TREATMENT**

Treatment of seed is needed for many tree species just before sowing to allow fast entry of water for good germination. These are certain conditions to which seeds are exposed to in order to break dormancy and increase germination. This can be improved necessarily by artificial means thus breaking dormancy. In the natural environment the conditions may be exposure to fire or being eaten by animals. When the seeds are eaten by animals, they are exposed to the hydrochloric acid in the stomach of the animal, and this breaks the dormancy without damaging the seed. Similar methods are used by man to treat seeds and break the dormancy of seeds to be germinated. There are several methods of pre-treating tree seeds, but knowledge of a few simple techniques is sufficient to get reasonable germination of almost all species.

### **2.5.1 HOT WATER TREATMENT**

The seeds are usually placed into boiling water which is immediately removed from the heat source and left to cool gradually, the seeds remaining in the water for reasonable hours (Kemp 1975 c). The seeds will then imbibe and swell as the water cools. The proper relationship of the volume of water to volume of seeds can be determined by the desired experiment and it varies considerably according to species (Goor and Barney 1976), 4 – 5 times (Bonner *et al.* 1974) and 5 –10 times (Seeber and Agpaoa 1976) as much water as seed have been suggested. Responses to hot water treatment differ with the tree species. Some species respond better to an initial temperature well below boiling for example *Albizzia falcataria* (Valencia 1973) which recorded high germination percentage. An initial temperature of 90° C, cooled to ambient 20° C, has given good results with *Parkinsonia aculeata* and *Ziziphus spina-christi* (Kisou *et al.*, 1983). Of various initial water temperatures and periods of soaking and cooling tested for *Leucaena leucocephala* in the Philippines, one minute soaking from an initial temperature of 80° C gave the best result 90 % germination (Laurent and Chamshama 1987). The period of soaking and cooling appeared to have little effect, for example an initial 80° C and 6 hours' soak and cool gave 89 % germination. The initial water temperature had a big effect; germination was only about 30 % after an initial temperature of 100° C and only about 25 % after an initial 40° C.

Prescriptions for hot water treatment have to be applied meticulously if seed coat dormancy is to be removed without killing the seeds through excessive heating. Hot water treatment is relatively easy and safe to apply, as well as being effective with some species. It is not well adapted to large lots because of the difficulty in handling and sowing the swollen seeds (Heit, 1967).

### **2.5.2 ACID TREATMENT**

Seed coat dormancy can be broken with concentrated sulphuric acid. For some species it is more effective than hot water treatment. Seed which has been kept for a long period in store may require a longer period in the acid than fresh seed, which could be severely damaged by the same length of treatment (Kemp 1975). Toughness of seed coat varies between individual trees in most species. Pre-treatment with sulphuric acid in Nigeria proved to be successful and increased germination percentage of *Acacia. Auriculformis*. It also enhanced growth performance (Olatunji *et al.*, 2013). Germination of seeds tends to differ with increased time of acid treatment with the example of *Leucaena leucocephala* (Duguma *et al.*, 1988). Besides better germination of *A. acuriculformis* with acid scarification other species such as *Calligonum bengalensis* recorded significant germination with sulphuric treatment (Kim *et al.*, 1990). El- Juhany *et al.*, (2009) argued that seedlings raised from seed pre-treated with sulphuric acid for a period ranging from 2 to 10 minutes in Tanzania produces the best vegetative characteristics such as height and stem diameter with the example of *Juniperus procera*.

## **2.6 FACTORS AFFECTING GERMINATION**

Life cycle of plants start with the germination of seeds and this keeps. Light requirement for germination differs with species. Some require light to break dormancy whilst others germinate fine in dark.

### **2.6.1 TEMPERATURE**

Temperature has a significant effect on germination of seeds. Cardinal temperatures at which germination occurs and the nature of germination response varies with species. Baskin and

Baskin (2001) outlined that seeds are exposed to alternating temperatures in their natural habitat.

### **2.6.2 LIGHT**

Light has been recognised as a germination controlling factor since the mid nineteenth century (Pons, 2001). Light interact with temperature hence the recent studies found that it has a crucial role in dormancy induction. Response to light differs with species. According to Baskin and Baskin, 2001 seeds of many species if not dormant germinate well in both light and darkness while other species germinate well in light than darkness.

### **2.6.3 AIR (Oxygen and Carbon dioxide)**

Seed germination can also be affected by air as another factor. Air is composed of about 20% oxygen, 80% nitrogen and 0.03% carbon dioxide with oxygen being regarded as the important gas because of its role in oxidation process as reported in other studies. Copeland and MacDonald, (1994) reported that low oxygen concentration results in reduced germination.

