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COLLEGE OF BASIC AND APPLIED SCIENCES

OPTIMIZATION OF PROPAGATION METHODS FOR THE PRODUCTION OF QUEENS FLOWER (*LAGERSTROEMIA SPECIOSA*)

By

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DECLARATION

I, **Bervelyn Okyere** hereby declare that except for references to other people's research which have been duly cited, this thesis submitted to School of Research and Graduate Studies, University of Ghana for the award of MPhil degree in Crop Science, Horticulture Option is my own investigation and that it has neither in whole nor in part been presented elsewhere.

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ABSTRACT

This study consisted of two experiments that evaluated the effect of Indole-3-butyric acid (IBA) treatments on the rooting of *Lagerstroemia speciosa* stem cuttings and the effect of seed germination pre-treatments (sodium hydroxide, hydrogen peroxide and sulphuric acid) on the germination of *Lagerstroemia speciosa* seeds. In the rooting experiment, 15 cm long stem cuttings from softwood, semi-hardwood and hardwood were used. The treatments comprised six IBA concentrations namely, 0 ppm (control), 500 ppm, 1000 ppm, 1500 ppm, 3000 ppm and 4500 ppm for 10 seconds. The semi-hardwood and hardwood were subjected to the 0 ppm (control), 1500 ppm, 3000 ppm and 4500 ppm treatments while the softwood cuttings received 0 ppm (control), 500 ppm, 1000 ppm, 1500 ppm and 3000 ppm. There were 20 cuttings per treatment. Each treatment was replicated three times. The 1500 ppm IBA treatment outperformed the other IBA treatments in all the different stem cuttings producing 14.8 % rooting in softwood cuttings, 14.8 % in semi-hardwood cuttings and 16.6 % in the hardwood cuttings. Hardwood stem cuttings generally produced better response to rooting than the semi-hardwood and hardwood cuttings. Percent rooting, number of roots per cutting as well as length of longest roots improved with age of cuttings for the best IBA concentration as well as the control. Hence the combination of hardwood cuttings at 1500 ppm IBA rooting hormone produced the best rooting in *Lagerstroemia speciosa*. Mature seeds (820) from healthy looking trees were used in the germination experiment. Freshly harvested seeds (20 each) were treated with hydrogen peroxide (5% and 10%), 3.5% sodium hypochlorite (50% and 75%) and 90% sulphuric acid (5 min., 10 min., 15 min., and 20 min). The treatments consisting of a control and the pre-treatments mentioned were replicated five times. The evaluations performed for 34 days starting from seed sowing, showed that sulphuric acid had the highest germination percentage (3.7 %) than those

treated with hydrogen peroxide (2.6 %) and sodium hydroxide (Clorox) 0.0 %. The most effective sulphuric acid treatments were for 10 min. and 20 min. Strategies for further improvement of germination as well as rooting of cuttings of *Lagerstroemia speciosa* are suggested

DEDICATION

The thesis is dedicated to my late parents Mr Peter Opoku and Madam Victoria Aduaso. May their souls rest in peace.

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LIST OF ABBREVIATIONS

NRPC	Number of Roots per Cuttings
LLR	Length of Longest Roots
IBA	Idole-3-Butyric Acid
SWC	Softwood Cuttings
SHWC	Semi-hardwood Cuttings
HWC	Hardwood Cuttings
G (%)	Germination Percentage
MT	Mean Time Germination
CV	Coefficient of Variation
SOG	Speed of Germination

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Ornamental plants are important in many cultures and societies (Heywood, 1999). Queen's flower (*Lagerstroemia speciosa*) is an ornamental plant used worldwide in home landscaping and avenue planting for public and private use. It is a deciduous multipurpose, ornamental tree which grows in tropical and subtropical countries and belongs to the *Lythraceae* family. It serves as a wind break and can also be grown along rivers, streams and in swampy lands (Zabala, 1990). It is a tree that grows upright and deciduous. The leave of the tree is 12-inch-long, dark green, oblong, leathery and turns attractively red before shedding. It thrives on well drained clayey, loamy or sandy; acidic or alkaline soils (Lichtenhan *et al.* 1993).

As an ornamental species, the captivating architecture of the tree and the flowers makes it ideal for shading and the incorporation of colour into the landscape. It is used for timber and for construction. It is also used in building boats and in construction of carts. The tree is also used for afforestation of comparatively moist localities as it is able to become established and spreads in natural ecosystems. It also controls erosion due to its dense and wide spreading root system.

In Ghana, *Lagerstroemia speciosa* seeds germinate poorly as a result of seed dormancy challenges (Personal contact with Mr. Mawuli, nursery owner, Spintex Accra). The problem has also been confirmed by Azad *et al.* 2010. Khurana and Singh (2001), have also indicated in their article that variability in germination and seedling growth rate in *Lagerstroemia parviflora* were affected by seed dormancy. This causes a delay when establishing a plant nursery which limits the planting of *Lagerstroemia speciosa* in horticultural nurseries, forestry plantations and home (Hossain *et al.* 2007; Azad *et al*. 2010).

Unfortunately, the rooting of *Lagerstroemia speciosa* cuttings is also difficult and therefore investigation into ideal auxin treatments for its propagation is required (Yakandawala and Adhikari, 2014). This research focused on optimizing the propagation methods for the production of Queens Flower (*Lagerstroemia speciosa*).

Specific objectives are to:

- Determine the effect of rooting hormone (IBA) on the rooting ability of *Lagerstroemia speciosa* cuttings.
- Identify a suitable vegetative propagule (soft wood, semi-hardwood or hard wood cuttings) for the propagation of *Lagerstroemia speciosa.*
- Determine the effect of seed pre-treatments on germination of *Lagerstroemia speciosa* seeds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Geographical Distribution

Lagerstroemia speciosa is indigenous to Western Ghats from Belgaum, North and South Kanara, Malabar and Travancore, and in evergreen forests. It is primarily found in the neighbourhood around rivers and streams. *Lagerstroemia speciosa* is also found in Ceylon, Burma and eastwards to the Malay Peninsula, Australia and northwards to China (Santapau, 1966). In Ghana, *Lagerstroemia speciosa* is mostly found in the southern part and usually grown in the cities such as Greater Accra, Kumasi, Koforidua and Sunyani.

2.2 Botanical Description

Lagerstroemia speciosa is a deciduous tree. It grows into small to medium size, up to 40 (45) m tall. The tree is quite straight to and does not give out branches for up to 18 m with a diameter of 100-150 cm. It is often shallow curved in with small buttresses and a smooth bark surface with small thin flakes. The tree mostly has pale yellowish-brown covered with patches of different colours which do not form a regular pattern and turns dirty purple due its environmental exposure (Orwa *et al.* 2009).

The crown is mostly bushy and spreads. The leaves have opposite form of arrangement and simple. Flowers with a broad, axillary or terminal panicle are showy with a bell shaped calyx. The flowers have six to nine lobes, often six petals, which are found close to the tip. The flowers also have clawed, wrinkled, many stamens in several rows, superior ovary, three to six locular with many ovules in each locule. “The common name honours M. Lagerstroemia, 1691 to 1759, a Swedish benefactor of discipline and the precise nickname 'speciosa' is a Latin term for

attractive, which refers to the flowers''(Orwa *et al.* 2009).The fruit of *Lagerstroemia speciosa* has a large woody capsule with an apical winged seed.

2.3 Uses

Lagerstroemia speciosa is broadly used for home landscaping and avenue both for public and private landscaping. It can as well be planted along rivers and streams and in swampy lands and also serves as wind breaks (Zabala, 1990).

As an ornamental species, the captivating form of the tree and flowers makes Lagerstroemia ideal for shade and the incorporation of colour to the landscape. The tree is also used for afforestation of comparatively moist localities as it able to become established and spread in natural ecosystems. It also controls erosion due to its dense and wide spreading root system.

Lagerstroemia speciosa is used as medicinal plant. The seeds are narcotic, the bark is used as purgative, and the roots are used for treatment of fevers and dysentery. Also, all parts have been reported useful for diabetes treatments (Kramer and Kozlowski, 1960).

In Ghana, *Lagerstroemia speciosa* are used in home gardening and for road-side plantation mostly in the cities. It can also be grown along the low-lying swampy lands in the coastal areas in some of our cities. Fire wood and charcoal problems can also be solved by the use of *Lagerstroemia speciosa* in Ghana.

2.4 Methods of Propagation

Most ornamental plants can be propagated easily to bring high number of plants with ideal characteristics thus reducing the costs associated with landscaping (Ingram *et al*. 1993). However ornamental plants like *Lagerstroemia speciosa* can be propagated with difficulty by seed or

asexually using techniques such as stem cuttings and tissue culture (Yakandawala and Adhikari, 2014). In Ghana, lagerstroemia speciosa is mostly propagated by seed by nurseries especially the nurseries located in the cities of the country.

2.5 Seed Germination

Germination involves imbibition of H_2O by the dormant dehydrated seed, changes with the elongation of the embryo and completed when the developed features surrounding the embryo by the radicle are clearly seen (Bewley, 1997). Sexual propagation is a means of reproducing plants using the seeds so that plants that are difficult or impossible to propagate through vegetative means can be propagated. Sexual propagation may cost less for commercial production. However, seedling characteristics mostly vary which may be a disadvantage especially when growth uniformity is the desired outcome.

Lagerstroemia speciosa may be propagated by seed. However, seed germination ability is mostly low due to dormancy of seeds (nursery owners). The seeds are sown when dried. Azad *et al.* (2010) confirmed that dried seeds enhanced germination as the viability of seeds was intact after 2 years of air tight storage at room temperature. Germination of seeds however improves in first 3-12 months of storage. Germination takes place within 15-56 days. Pricking out may be carried out after germination of small seedlings and transplanted. However, upon several observations about the dryness of *Lagerstroemia speciosa* seeds, this research may indicate that the seeds break easily when properly dry due to their light weight.

2.6 Seed Pre-treatment

Seed pre-treatment is the method of reducing seed dormancy for optimum germination (Azad *et al.* 2010).

2.6.1 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) promotes germination of seeds. Ogawa and Iwabuchi (2001), confirmed that H_2O_2 promotes germination of seeds in a small manner as respiratory inhibitors functions, which indicates that hydrogen peroxide aforementioned perhaps enhance germination of seed somewhat than oxygen. In an experiment by Ogawa and Iwabuchi (2001), they found that seeds that germinated using *Zinnia elegans,* was increased as the pericarp was taken from seeds and ethanol-soluble compounds were removed from seeds. The ethanol-soluble compounds prevented seeds from germinating which have no pericarp and this eventually was changed by the use of H_2O_2. Hence, the outcome indicates that corrosion of the sprouting inhibitor(s) in the pericarp by hydrogen peroxide enhanced sprouting of seeds. Seeds were treated with 1% and 2% H_2O_2 for 12h and 24h respectively. Kannan *et al.* (1996) conducted an experiment to investigate the best pre-treatment for improvement of germination of seeds and vigour of *Albizia* species (*Albizia odoratissima*, *Albizia procera* and *Albizia falcataria*). Contrarily, in an experiment by Kannan *et al.* (1996) they found hydrogen peroxide gave poor performance (below 10%) indicating its ineffectiveness to penetrate the seed coat. This research also confirmed that hydrogen peroxide performed poorly (2.6%) which is also below 10%.

2.6.2 Cytokinin

Cytokinins found in growing seeds in liquid endosperm is essential for enhancement of cell separation the embryo (Kucera *et al.* 2005). Cytokinins play important roles in embryogenesis,

embryonic pattern formation, and enhancement sink strength. Yahiro (1980), confirmed that lower concentrations of cytokinins (1, 5 and 10 ppm) enhanced germination of Papaya seeds. Iqbal and Ashraf (2005) have reported that a considerable amount of kinetin had a steady consequence in changing the progress and grain produce of wheat cultivars. Marsh *et al*. (2017) also suggested that the breaking of seed dormancy at little temperature involves the variations in developed hormones needed to define the sequence of improvement for the removal of seed inactivity. Van-Staden and Wareing (2017), reported that in seeds that are light-sensitive, cytokinin activity may perhaps be under the regulation of phytochrome.

2. 6.3 Gibberellic Acid

Gibberellic acids (GAs) perform a part in inactivity of seeds discharge then also promotes seed germination. Gibberellic acid biosynthesis in the developmental stage in seeds of several plant species primes to the build-up and storing of either bio inactive gibberellic acid precursors or bioactive gibberellic acid (Kucera *et al*. 2017).

Çetinbaş and Koyuncu (2006), stated that an improvement in germination of *Prunus avium* L. seeds after seeds were treated with GA_3. Keys *et al*. (1975) confirmed that gibberellic acid and kinetin when combined by ethylene and carbon dioxide was ideal in incapacitating thermo-dormancy of *Lactuca sativa* L. seeds, by alleviating any induced light requirement.

Jarvis *et al.* (2017) also reported that gibberellic acid (GA_3) increases the synthesis of nucleic-acid in breaking dormancy of Hazel seeds.

2.6.4 Bleach (Clorox)

Sodium hypochlorite (NaOCl) is the dynamic element of domestic bleach. It is the greatest common form of chlorine used in plant propagation and will continue to be used as an important

disinfectant in propagation (Hartmann *et al.* 2002). Badawy *et al.* (2005), stated that highest average value (2.66) of healthy free of contamination explants of *Draceana fragrans* was recorded by using 15% (v/v) Clorox. Laude (2017), reported that the use of Clorox (30%) as a sodium hypochlorite solution (2.5%) for Smilo grass seed treatment increased the speed of emergence. In this experiment, the greatest increase in speed of emergence and in numbers of seedlings emerged was found in seeds treated for 1.5 hours (86.7%). Chun *et al.* (1997), similarly showed that sodium hypochlorite (5.25%) caused rice plantlet growth, due to an unintended outcome to eliminate microbial pollutants or to reduce dormancy. On the contrary, this research found that *Lagerstroemia speciosa* seeds treated with chlorox had no germination.