## **2.7 VEGETATIVE PROPAGATION STEM CUTTING**

Throughout time man discovered the ability of plants to regenerate and started to use different vegetative propagation techniques to propagate a variety of plants nearby (Hartmann & Kester, 1983). Tchoundjeu *et al.*, (2002) and Amri, (2010) defined vegetative propagation as a process whereby new individual plants that are similar to the parent are regenerated using plant organs. This can be through stems, roots, rhizomes and leaves. A cutting can be defined as any vegetative plant part which, when detached from the parent, is capable of regenerating the missing organ or organs. It can be described as a method of propagating plants by the use of detached vegetative plant parts which, when placed under conditions favourable for regeneration, will develop into a complete plant, similar in all characteristics to the parent plant (Hartmann & Kestrel, 1983). According to Hartmann *et al.* (1997) cuttings can be made from the vegetative portions of the plant, such as stem, modified stems (rhizomes, tubers, corms and bulbs), leaves or roots. Based on the part of the plant taken, cuttings can be classified as stem cuttings (hardwood, semi-hardwood, softwood and herbaceous), leaf cuttings, leaf-bud cuttings (single-eye or single-node cuttings) and root cuttings. Vegetative propagation is becoming increasingly important in forestry and agroforestry for the multiplication of limited seed material and for the production of genetically uniform stock for planting. The value of

vegetative propagation to multiply selected planting material and to capture genetic potential has long been known (Libby 1973; Nobel & Talbert 1984; Leakey 1987). Vegetative propagation gives the tree improver the ability to multiply test, select from and utilise the large genetic diversity present in most tree species. In this way selected, highly productive but unrelated clones can be used commercially for reforestation and agroforestry (Leakey 1991). Woody plants used for ornamental are the ones reportedly to have been cultivated through vegetative means for example *Bougainvillea* through cuttings.

Studies to determine the best propagation environment for semi-arid species have indicated that a non-mist propagation system is generally more effective than conventional mist propagation (Dick *et al.*, 1991). In particular, the better rooting of cuttings in non-mist propagators seems to be related to a lower susceptibility to rotting and consequent mortality. The low-technology non-mist propagator currently used in Kenya has been described by Leakey *et al.*, (1990). This design does not require electricity or piped water, and is therefore particularly suitable for rural areas in the tropics. Several groups working in Kenya have successfully utilised this technology to root a variety of species. Trials have started in Kenya to determine protocols for the larger-scale propagation of semi-arid zone species. Initial work has shown that *Acacia tortilis* and *Prosopis juliflora* both root well from pollarded material.

In propagation by stem cuttings, segments of shoots containing lateral or terminal buds are obtained with the expectation that under the proper conditions adventitious roots will develop and thus produce independent plants. The formation of adventitious roots and buds is dependent on plant cells to differentiate and develop into either root or shoot system. The process of differentiation is the capability of previously developed, differentiated cells to initiate cell divisions and form a new meristematic growing point (Hartmann *et al.*, 1997). However, the development of adventitious roots in a variety of plant species can be influenced by different factors such as position of cutting, rooting hormone, rooting medium, environmental and physical factors (Wilson, 1993). There are many advantages for plant species that can be propagated early by cutting. Numerous new plants can be propagated in a restricted area from a few stock plants. Unlike the other asexual methods like grafting, budding and micro propagation, cutting is easy, cheap and quick (Hartmann *et al.*, 1997).

## **2.8 PHYSICAL FACTORS AFFECTING ROOTING ABILITY OF CUTTINGS**

A number of factors that affects the rooting ability of cuttings include cutting type, stem diameter, cutting length, season, and stock plant from which the cuttings were taken (Leakey, 1983).

### **2.8.1 CUTTING LENGTH AND DIAMETER**

The importance of internode length on rooting ability of cuttings has been shown in recent studies. Cuttings differ in lengths according to species. According to Leakey, (1983) and Leakey and Mohammed (1985), there is a relationship between cutting length and percentage of rooting when cuttings increase in length acropetally than basipetally. Rooting percentage of *Azadirachta indica* was higher from apical than basal node (Palanisamy and Kumar, 1997)

### **2.8.2 SIZE OF STEM CUTTING**

The rooting ability of cuttings can be affected by stem size .The diameter of the stems varies along the shoot (Wilson, 1993).

### **2.8.3 TEMPERATURE**

Minimum, maximum and optimum temperatures for germination differ with species. Optimum temperature for germination of *Eucalyptus globulus* has been reported to be 25degrees Celsius (ISTA, 1999).

### **2.8.4 AGE OF THE PARENT PLANT**

Studies had indicated that cuttings taken from juvenile plants have low rooting ability as compared to those from mature plants. Smith, (1995) noted that cuttings made from mature apple trees rooted well in comparison to cuttings taken from fruiting branches. Danilov, (1968) also noted that cuttings from the lower parts of mature apple trees rooted well as compared to cuttings taken from the upper part.

### **2.8.5 ROOT HOMORNE AND MEDIUM**

Rooting, shooting and growth of cuttings are encouraged by natural hormones contained in plants (Leakey, 2004).These natural hormones help the cuttings to adapt to the environment.

However a number of plants require exogenous hormones to stimulate their rooting, shooting and growth of cuttings (Amri, 2010) and this differ with tree species (Egbe *et al.*, 2012. ). Commercial powders such as Seradix, acids like Indole-3-butyric (IBA) and naphthalene acetic acid (NAA) are the most common exogenous hormones used. *K.ivorensis* that was treated with IBA recorded 80-90% rooting as reported by Tchoundjeu and Leakey (1996). Stem cuttings without exogenous hormone treatment are difficult to root and the application of exogenous hormones on plant species to root, grow and survive may fail in some species for example *L. leucocephala* (Tchoundjeu and Leakey, 1996, Danthu *et al.*, 1992; Saifuddin *et al.*, 2013).

#### **2.8.6 PROPAGATION MEDIUM**

Rooting capacity, shooting capacity, growth and survival are a result of rooting medium (Tchoundjeu *et al.*, 2002). Various media can be used for vegetative propagation and this includes sandy soil, forest soil, a mixture of sandy and peat soil, saw dust and a mixture of sand and saw dust (Ofori *et al.*, 1996; Tchoundjeu *et al.*, 2002; Owusu *et al.*, 2014). Studies on rooting success of *Prunus Africana* reported 71% rooting in sand saw dust mixture, 72% in sandy alone and 80% in sawdust. However, rooting success also depends on ecological regions besides rooting media and other factors Tchoundjeu *et al.*, (2002).

#### **2.8.9 SEASON AND LEAVES**

Rooting ability of cuttings can be affected by season. Plants propagated during the winter season differ in rooting success to those propagated in the summer in accordance to tree species. This is perhaps because when towards winter deciduous trees lose their leaves making it difficult to root. The study carried out by Danthu *et al.*, (1992) discovered that rooting of *Senegalia senegal* was greatest in June to October at 70% , 30-40% for May and 10% from January to March. According to studies done by Elgimabi (2008), hardwood cuttings of *Ixora cocinea* rooted well in summer. However, studies done by Magingo and Dick (2001) reported that *B. spiciformis* and *P. angolensis* successfully rooted in May with 59% and 60% respectively. Some species are naturally difficult to shoot and survive despite season and presence of leaves (Leakey, 2004).

Leaves play a vital role in the manufacturing of carbohydrates and water uptake essential for plant growth (Owusu *et al.*, 2014). Their presence in cutting has an effect on rooting ability. Studies carried out by Tchoundjeu *et al.* (2002) and Swamy *et al.* (2002) reported that leafless



cuttings were difficult to root. The leaves however have to be trimmed to reduce water loss (Owusu *et al.*, 2014). Previous studies discovered that there is no universal optimum leaf area. However, *Tectonis grandis* reported 68% rooting (Palanisamy and Subramanian, 2000)

## **2.9 SEEDLING GROWTH**

Growth in plants can be defined as an irreversible increase in volume. The largest component of plant growth is cell expansion driven by turgor pressure. During this process, cells increase in volume and become highly vacuolated (Taiz and Zeiger, 2002). Growth can also be measured in terms of change in fresh weight: that is, the weight of the living tissue over a particular period of time. However, the fresh weight of plants growing in the soil fluctuates in response to changes in the water status, so the criterion may be a poor indicator of actual growth. Thus, measurements of dry weight are often more appropriate than the fresh weight (Taiz and Zeiger, 2002).

### **2.9.1 GROWTH MEASUREMENT TECHNIQUES**

Measurements of above-ground biomass could be made through two basic ways, *destructively* and *non-destructively*. Among the various methods for evaluating forest biomass, the most widely used is complete harvest of randomly selected plots (destructive method). However, such methods are not suited to the natural environment, especially if the environment is highly degraded and also with threatened species (Montes *et al.*, 2000). Destructive method is expensive in terms of time and expended for collecting the data when compared with the non-destructive method. The other alternative way of measurement of biomass is non-destructive sampling. Total harvesting is generally impractical or inappropriate in forest studies; so allometric methods have been developed to estimate total biomass from non-destructive surrogate measurements such as diameter of the bole at breast height (dbh) or recording the height of selected plants (Vann *et al.*, 1998). The non- destructive measurement techniques allow determination of biomass and leaf area of a tree throughout the growing cycle. Periodic measurements of tree productivity are easily done (Lott *et al.*, 2000)

## CHAPTER 3: MATERIALS AND METHODS

### 3:0 DESCRIPTION OF THE STUDY AREA

The study was carried out in Bindura Mashonaland Central, Zimbabwe at the Bindura University of Science Education (BUSE) Astra Campus Nursery. Its geographical coordinates are 17° 17' 47" South, 31° 19' 47" East. Bindura is located in the agro-ecological region II (Agritex 1996) with rainfall ranging from 750 to 1 000 mm/year, (Moyo, 2000; Vincent and Thomas, 1961) and temperatures above 20°C. The area receives rainfall in summer between November and April with cold winters extending from May to August. Bindura is characterised with clay red soils.



Figure 3.1 A map showing Astra campus Nursery at Bindura University of Science Education Zimbabwe.