2.6.5 Sulphuric Acid

Azad *et al.* (2010) reported low percent germination in *Lagerstroemia speciosa* seeds which they attributed to seed dormancy. In their experiment, H_2SO_4 (80%) treatment recorded the highest germination percentage of (79%). Aref *et al.* (2011) also showed that seed dormancy in *Acacia spp.* may prolong germination over a period of a month or a year, and therefore it is important to treat seeds before sown in order to maintain largest and faster germination. In the experiment, seeds treated with sulphuric acid (H_2SO_4, 98%) for 5 min, 10 min. and 15 min, respectively recorded the maximum germination percentage (92.0 to 96.0%). Missanjo *et al*. (2014) however confirmed that to preserve plant biodiversity for posterity and continuity of plant heritage there is the need for seed pre-treatments to increase germination rate, seedling growth, and survival percentage to provide information for mass production of seedlings. In their experiment, it was reported that the immersion of *Acacia* seeds in (0.3 M H_2SO_4) recorded the second highest

germination percentage (97.4 %). In this experiment also, Lagerstroemia seeds treated with 90% of H_2SO_4 for 10 and 20 minutes gave the highest germination percentage (3.7%).

2.7 Factors Affecting Seed Germination

Factors affecting seed germination reviewed in this section are: seed dormancy, seed viability, oxygen/respiration, temperature, moisture/water, light and growth medium.

2.7.1 Seed Dormancy

Seed dormancy is when an undamaged feasible seed fails to thoroughly germinate under positive environments (Bewley, 1997). It is a multifaceted adaptive attribute of advanced plants that is predisposed by amount of genes and ecological factors such as temperature, moisture, oxygen and light. Studies have revealed that plant hormones, abscisic acid and gibberellins play essential characters in the directive of dormancy (Marsh *et al*. 2017).

2.7. 2 Primary Dormancy

Primary dormancy is importantly connected to the development and maturation of the seed (Hilhorst, 2008). Exogenous dormancy is a type of primary dormancy imposed by factors outside the embryo. These factors are; inhibition of water up-take due to hard seed coat and limited oxygen to the embryo respectively. These constitute physical dormancy. Preventing the percolating of inhibitors from the embryo and providing the inhibitors to the embryo also constitute another type of dormancy referred to as chemical dormancy (Bewley, 1997). In an experiment by Azad (2010), hot water and scarification treatments were used to overcome primary dormancy in *Lagerstroemia speciosa* seeds. In the experiment, seeds treated with hot water recorded 64% germination and scarification recorded 62% germination.

Nature's way of breaking physical dormancy is by high temperature, temperature fluctuations, cracking of the seed coat due to mechanical abrasions, fire, action of soil micro-organisms, passage through the digestive tracts of birds and mammals.

Endogenous dormancy is a primary dormancy imposed by factors within the embryo (Marsh *et al.* 2017). These factors may be morphological or physiological. Morphological when the embryo is not developed and physiological when the embryo lacks the growth potential for germination. Combinational dormancy is a combination of two kinds of dormancy such as morpho-physiological and exo/endo-dormancy.

2.7. 3 Secondary Dormancy

Secondary dormancy is the type that can solitary happen after seed scattering and is mostly related to yearly dormancy cycles in the seed and is revocable (Hilhorst, 2008). Secondary dormancy takes place if seeds fail to germinate after primary dormancy is broken and environmental conditions are not favourable.

Thermo-dormancy is the mechanism that prevents seeds from germinating at high temperatures and conditional dormancy is the transitional phase where seeds germinate but only over small range of temperatures.

2.8 Seed Viability

The staining of seeds to determine percentage seeds with viable embryos is an essential step in plant conservation. Chemical staining is used more often than seed germination count (SGC) as a direct method to measure viability (Vujanovic, 2000). Triphenyl tetrazolium chloride (TTC) and Fluorescein diacetate (FDA) discolouration procedures are used for testing viability in seeds

(Bandurski, 2017). In this experiment, TTC was used to test the viability of the *Lagerstroemia speciosa* seeds and the viable seeds got stained within 3 days.

2.9 Environmental Factors

Germination is the most critical phase in a plant's development. It is the outcome of complex interactions between numerous internal as well as external control factors. Internal control of germination relates to the state of the seed itself whereas the external control relates to environmental factors that breaks seed dormancy and causes germination (Zhou *et al*. 2017).

2.9.1 Oxygen/Respiration

Increasing the partial oxygen pressure of the atmosphere brings about or improves the germination of intact seeds of several plants (Morinaga *et al*. 2017). It has been revealed that reducing the oxygen pressure has beneficial effects on germination at a varying temperatures. Due to enhanced oxygen entry, germination of intact seeds increases in the dark with seeds that have gone through scratching, pricking, and cutting. Thus, inhibitors in the seed coat increases the oxygen requirement of the embryo. Also, an oxygen enriched atmosphere increases germination in short day plants (Black and Wareing, 2017).

2.9.2 Temperature

Temperature is critical in regulating the occurrence and speed of germination (Zhou *et al.* 2017). Seeds mostly germinate at relentless temperatures from 19 to 39 ^{0}C, with uppermost germination between 27 and 33^{0}C. Temperatures below 25/15 ^{0}C or above 40/30 ^{0}C are unfavourable for seed germination (Chachalis and Reddy 2017). There is a greater demand for light at lower temperatures (10 – 15 ^{0}C) such that day-to-day temperature variation increases sprouting level once seed radiates with far-red light which suggests that there is a lesser claim for the far- red-

riveting system of phyto-chrome (Benvenuti *et al.* 2000). Low temperature (<15 °C) may also be involved in the inhibition of germination. Research done by Ellis (2004), indicated that alternating temperatures promoted germination of *Lagerstroemia speciosa* considerably. In the experiment, a two-dimensional temperature gradient plate (35/22 ^{0}C, 16/8 h) showed that *Lagerstroemia speciosa* germinated most rapidly within 14 days. In this research temperature (31.4/29 ^{0}C) also ensured germination of *Lagerstroemia speciosa* seeds during the experiment period.

2.9.3 Moisture/Water

Moisture is an essential environmental condition for seed germination (Rinaldi *et al*. 2005). Absorption of water is the beginning of seed germination where the metabolic functions needed for germination get activated (Beal, n.d). After sufficient absorption of water, the embryo enlarges which become too large for the seed and rapture the seed outer layer. The radical submerged to form roots in the soil and the plumule emerges into a small plant. There may be rapid fluctuations of soil moisture around seeds since most seeds are planted at shallow depths. Seeds of several tree species respond differently to various amounts of supplementary water in addition to natural rainfall.