### 3:1 EXPERIMENTAL DESIGN

Two propagation experiments and seedling growth experiment were carried out in completely randomized design at the laboratory and nursery garden at BUSE Zimbabwe, Astra Campus. The study comprised the following experiments: sexual propagation using seeds, vegetative propagation using leafless stem cuttings and leafy branch cuttings. Seedling and cutting growth were using results from seedlings and cuttings established

### 3.1.1SEXUAL PROPAGATION

Seeds of *E.abysynica* were collected from mature trees in Bindura, Zimbabwe in September. The seed of *E.abysynica* is bright red, ellipsoidal and 9-12 mm long and 6-7 mm in diameter. Three different pre-treatments were tested. In each treatment a total of 60 seeds were used with three replicates. The seeds were tested for seed viability using traditional methods before pre –treating them .They were placed into cold water, and all seeds that floated were removed. Floating was regarded as a sign of destroyed embryo therefore unable to germinate (Malone, 1967).

### 3.1.2 TREATMENTS USED

**Table 3.1 Treatments used during the study.**

Treatment	Scarification
1	Hot water
2	Sulphuric acid
3	Cold water
4	Control

#### i) Hot water treatments

Seeds were soaked in boiling water at 100 degrees Celsius for 5 minutes. The seeds were then removed from the water and soaked in cold water for 30 minutes and then sown.

#### ii) Lukewarm water treatment

Seeds were immersed in cold clean tap water at room temperature for twenty four hours and were planted after being subjected to this treatment.

#### iii) Concentrated Sulphuric acid treatment

The acid treatment for *E .abysynica* involved placing seeds in beakers containing 150 ml of 98% concentrated sulphuric acid and stirred for period of 10 minutes. The seeds were then washed thoroughly in cold water to remove the acid and rinsed and were left in cold water to soak for 30 minutes then sown.

#### iv) Control

The seeds were planted directly into the soil without them being subjected to any pre-treatment.

The pre-treated seeds together with the control were sown at a depth of 2cm (Missanjo et al., 2014) in 100mm x150mm x 40 mm black polythene bags. A commonly used growing media in nurseries humus (murakwani) was used for planting all treatments in polythene bags.

### **3.1.3 VEGETATIVE PROPAGATION**

An experiment on vegetative propagation by stem cuttings of *E. abyssinica* was also carried out at a nursery located at the BUSE Astra campus. Four mature *E. abyssinica* plants were selected randomly from the mature trees in Bindura area. Selection of the mother plants was done on the basis of true-to-name and type. The cuttings were taken early in the morning when transpiration is low. This was to reduce the risk of drying out. A total of 60 stem cuttings with 3 replicates each, 30 leafy and 30 leafless (55 cm height; 2cm in diameter) were planted in sand beds and treated with used oil to avoid decaying. The plants were watered every day to field capacity.

## **3.2 MANAGEMENT OF SEEDLINGS**

### **3.2.1 WATERING**

The seedlings were watered thrice a day using taped water to avoid water stress and keep abundant moisture. After germination, watering was done twice a day, early in the morning and late before sunset.

### **3.2.2 WEEDING**

Weeding was done to avoid competition for moisture and nutrients. This was done when weeds were noticed in the pots and beds. Hand picking was the only method used to avoid damage to the seeds and seedlings.

## **3.3 DATA COLLECTION**

- Date of sowing-17/12/17

Data were collected from the sample plants at weekly intervals measuring the parameters in table below using non-destructive methods to allow periodic measurements of tree growth.

**Table 3.2 Variable measures**

Variable	Material/method
<b>Germination percent</b>	Mathematical calculation( number of seeds sown /number of tested seeds)
<b>Tree survival rate</b>	Physical counting of the trees Mathematical calculation (trees survived/trees planted *100)
<b>Height</b>	Tape measure
<b>Root collar basal diameter (RCD)</b>	Veneer caliper
<b>Number of shoots</b>	Physical counting

### 3.3.1 GERMINATION

Seedling emergence was recorded daily according to the day each seed emerged. Daily observation on germination was done, counted and recorded in accordance to the number of days. The germination percentage was determined after 10 days from sowing day, using the following formula:

$$GP = [\text{Number of seeds germinated} / \text{Number of seeds tested}] \times 100 \quad \text{Equation 1}$$

Where GP is the germination percentage

### 3.3.2 SEEDLING HEIGHT

Seedlings height was measured from the base to the highest point of the plant every 7 days (from December 2017 to February 2018) using a flexible tape measure and the average height for each species was recorded.

### **3.3.3 ROOT COLLAR DIAMETER**

Root collar diameter of each seedling was taken using veneer calliper every 7 days (from Decembers 2017-February 2018), and the diameter was computed.

### **3.3.4 SURVIVAL PERCENTAGE**

Number of wilted or dead seedlings from each species was counted and recorded every 7 days. Then at the end of the study period (using total number of dead seedlings) survival percentage was calculated.

$$[\text{Total number of trees survived} / \text{trees survived} \times 100] \quad \text{Equation 2}$$

### **3.3.5 Number of sprouted shoots**

Number of sprouted shoots from both leafy and leafless cuttings were physically counted and recorded from week two of planting.