Seeds normally germinate at 100% relative humidity (RH) and decline when relative humidity is reduced from 100 to 92% (Arauz and Sutton, 1989). Work done by Arauz and Sutton (1989), reported maximum germination (80%) of Conidia of *Botryosphoeria obtusa* in free water which declined (23%) as relative humidity reduced from 100% to 92%. In an experiment Vieira *et al.* (2008), found that germination and seedlings of *Aspidosperma pyrifolium, Cavanillesia arborea, Cedrela fissilis, Amburana cearensis* and *Anadenanthera colubrina* that survived early improved under shade since soil retained moisture over a long period of time and the

microclimate was less severe than in areas of sunshine. In this research, moisture increased the relative humidity (93.4/62.8%) during the experiment period which ensured germination of *Lagerstroemia speciosa* seeds.

2.9.4 Light

Seeds that are viable of many plants after imbibition may not be able to germinate due to limited availability of light required. However, this response can be changed by substitute acquaintances to red and far-red radiation which likewise panels photoperiodic flowering responses, etiolation, bulbing, and other growth retorts (Toole *et al*. 1955). Far-red light is involved in germination.

After several months of seed storage, there is gradual loss of dormancy and the seed becomes photosensitive (Benvenuti *et al*. 2000). Thermal targets for seed sprouting is usually amid 20°C and 25°C in light or in the dark. Also demand for light increases at lower temperatures.

Spectrophotometric measurements show that a percentage less than one of the incident light penetrated 2.2 mm at any wavelength between 350 and 780 nm up to 1 mm. Biological measurements with light sensitive seeds also show that an acquaintance to light is correspondent to about a bright day induces sprouting of seeds that is 2 mm underneath the soil superficial, but would not disturb seeds which are 6 mm below the soil surface (Baskin and Baskin 2009).

2.9.5 Growth Medium

Rooting medium such as top soil, peat or sphagnum moss, coconut husk compost, perlite, vermiculite or sand, compost etc. promote seed germination when used singly or in ratios (Pijut *et al.* 2011). The large total pore space of peat makes increases its water holding capacity. Drainage of rooting media is improved by use of perlite, vermiculite or sand. Work done by Azad (2010) reported that growing medium of a ratio of 1:1:4:3 3:4:1:1 of fine sand and compost, coarse sand, coconut husk and top soil respectively was used for germination of

Lagerstroemia speciosa seeds and germination started 2 days after sowing. Compost as a growth medium promotes sprouting, growth, and harvests of horticultural florae (Arancon *et al*. 2008). Features such as enhancement of soil physical structure, increased numbers of useful microorganisms, and the accessibility of plant growth-influencing-substances such as hormones contribute to germination, growth and flowering of ornamentals.

Compost also has adequate water holding capacity to enhance seed germination (Medina, *et al.* 2009). In this work also, compost prepared from poultry manure (2 Rice Husk + 2 Coco Peat + 1 Poultry Manure; v/v) was able to hold water to enhance germination of *Lagerstroemia speciosa* seeds.

2.10 Factors Influencing Propagation of Cuttings

Factors influencing propagation of cuttings of Lagerstroemia speciosa reviewed in this section are: environmental conditions, propagation structure, rooting media, season, effect of carbohydrates and phenol levels, rooting co-factors, auxins and stock plant juvenility.

2.10.1 Environment

Rooting and growth of cuttings can be influenced by environmental conditions (Newton, 2001). The propagation environment encourages physiological activities such as photosynthesis and transpiration. The right environment also influences the physiological stress skilled by the tissues of cuttings from transpiration and respiration processes and inspires action of meristem such as differentiation of cells and mitosis in stems (Mesen *et al*. 1997). Propagation environment when controlled minimizes the period of physiological shock that arises from taking a cutting from its stock plant and implanting it into a propagator.

2.10.2 Propagation Structure

Propagation of cuttings is affected by physiological, biochemical and environmental factors. Specialized propagating structures such as polythene propagators, propagation bins, tunnels and mist propagators are required to achieve an acceptable rooting percentage (Yeboah *et al.* 2011). In their study propagation bin and polythene propagator were used and the results indicated higher rooting from cuttings set in the propagation bin (63.3%) as compared to the polythene propagator (57.5%). The lower percentage rooting in the polythene propagator was attributed to high temperatures ranging from 23ºC - 27ºC and 30ºC – 32ºC for night and day temperatures respectively. In this research, the propagation structures were shaded with an overhead layer of shade net (25% light exclusion) which enhanced rooting of *Lagerstroemia speciosa* cuttings.

2.10.3 Rooting Media

Rooting media plays a very significant role in vegetative propagation of plants. Appropriate rooting media may depend on the plant species, propagation techniques, season and the propagating system. The characteristics of ideal rooting media are; ability to hold cuttings firmly in place, provide sufficient porosity for good aeration at the base of cuttings, have good water holding capacity and drainage, free from bacteria and fungi, opaque enough to exclude light penetration to the base of cuttings and capacity to maintain suitable temperatures for adequate root formation (Hartmann *et al*. 2002). Also, the appropriate rooting medium is one which minimises physiological stress in the cuttings by lowering the air temperature, providing high humidity and reducing transpiration losses from the leaves of cuttings (Rogers *et al*. 2017). Earlier work done on stem cuttings using different rooting media such as sand and coir, sand dust 1:1 (v/v) showed uppermost rooting proportion (71%) from cuttings rooted in sand

(Yakandawala and Adhikari, 2014). According to Ofori-Gyamfi (1998), rice husk used as a rooting medium recorded the highest rooting percent in sheanut tree which was attributed to its ability to regulate temperatures leading to the enhancement of metabolic activities especially auxins biosynthesis and promotions of gaseous exchange at the base of the cuttings. In this work also, compost prepared from poultry manure (2 Rice Husk + 2 Coco Peat + 1 Poultry Manure; v/v) was able to hold water to enhance rooting of *Lagerstroemia speciosa* cuttings.

2.10.4 Season

Timing of season when cuttings are taken plays an essential role in cuttings with roots (Hartmann *et al.* 2002). Seasonal variations of woody cuttings to develop roots are determined by environmental features, genotypes, nourishing status and phenological stage (Hartmann *et al.* 2002; Loreti and Pisani, 1982). The Cuttings of some plants root well irrespective of the time of the year, while others root efficaciously only at specific periods of time (Sebastiani and Tognetti, 2004). Seasonal variations of cuttings to develop roots is not well unspoken of, but at great heights of irradiance, water strain, and occurrence of flowering may cause a decrease in rooting ability (Leakey, 2004).

2.10.5 Effect of Carbohydrates and Phenol levels on Rooting and Growth of Plants

Rooting of cuttings obtained from hardwood depends on availability of hydrolysis of carbohydrates stored within stem tissues of cuttings (Leakey, 2004).

The quantity of carbohydrates given and shared within cuttings may limit root formation and this limitation may be due to the absence respiration in roots (Haissig, 1984). Also, auxin improves starch hydrolysis. A study has shown that there is a relationship between rooting ability of cuttings and fathomable carbohydrates content (Leakey and Storeton-West, 1992). This recommends that ability to root is improved through making of exact quantum of sugars when

the cuttings are in a propagator (Leakey *et al*. 2017). Rooting ability of many cuttings has been correlated with the amount of carbohydrates present (Aslmoshtaghi and Shahsavar, 2016). Researchers have commented that carbohydrates of allowed plummeting sugars as well as storing carbohydrates are essential for root formation which serve as vigor and operational resources of a cell to introduce the root primal (Delrio *et al.* 1991; Bartolini *et al.* 2008). In an experiment by Aslmoshtaghi and Shahsavar (2016), they found that Olive cuttings showed a maximum soluble sugars and starch contents (125.6 mg g-1DW) at the beginning of the experiment but reduced (43 mg g-1DW) at 60 days later. Henrique *et al.* (2006) also reported in their experiment that *Pinus* cuttings decreased in total sugar contents from start of rooting (14.63 %) to end of the analyses period (5.71 %) which coincided with the highest rooting percentage (95.31 %). An indication that sugar content in the cuttings was used in the rooting process.

Phenolic compounds play crucial roles in the complex metabolism of plants. They are convoluted in physiological procedures of plants progress and improvement (Usenik *et al.* 2006). Phenolic composites perform a significant part in internal control mechanisms of rooting process (Aslmoshtaghi and Shahsavar, 2016). Phenolic compounds found in olive leaves have been realized to partake different biological actions and accounts for the pharmacological activities of the leaves of olive for strengthening those activities (Bartolini *et al.* 2008). In the experiment by Aslmoshtaghi and Shahsavar (2016), they found that the amount of phenolic compounds in olive cuttings increased from day zero (9.8 mg/g) to (18.56 mg/g) at 120 days. However, work done by Henrique *et al*. (2006) reported that total phenol contents analyzed in *Pinus* cuttings showed that phenols in *Pinus* cuttings did not reflect in rooting process. The total free phenols analysed before the formation of roots was 1.92 % and did not change after the rooting process. In this experiment, it was realised that total sugars and total free phenols were significantly different

among softwood, semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa* with hardwood having the highest total free phenols content.

2.10.6 Rooting co-factors

Rooting co-factors is a complex form of indole and phenolic substances. Initiation of root primordia may directly be affected by Co-factors with their oxidative enzymes. Co-factors are required to enhance auxin activity in the rooting process. There may be complicated connections amid co-factors, auxins and other materials (Leakey 2004).

According to Fadl and Hartmann (1967), there is an indication that indole-phenolic multiplexes exist in cuttings. Polyphenyl oxidase act, and stages of phloridzin increase preceding the increase in the sum of co-factors that are endogenous in turn relate to enhancements in the root aptitude (Bassuk and Howard 1981).

Equilibrium of enzymes essential to establishment of indole phenolic multiplexes might occasionally be complex through thoughtfulness to the plant growth regulators and can also be pretentious by the environment and stock plant circumstance (Leakey, 2004).

2.10.7 Auxins

Auxins are basely distributed in stems of plants, and accountable aimed at the schism of young plants. Auxins greatly increase ability of cuttings to yield roots in many plant species. In horticulture and forestry industries, auxins are collectively used unaided, or in a blend with new chemicals as an assistance to propagation (Leakey 2004).

Synthetic auxins such as indolebutyric acetic (IBA), are typically chosen more than Indole acetic acid (IAA) that is endogenous. IBA is the best commonly used and is often joint with NAA or one of the phenoxyacetic acids. Work done by Henrique *et al.* (2006) testified that *Pine* cuttings pickled with 4000 mg/L IBA showed a higher root formation (95.31%) than *Pinus* cuttings

(14.08 %) pickled with 4000 mg/L NAA, indicating that IBA as a regulator of growth for plants remained real in supporting root formation in the cuttings.

As a difficult-to-root plant, the use of rooting hormone enhanced rooting in cuttings of *Lagerstroemia* (Haissig, 1974). Work done by Yakandawala and Adhikari (2014), confirms *Lagerstroemia* cuttings do not root readily unless they are treated with a rooting hormone (s). At concentration of 0.3% IBA, this auxin was found to have highest percentage (71%) on rooting in Lagerstroemia cuttings. Similarly, research done by Amissah *et al.* (2013) showed that at higher concentration (10,000 ppm IBA) auxin produced the highest rooting percentage of 53.3% in sheanut tree. Work done by Aslmoshtaghi and Shahsavar (2016) also reported that Olive cultivar, Roghani cuttings pickled with IBA (4000 mg/L) showed a higher rooting percentage (66.3%) as compared with cuttings without IBA treatment (15%) indicating that the usage of rooting hormone enhances rooting in cuttings.

The special properties of auxins on root formation aptitude might be determined by the way of use (Howard 1973). Over the years, the quick-dip method, the powder application technique and the diluted soak technique have been used mostly for smearing auxins to cuttings (Merhaut *et al*. 2006).

Auxins are ordinarily used at the base lot of the cuttings. The quick-dip technique is frequently ideal for the use of liquid auxin that of liquid preparations for rapidity, affluence, and consistency of use and outcomes (Hartmann *et al.* 2002).

The concentration and type of carrier or solvent are important factors to be considered in the preparation of the 'quick dip' hormone solutions. The kind and amount of auxins can promote rooting, inhibit shoot growth, or yield herbicidal effects (Merhaut *et al*. 2006). The effectiveness of a particular rooting hormone depends on its formulation such as salt or acid, time or duration

of treatment, concentration and the forms of solvents and wetting agents used. Hartmann *et al.* 2002, however, recommended 50% concentrations for carriers such as ethanol, methanol and acetone for the preparations of concentrated rooting hormones.

Work done by Blythe *et al* (2007), in cutting planting of foliage crops by means of a leaf application of auxin showed that the entire length of root on cuttings of *Ficus benjamina* was 332 mm long when raw, 400 mm once pickled with a base dip, as well as 280–355mm while sprayed with the auxin at amounts of 49.2 µM IBA + 26.9 µM NAA. Thus the auxin posies at lesser degrees made minor entire length of root (189–218 mm) more than with base dip method. These authors also reported that the basal quick-dip method involves dipping the basal portion of stem cuttings into rooting hormone solution for 1 to 5 seconds or lengthier, preceding to sticking of cuttings into the rooting substrate. The powder use technique includes sinking the base parts of stem cuttings (frequently pre-moistened for hold) to a mixture of auxin and talc powder, tailed through a light hit to eliminate extra talk aforementioned to the sticking of the cutting into the substrate for root formation. The diluted infuse method likewise includes inserting the base part of the stem cuttings in a diluted solution of auxin aimed at a lengthy epoch of 2-48 hours in a lukewarm [20 ^{0}C (68 ^{0}F)] site with unintended brightness.