## **3.4 DATA ANALYSIS**

### **3.4.1 STATISTICAL ANALYSIS**

The Statistical Package Software for Social Sciences (IBM SPSS Windows version 20 of 2011) was used to analyse all data collected for different parameters. Data on height and root collar diameter were subjected to Analysis of Variance (ANOVA) to determine differences among the seedlings for their growth using the Least Significance Difference test at 5% level. Analysis of variance was also carried out to test the effect of seed treatments on the seeds (Aref *et al.*, 2011) using the data collected on germination. Data were presented through the use of tables, graphs and pie charts.

## **CHAPTER 4: RESULTS**

### **4.1 GERMINATION OF *E. ABYSSINICA* ACROSS DIFFERENT PRE-TREATMENT METHODS**

Germination was observed in seeds pre-treated with sulphuric acid at 7 days after sowing while seeds under hot water treatment and cold water sprouted at 9 days. Seeds without any pre-treatment were the last batch to germinate at 12 days. There was a significant difference in

germination of *E. abyssinica* across the treatments ( $p=0.005$ ). Percentage germination was highest with hot water treatment recorded the highest germination (49%) with the lowest germination percentage recorded from control (9%) (Figure 4.1)

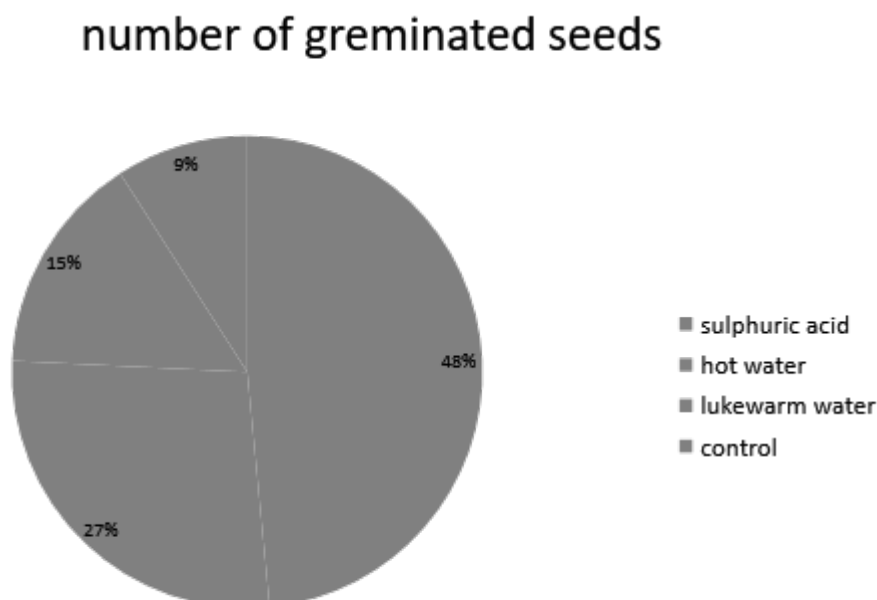


Figure 4.1 Germination percentage of *E. abyssinica* under different pre- treatment methods.

## 4.2 NURSERY GROWTH PERFORMANCE OF SEEDLINGS ACROSS DIFFERENT PRE- TREATMENT METHODS.

There was significant difference in height across the eight weeks  $p<0.0001$ . Mean total height was high on seedlings pre-treated with sulphuric acid ( $16.33 \pm 0.65$ ) with significant difference  $p<0.01$  against other pre-treatments from week one until week eight. Height decreased in the order sulphuric acid ( $p<0.05$ ) > control ( $p<0.05$ ) > cold water ( $p<0.05$ ) > hot water ( $p<0.05$ ) (Table 4.1). There was significant difference in root collar diameter of *E. abyssinica* seedlings across different pre-treatment methods. Seedlings of seeds treated with sulphuric acid presented the highest mean total root diameter ( $1.29 \pm 0.08$ ) and reasonable growth from week one until week 8. The root diameter of pre-treatment 2 and 3 changed gradually over the period but remained below 0.5 cm (table 4.1) Seedlings from sulphuric acid pre-treatment shown significant difference in both height and root collar diameter against the other three pre-treatments. In terms of height and root collar diameter among hot water, cold water and sulphuric acid pre-treated the seedlings did not exhibit a significance difference ( $p<0.05$ ).