In this work, it was generally observed in the propagation experiment that IBA showed a significant effect on rooting performance of softwood, semi-hardwood as well as hardwood cuttings of *Lagerstroemia speciosa.* Indolebutyric acid (IBA) also contributed significantly to rooting by increasing the total sugar levels and total free phenols in *Lagerstroemia speciosa* cuttings.

2.10.8 Stock Plant Juvenility

Plant juvenility is crucial in propagating difficult-to-root plant species. Plant juvenility is the phase in plants characterized by the plant's inability to form flowers or be induced to form flowers under favourable environmental conditions (Wendling *et al.* 2014). These characteristics are retained in ontogenetically young tissues near the central axis at the tree base (Hartmann *et al*. 2002).

The length of the juvenile period varies among different plants (Kibbler *et al.* 2004) and can be predisposed by nutritive rank, ecological and hereditary features (Hackett, 1985) and the epoch is contrariwise associated to the refinement proficiency of perennials which are woody such that some plants lose their rooting ability long before reaching the maturity phase (Hansche and Beres 1980).

In several plants, desirable characteristics such as the form, flower are not realized until the mature phase which makes vegetative propagation difficult (Wendling *et al.* 2014). According to Husen and Pal (2006), rooting aptitude of cuttings reduction as stock plants developed which may be caused by; (i) build-up of rooting inhibiters (ii) reduction in the content of auxin that are endogenous and root supporters, and (iii) reduced tissues sensitivity.

Stock plant stage has a significant consequence on cuttings which form roots (Eshed *et al.* 1996). Ontogenetic aging and chronological aging are identified with juvenility in plants (Hartmann *et al*. 2002). Ontogenetic aging is the developmental phase from embryonic to maturity of a seedling plant where as chronological aging refers to the number of years plants have grown from seed or vegetative propagule (Wendling *et al.* 2014).

Kibbler *et al.* (2004), suggest that stem cuttings taken from ontogenetically juvenile plant parts have a higher tendency for adventitious rooting. A reverse to the juvenile stage through

coppicing or severe pruning as the plants produce the most juvenile plant parts, the embryos, through gametogenesis and sexual reproduction (Wendling *et al.,* 2014) may cause difficult-to-root plant species to root easily. Also, reports have been made on the use of gibberellins on a number of mature plants species that have brought back juvenile features (Hackett, 1985).

Work done by Eshed *et al.,* (1996) showed that root formation on cuttings taken from oak stock plants treated with gibberellin were better than that of cuttings from non-treated stock plants. Cuttings from non-treated stock plants dropped from 30% in cuttings from 1-year-old plants to 7% in cuttings from 3-year-old plants. Also, GA_3 concentrations (500, 1000, and 2000 mg/L) useful as bark action to 3-year-old stock plants increased rooting over the control by 6 to 7 fold. Husen and Pal (2006), confirmed from development of adventitious roots in the cuttings of *Backhousia citriodora* F. Muell varying in, juvenility, plant genotype and features of cutting materials, that aging of giver plants bottled-up of root formation and shooting of cuttings, but amplified formation of callus. Percent rooting declined with age of donor [2-months (67.35%), 15-years (46.37%), 30-years (35.56 %)] similarly % sprouting declined with donor plant age [2-month (61.46 %), 15-years (47.53 %), 30-years (27.84 %)]. Rooting of cuttings declined as donor plants aged from two months to fifteen years and then to thirty years. Moreover, work done by Amri *et al*. (2010) in vegetative propagation of *Dalbergia melanoxylon* Guill. and Perr. showed that cutting materials from young parent plant made improved (71.11%) in all factors of root than from old parent plant (24.42%).

Based on the research work reviewed, it is recommended that more research should be conducted on the use of IBA (4000ppm-10000ppm), hydrogen peroxide (1% -2%) and H_2SO_4 (92% - 98%)on propagation of *Lagersroemia speciosa*. Therefore, this research was conducted to

investigate the ideal IBA, Hydrogen peroxide and H_2SO_4 for optimum germination and propagation of *Lagerstroemia speciosa*.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

Two experiment consisting of a *Lagerstroemia speciosa* cutting and seed germination pre-treatment experiments were done in Department of Crop Science, University of Ghana, 0.5^0 39 N^1, 00^0 10 W^0, 69 m above sea level. The rainfall for this area ranges from 74.1mm to 95 mm and annual maximum and minimum temperatures means are 31 ^{0}C and 24.3 ^{0}C respectively.

3.2 Types of propagules and materials used

3.2.1 Cuttings

Cutting materials were taken from the juvenile, strong, dynamically growing parent plants of *Lagerstroemia speciosa* in the University of Ghana-Accra.

3.2.2 Rooting hormone

Indole 3- butyric acetic acid (IBA)

3.2.3 Growing medium

Compost prepared from poultry manure was used for the germination of *Lagerstroemia speciosa* seeds (2 Rice Husk + 2 Coco Peat + 1 Poultry Manure; v/v).

3.3 Vegetative Propagation Experiment

3.3.1 Cuttings

The soft wood, semi hard wood and hard wood cuttings of *Lagerstroemia speciosa* were harvested and trimmed to a length of 15 cm with at least two nodes.

The cuttings were kept moist by placing the ends into a bucket with some water at the bottom to prevent them from drying up.

Figure 1: a) Semi-hard and **b)** hardwood cuttings of *Lagerstroemia speciosa*

3.3.2 Poly Propagator

The cuttings of *Lagerstroemia speciosa* were rooted in a poly propagator constructed with a Styrofoam frame measuring 3 by 4ft with a white transparent polythene covering. (To keep relative humidity high ~ 95 %).

3.3.3 Rooting/ Growing Medium

Compost prepared from poultry manure was used as the growing medium for the propagation of *Lagerstroemia speciosa*. The medium was sterilized in oven at 105°C for two hours after which it was left to cool. The medium was drenched with fungicide for 72 hours in the poly propagators. The medium was then filled into the poly propagator (3/4) and watered thoroughly.

3.3.4 Planting Process

The basal portion of the cuttings of *Lagerstroemia speciosa* were cut and about 2-3 cm of the basal length dipped into the appropriate IBA concentrations for 10 seconds. Five to ten minutes

were allowed for the drying of the hormone before sticking into the growth medium at depth of 2.5 cm. There were 20 cuttings per treatment. The IBA used were 500 ppm, 1000 ppm, 1500 ppm, 3000 ppm and 4500 ppm respectively.