**Table 4.1 Nursery growth performance of *E. abyssinica* across different pre treatment methods for a period of eight weeks**

Time	Parameter	Sulphuric acid	Hot water	Luke warm water	Control
	Height (cm)				
	RCD (mm)				
Week 1	Height	3.54±0.17 <sup>a</sup>	2.93±0.14 <sup>b</sup>	2.50±0.15 <sup>b</sup>	2.93±0.44 <sup>b</sup>
	RCD	0.33±0.02 <sup>abc</sup>	0.11±0.16 <sup>b</sup>	0.11±0.18 <sup>b</sup>	0.16±0.01 <sup>c</sup>
Week 2	Height	5.56±0.29 <sup>a</sup>	3.75±0.36 <sup>b</sup>	3 <sup>b</sup>	4.33±0.33 <sup>c</sup>
	RCD	0.42±0.01 <sup>a</sup>	0.28±0.01 <sup>b</sup>	0.26±0.03 <sup>b</sup>	0.32±0.04 <sup>b</sup>
Week 3	Height	7.56±0.21 <sup>a</sup>	4.93±0.31 <sup>b</sup>	4±0.01 <sup>b</sup>	4.62±0.28 <sup>b</sup>
	RCD	0.58±0.02 <sup>a</sup>	0.3±0.02 <sup>b</sup>	0.3±0.03 <sup>b</sup>	0.42±0.04 <sup>b</sup>
Week 4	Height	10.1±0.31 <sup>a</sup>	5.4±0.02 <sup>b</sup>	5.3±0.33 <sup>b</sup>	4.9±0.32 <sup>b</sup>
	RCD	0.62±0.02 <sup>a</sup>	0.39±0.02 <sup>b</sup>	0.34±0.03 <sup>b</sup>	0.42±0.04 <sup>b</sup>
Week 5	Height	11.7±0.46 <sup>a</sup>	6.21±0.02 <sup>b</sup>	6 <sup>b</sup>	6.51±0.05 <sup>b</sup>
	RCD	0.74±0.02 <sup>a</sup>	0.39±0.03 <sup>b</sup>	0.34±0.03 <sup>b</sup>	0.48±0.07 <sup>c</sup>
Week 6	Height	13.78±0.43 <sup>a</sup>	8.40±0.42 <sup>b</sup>	7.33±0.32 <sup>b</sup>	9±1 <sup>b</sup>
	RCD	0.9±0.05 <sup>a</sup>	0.43±0.03 <sup>b</sup>	0.39±0.01 <sup>b</sup>	0.58±0.03 <sup>c</sup>
Week 7	Height	16.33±0.64 <sup>a</sup>	9.8±0.58 <sup>b</sup>	10 <sup>b</sup>	11±1 <sup>c</sup>
	RCD	1.05±0.05 <sup>a</sup>	0.47±0.03 <sup>b</sup>	0.43±0.03 <sup>b</sup>	0.62±0.03 <sup>ab</sup>
Week 8	Height	16.33±0.65 <sup>a</sup>	9.8±0.58 <sup>b</sup>	10±0.01 <sup>b</sup>	11±1.00 <sup>c</sup>
	RCD	1.29±0.08 <sup>a</sup>	0.52±0.24 <sup>b</sup>	0.49±0.04 <sup>b</sup>	0.82±0.15 <sup>b</sup>

\*Means with different superscripts within the same row are significantly different at  $p < 0.05$ .

### 4.3 SURVIVAL OF *E. ABYSSINICA* SEEDLINGS ACROSS DIFFERENT PRE-TREATMENT METHODS

During early nursery performance high mortality was observed in seedlings. Hot water treatment had the highest survival percentage of 27 % (Figure 4.4) followed by sulphuric acid



with 15 % .The cold water had 9% whilst control 4% and the lowest in terms of survival. It was 100% during the first two weeks and it declined from the third week .Survival therefore remained constant from week 4 until week 8 (figure 4.4) .

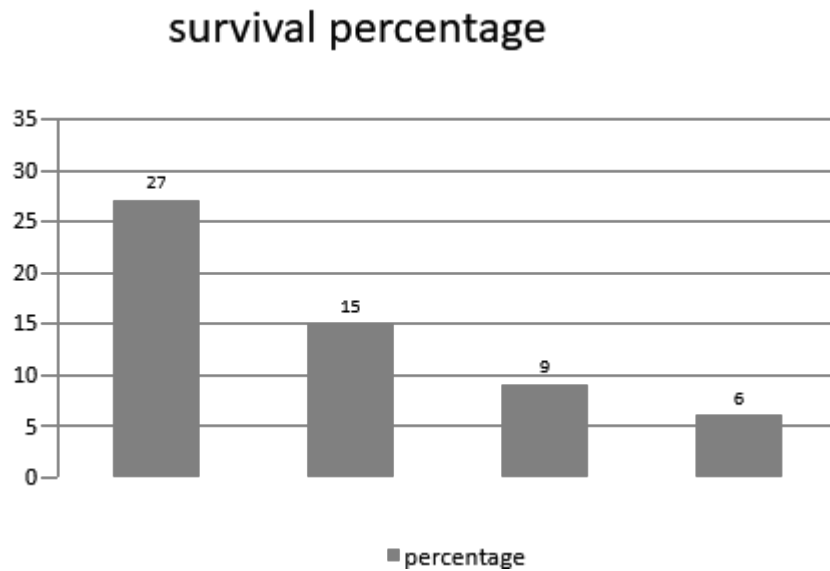


Figure 4.3 Survival of *E.abbyssinica* seedlings across different pre- treatments.

## **CHAPTER 5: DISCUSSION**

### **5.1 THE EFFECTS OF PRE- TREATMENTS ON GERMINATION O *E. ABYSSINICA***

The effects of pre-treatment on germination percentage of *E. abyssinica* were shown in fig 4.1. the highest germination percentage was recorded on sulphuric acid pre-treated seeds followed by hot water treated seeds. Similar results were reported by Laurent and Chamshama (1997) in Tanzania where *E. abyssinica* recorded 84% germination. The lowest germination percentage was recorded on lukewarm water and control. This differs with the study by Laurent and Chamshama (1997) where no germination was recorded on seeds without any pre-treatment. Both hot water and sulphuric acid are known to break dormancy that inhibit water uptake hence germination. The fact that *E. abyssinica* seed were 80% viable through hot water and sulphuric acid pre-treatments shows that dormancy is the limiting factor of germination of *E. abyssinica*. Seed dormancy of leguminous plants is related to permeability of the seed coat to water. This hard seed coat structure restricts entry of moisture into the seeds therefore pre-treatment with sulphuric acid facilitates germination. The results of this study are similar to the results that were obtained in Ethiopian on experiments of acid treatments in variation of duration for several Ethiopian species (Teketay, 1996). However, Schmidt, (2000) reported that time of exposure to acid treatment is critical and needs to be quantified as hardness of the seed coats differs with species. Hot water treatment recorded the second largest germination percentage. Hamilton and Midcap (1999) reported that hot water temperatures have to differ with species since some exposed embryo might become denatured in excessive hot temperatures. However, low germination across the different pre-treatment methods might be a result of low moisture content.

### **5.2 NURSERY GROWTH PERFORMANCE OF *E. ABYSSINICA* ACROSS DIFFERENT PRE- TREATMENTS**

Values obtained for root collar diameter and height across the eight weeks demonstrated the effective development of the seedlings (Figure 4.3) Seedlings pre-treated with sulphuric acid presented highest root collar diameter and height. Seedlings which presented superior root collar diameter and height development assure better quality plantlets since collar diameter is very important in evaluating the potential of the seedlings to survive after transplanting to the field. Results from this study conformed slow growth on seedlings pre-treated to lukewarm

water, hot water and control .Growth performance in the nursery is affected by a number of factors including pest and disease control, watering regimes, weeds among others. According to Olatunji *et al.*, (2013) sulphuric acid has effects on root collar diameter and height and this might be due the high desiccant effect of the acid on the coat of the seed which therefore allows easy uptake of water and oxygen diffusion .El- Jahany *et al.*, (2009) argues that seedlings raised from seeds treated with sulphuric acid has best vegetative characters in terms of root collar diameter and height. Lack of significant differences across the other three pre-treatments suggests that the pre-treatment methods do not have effects on growth performance of seedlings except on germination. The results obtained from this study accept the null hypothesis that there is no significant difference in nursery growth across different pre-treatment methods.

### **5.3 SURVIVAL OF ERYTHRINA ABYSSINICA**

Artificial regeneration of *E. abyssinica* species has been perceived to be difficult due to high mortality rate of indigenous tree seedlings in nursery (Magingo and Dick, 2001) in their study focused on miombo species. Mortality rate of *E. abyssinica* seedlings was minimal hence artificial regeneration proved to successful. The tree seedlings were exposed to weather effects implying that the nursery at BUSE, Zimbabwe Astra campus was in open air. Mortality was a result of pests and diseases and poor nursery management in terms of watering and weeding. Bognounou *et al.*, (2010) stated that watering and weeding around the seedlings and protection against pests enhances seedling growth.

### **5.4 VEGETATIVE PROPAGATION OF ERYTHRINA ABYSSINICA**

In this study propagation of *E. abyssinica* using cuttings both leafy and leafless using sand beds as rooting medium failed completely after being monitored for a period of 4 weeks. Five cuttings (3 leafy and 2 leafless) exhibited shootings after 14 days of planting and the shoots died within a week. Results of this study contrast with the findings of Meunier, (2005) and Meunier *et al.*, (2006) in Uganda which found that it is possible to propagate *E. abyssinica* through cuttings. This may be due to the fact that Meunier (2005) and Meunier *et al.*, (2006) exposed the cuttings under conditions of a mist propagator. The results of this study also have shown that establishment of *E. abyssinica* cuttings without application of rooting hormone is not successful .Taiz and Zeiger (2003) reported that auxins (rooting hormones) are required for root induction and establishment of cuttings.

## **CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS**

### **6.0 CONCLUSIONS**

From the results obtained from this study it can be concluded that germination of *E. abyssinica* can be increased through pre-treatments. Hot water pre- treatment and sulphuric acid proved to be most effective pre-treatment with high nursery growth performance .Sexual propagation proved to be more successful than vegetative propagation. Factors such as rooting hormones, season and age of mother tree for cutting has to be considered to improve the vegetative propagation results.