3.3.5 Experimental Design

Completely Randomised Design (CRD) was used for the experiment consisting of two types of cuttings (semi-hard wood and hard wood) and three levels of 1500 ppm, 3000 ppm, and 4500 ppm (Yakandawala *et al*., 2014) of IBA, dissolved in 50 % of ethanol plus a control. There were three replications and a total of 480 cuttings consisting of both semi-hard and hard wood were used. With the soft wood cuttings, there were four levels (500 ppm, 1000 ppm, 1500 ppm, 3000 ppm) of IBA concentration, dissolved in 50 % of ethanol and a control (no IBA application). 300 soft wood cuttings were used. All cuttings treatments had their control replicated three times.

3.3.6 Treatments

The treatments for Lagerstroemia experiment consist of:

- 20 semi-hard wood cuttings each were dipped in 1500 ppm, 3000 ppm or 4500 ppm of IBA for 10 seconds. Each level was replicated three times.
- 20 hard wood cuttings each were dipped in 1500 ppm, 3000 ppm or 4500 ppm of IBA for 10 seconds. Each level was replicated three times.
- 20 soft wood cuttings each were dipped in 500 ppm, 1000ppm, 1500ppm or 3000 ppm of IBA for 10 seconds. Each level was replicated three times.
- Control (no IBA application) which was also replicated three times for the hardwood and semi-hardwood cuttings.

3.3.7 Environmental Conditions during the Rooting of *Lagerstroemia speciosa* Stem Cuttings

Minimum, maximum temperatures and the relative humidity of factors of external environment and in propagating constructions were measured using data loggers (Voltcraft DL-121TH USB Temperature & Humidity Data Loggers). Maximum mean ambient temperature for the period of experiment was 31.4 ^{0}C and minimum mean temperature was 29 ^{0}C, while temperatures mean in the propagation constructions were 25 ^{0}C (minimum) and 40.9 ^{0}C (maximum). The relative humidity of the environment in the experiment surroundings during the experiment period was between 93.4% in morning (8.00am) to 62.8% around noon (4:00 pm) (Appendix 1). The propagation structures were shaded with an overhead layer of shade net (25% light exclusion).

Figure 2: Cutting propagation experimental set-up.

3.3.8 Determination of Carbohydrates and Phenols

Samples of plant materials from the various experiments were analysed for total carbohydrates Ghasemi *et al.* (2009), (soluble and insoluble sugars), and total free phenols at the Noguchi Memorial Institute for Medical Research, University of Ghana. A cutting each from the replicates

was analysed before and after IBA application. The basal portions (approximately 6cm) of cuttings was taken through biochemical analyses. The samples were analysed according to Anim-Tawiah *et al.* (2016), Dubois *et al.* (1956), Ghasemi *et al.* (2009) and Usenik *et al*. (2006) for sugars and phenols correspondingly.

3.3.9 Carbohydrate Determination

The Anthrone as a method was used to find the aggregate carbohydrates of the cuttings (Ghasemi *et al*, 2009). The cuttings were dried and grounded into powder. 10-20 mg of the powder were weighed into a centrifuge tube. The interfering pigments were extracted with 100 % acetone, e.g. using ultra-turrax and filtering/centrifuging. 200 mL of the samples was used for sugars extraction. The sugars were then extracted with 2.5 mL aliquots of 80 % ethanol and centrifuged. The supernatant was then kept for soluble sugar analysis.

Five mL of 1.1 % HCL was included to the residue and heated in a water bath at 100 ºC for 30 minutes which was further diluted to 10 mL with deionized (DI) water.

3.3.10 Absorbance Determination

The spectrophotometer was turned on and allowed to warm up. The Anthrone reagent was made by dissolving 1 g of Anthrone in 500 mL of 72 % sulphuric acid. 1.0 mL of test solution was then pipetted into a 10-mL test tube and cooled to 0 ºC on ice. 5 mL of ice-cold Anthrone reagent was then added to the soluble and insoluble sugars (Ghasemi *et al.*, 2009).

The samples were then heated for exactly 11 minutes at 100 ºC (in a water bath) and air-conditioned rapidly to 0 ºC on ice. The samples were placed in the spectrophotometer to read the absorbance at A_{630} (against water) within an hour for both soluble and insoluble sugars for all cuttings.

3.3.11 Determination of Phenols

Phenolic content of the plant extracts was evaluated by means of the process described by Ghasemi *et al.* (2009). To a volume of 10 µl of sample, 790 µl of distilled water was then added. An aliquot of 50 µl of Folin-Ciocalteau reagent was then added and thoroughly mixed. The mixture was incubated in the dark for 8 minutes. About 150 µl of Sodium carbonate (20 g of anhydrous Na_2CO_3 in 80 ml distilled water) was added and then incubated for 2 hours in the dark. Each sample was run in triplicates.

3.3.12 Determination of Total Free Phenolic Using Standard Gallic Acid

The absorbance reading was taken at 750 nm. Six concentrations (0.156 to 5 mm) of the standard phenolic compound, Gallic acid were also run alongside in triplicates to generate a standard curve

(Ghasemi *et al.*, 2009).

3.3.13 Statistical Analysis

Percentage rooting for each treatment was calculated. Microsoft excel was used to calculate the means of data collected and analysed using GENSTAT (12th Edition). The differences amid the means of various parameters studied were determined by the Least Significant Difference (LSD) and the significance was definite at $p<0.05$.

3.3.14 Data collected

- Relative humidity and temperature was determined in the tunnels over one-week period.

  One and a half months after sticking the cuttings:

- The percentage of cuttings that got rooted / treatment
- The number of roots per cuttings per treatment

- The average length of the longest root per cutting per treatment
- Carbohydrates and phenol contents were determined per cutting per treatment. Two samples per treatment per replicate were analysed before and after IBA treatment for total carbohydrates (soluble and insoluble) at the Department of Nutrition and Food Science and Total free phenols at the Noguchi Memorial Institute for Medical Research, University of Ghana. The basal portions (approximately 6cm) of cuttings was taken and stored in -80 ^{0}C for biochemical analyses. The samples were analysed using Dubois *et al.* (2013) and Usenik *et al.* (2006) methods for sugars and phenols respectively.

3.4 Germination Experiment

An experiment was done to show the effect of Hydrogen peroxide, Sodium hypochlorite (3.5 %) and conc. Sulphuric acid treatments on germination and seedling growth.

3.4.1 Seeds

Seeds of *Lagerstroemia speciosa* were collected from matured and healthy (8 year-old) trees one week aforementioned to the start of the experiments from the University of Ghana-Legon, in the Greater Accra Region, Ghana. Moisture content of seeds was reduced by air-drying within 5 to 6 days, after which the seeds were removed from the pods by hands for another week. The dried seeds were sorted to remove the discoloured and damaged seeds. The dried seeds at 10 % moisture level were then used in the experiments. Compost prepared from poultry manure was used for the germination of *Lagerstroemia speciosa* seeds (2 Rice Husk:2 Coco Peat :1 Poultry Manure; v/v).