### **6.1 RECOMMENDATIONS**

- The study has shown that highest percentage germination can be achieved by pre-treating *E. abyssinica* seeds with sulphuric acid and hot water. In rural areas concentrated sulphuric acid is not readily available and it requires careful handling to avoid damage. Therefore, hot water use is recommended since it is cost efficient.
- The study recommends attempts on other vegetative propagation method

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## APENDICES

### Oneway

#### Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		
					Lower Bound	Upper Bound	
week	1	9	8.00	.000	.000	8.00	8.00
	2	5	8.00	.000	.000	8.00	8.00
	3	3	8.00	.000	.000	8.00	8.00
	4	2	8.00	.000	.000	8.00	8.00
	Total	19	8.00	.000	.000	8.00	8.00
rcd	1	9	1.2933	.23076	.07692	1.1160	1.4707
	2	5	.5180	.05357	.02396	.4515	.5845
	3	3	.4867	.06658	.03844	.3213	.6521
	4	2	.8150	.20506	.14500	-1.0274	2.6574
	Total	19	.9116	.41732	.09574	.7104	1.1127

#### Descriptives

		Minimum	Maximum
Week	1	8	8
	2	8	8
	3	8	8
	4	8	8
	Total	8	8
Rcd	1	.99	1.62
	2	.48	.61
	3	.41	.53
	4	.67	.96
	Total	.41	1.62

#### ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
week	Between Groups	.000	3	.000	.	.
	Within Groups	.000	15	.000		
	Total	.000	18			
rcd	Between Groups	2.646	3	.882	27.093	.000
	Within Groups	.488	15	.033		

Total	3.135	18			
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## Post Hoc Tests

### Multiple Comparisons

LSD

Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.
Rcd	1	2	.77533*	.10065	.000
		3	.80667*	.12030	.000
		4	.47833*	.14106	.004
	2	1	-.77533*	.10065	.000
		3	.03133	.13178	.815
		4	-.29700	.15097	.068
	3	1	-.80667*	.12030	.000
		2	-.03133	.13178	.815
		4	-.32833	.16472	.065
	4	1	-.47833*	.14106	.004
		2	.29700	.15097	.068
		3	.32833	.16472	.065

### Multiple Comparisons

LSD

Dependent Variable	(I) treatment	(J) treatment	95% Confidence Interval	
			Lower Bound	Upper Bound
Rcd	1	2	.5608*	.9899
		3	.5503*	1.0631
		4	.1777*	.7790
	2	1	-.9899*	-.5608
		3	-.2495	.3122
		4	-.6188	.0248
	3	1	-1.0631*	-.5503
		2	-.3122	.2495
		4	-.6794	.0228

	4	1	-.7790*	-.1777
		2	-.0248	.6188
		3	-.0228	.6794

\*. The mean difference is significant at the 0.05 level.

## Oneway

### Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
week	1	9	7.00	.000	.000	7.00
	2	5	7.00	.000	.000	7.00
	3	3	7.00	.000	.000	7.00
	4	2	7.00	.000	.000	7.00
	Total	19	7.00	.000	.000	7.00
height	1	9	16.3333	1.93649	.64550	14.8448
	2	5	9.8000	1.30384	.58310	8.1811
	3	3	10.0000	.00000	.00000	10.0000
	4	2	11.0000	1.41421	1.00000	-1.7062
	Total	19	13.0526	3.53512	.81101	11.3488

### Descriptives

		Minimum	Maximum
Week	1	7	7
	2	7	7
	3	7	7
	4	7	7
	Total	7	7
Height	1	14.00	20.00
	2	9.00	12.00
	3	10.00	10.00
	4	10.00	12.00
	Total	9.00	20.00

## ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
week	Between Groups	.000	3	.000	.	.
	Within Groups	.000	15	.000		
	Total	.000	18			
height	Between Groups	186.147	3	62.049	23.988	.000
	Within Groups	38.800	15	2.587		
	Total	224.947	18			

## Post Hoc Tests

### Multiple Comparisons

LSD

Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.
Height	1	2	6.53333*	.89707	.000
		3	6.33333*	1.07221	.000
		4	5.33333*	1.25728	.001
	2	1	-6.53333*	.89707	.000
		3	-.20000	1.17454	.867
		4	-1.20000	1.34561	.387
	3	1	-6.33333*	1.07221	.000
		2	.20000	1.17454	.867
		4	-1.00000	1.46818	.506
	4	1	-5.33333*	1.25728	.001
		2	1.20000	1.34561	.387
		3	1.00000	1.46818	.506

### Multiple Comparisons

LSD

Dependent Variable	(I) treatment	(J) treatment	95% Confidence Interval	
			Lower Bound	Upper Bound
Height	1	2	4.6213*	8.4454
		3	4.0480*	8.6187

2	4	2.6535*	8.0132
	1	-8.4454*	-4.6213
	3	-2.7035	2.3035
	4	-4.0681	1.6681
3	1	-8.6187*	-4.0480
	2	-2.3035	2.7035
	4	-4.1294	2.1294
4	1	-8.0132*	-2.6535
	2	-1.6681	4.0681
	3	-2.1294	4.1294

\*. The mean difference is significant at the 0.05 level.