Figure 3: Seeds of *Lagerstroemia speciosa*

3.4.2 Viability Test

A seed viability test was conducted by immersing all seeds that were to be used in the experiment in tap water and those that were found to be floating were collected and discarded. The high density/non floating seeds were dried by sun for three days and kept at room temperature (24 ^{0}C). Dried seeds were selected at random at the start of the experiment to estimate the germination potential of the seeds using the tetrazolium chloride test (Vujanovic *et al*., 2000). Seeds used for the germination experiments were taken from selected seeds.

3.4.3 Treatments

- **Control**: Seeds with no treatment
- **Hydrogen Peroxide (5 % and 10 %):** 20 seeds were soaked in 5 % and 10 % of H_2O_2 for 30 minutes. The two treatment levels were replicated five times.

- **Clorox (3.5 % Sodium Hypochlorite):** 20 seeds were soaked in 50 % and 75 % of the bleach solution for 30minutes each. The two treatments and a control were replicated five times.
- **Conc. H_2SO_4 treatment:** 20 seeds were soaked in 90 % Conc. H_2SO_4 for 5, 10, 15 and 20 minutes. The four treatments and a control were replicated five times.

3.4.4 Experimental design

Completely randomized design was used for the experiment. The treatments were replicated five times and a total of 820 seeds were used for the experiment. The seed trays (55 cm x 28 cm) were arranged under a structure constructed with wood and covered with a layer of black shade net. Each tray consisted of 72 cells.

Figure 4: Seed germination experimental set-up

3.4.5 Data collected

The seeds germinated were recorded up to 34 days. Germination was realized when the plumule emerged from the coat of seeds.

Germination Percentage (%) = $\frac{\text{Number of germinated seeds x100}}{\text{Total number of seeds}}$ (Paul, 1972)

Speed of Germination (SOG): the determined germination daily gotten at a given time. Germinated seeds in each treatment were reckoned every day and speed of germination was examined by the formula;

$$SOG = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots\frac{X_n - (X_n - 1)}{Y_n}$$

Where; X_1, X_2 and X_n = germinated seeds on 1st, 2nd and nth day, respectively.

Y_1, Y_2 and Y_n = days from sowing to 1st, 2nd and nth count, respectively

Coefficient of Germination (COG) (%)

Coefficient of germination is the rate of germination of seeds or an index of rapidity and was examined by the formula;

$$COG\ (\%) = \frac{A_1A_2 + \ldots\ldots\ldots A_n}{A_1T_1 + A_2T_2 + \cdots A_nT_n}\ x\ 100$$ (Paul, 1972)

Where; A = germinated seeds

T = time (days) corresponding to A and

n = days to final count

Mean of Germination Time (MGT)

This is the average time of germinations.

$$MGT = \frac{\sum d_1}{\sum n_1}$$ (Paul, 1972)

Where; n_1 = number of seeds in every count

d_1= day of counting

Germination percentage (%), Germination mean time, germination speed and variation coefficient of the germination mean time were computed using an excel spreadsheet designed by Ranal *et al*. (2009). The data collected were analysed using ANOVA in GENSTAT (12th Edition). The Least Significant Difference (LSD) was used to determine differences among the means of the various parameters.

CHAPTER FOUR

4.0 RESULTS

4.1 General Observations from the Propagation Experiments and Germination Experiments

Sprouting of leaves was observed five (5) days after sticking of *Lagerstroemia speciosa* cuttings and was uniform among all treatments.

Root formation was observed to have started three weeks (21 days) after treatment of IBA. By the end of the experiment when root formation was assessed, the roots were vertically oriented close to the base of the stem (1mm). Both the young and old roots were white, fairly thick (~0.2 mm to 0.4 mm) with root hairs. Roots formed as close as 1mm and as far as 25 mm from the base of cuttings. Roots were also observed to grow along the axils of tender branches of sprouts in cuttings treated with 1500 ppm IBA.

Germination of *Lagerstroemia speciosa* seeds was observed eleven (11) days after nursing in Conc. sulphuric acid (H_2SO_4) treatment and thirteen (13) days in hydrogen peroxide (H_2O_2) treatment. There was no germination in seeds treated with bleach (Chlorox). It was also observed that the rate of germination was very slow.

Figure 5: a) Rooted cuttings of *Lagerstroemia speciosa* b) rooting on sprouted leaf petiole of *Lagerstroemia speciosa* cuttings (red- arrow).

4.2 Environmental Conditions during the Experiments

The maximum ambient temperature mean at the period of experiment was 31.4 ^{0}C and minimum temperature mean 29 ^{0}C, whereas the temperatures mean in the propagation structures were 40.9 ^{0}C (maximum) and 25 ^{0}C (minimum) (Appendix 1). The Relative humidity (R.H) of the environment surroundings at the period of the experiment which ranged between 93.4 % in the early morning relatively (8.00 am) to 62.8 % in the afternoon (4:00 pm) (Appendix 1). The structure for propagation was given a shade using a layer of shade net (25 % light exclusion).

4.3 Effect of IBA treatments on Rooting Number of roots per cutting Length of the longest root in the softwood cuttings of *Lagerstroemia speciosa*

Results presented in table 4.1 showed no significant difference in the percentage rooting and in the length of the longest root per shoot between different treatments. However, differences were found in the number of roots per shoot of the 1500 ppm IBA treatment and the other treatments. Rooting was observed to have occurred earlier in cuttings treated with 1500 ppm IBA compared to the others.

Table 4.1: Effect of IBA treatments on Rooting Number of roots/cutting Length of the longest root in the softwood cuttings of *Lagerstroemia speciosa*

Treatment (IBA)	% Rooting	NRPC	LLR (cm)
Control	0.0 ± 0.0a	0.7 ± 0.0a	0.0 ± 0.0a
500 ppm	4.3 ± 4.3a	0.9 ± 0.2a	1.8 ± 1.8a
1000 ppm	4.3 ± 4.3a	1.1 ± 0.4a	1.3 ± 1.3a
1500 ppm	14.8 ± 1.8a	2.1 ± 0.2b	6.1 ± 0.3a
3000 ppm	4.3 ± 4.3a	1.2 ± 0.5a	2.0 ± 2.0a

All data represent the means (±S.E) of the three replicates. Means that have different letters are significantly different at $p<0.05$. **(NRPC)** Number of roots per cutting, **(LLR)** Length of the longest root.

4.4 Effect of IBA on the levels of Soluble Insoluble total sugar Total free phenols in the soft wood cuttings of *Lagerstroemia speciosa*

In the softwood cuttings, 1500 ppm IBA had a significant ($p<0.05$) increase (4.0 mg/g) on the sugar levels and the total free phenols. Again, cuttings without IBA application had a significant result on total free phenols (4.6 mg/ mL) (Table 4.2).

Table 4.2: Effect of IBA on levels of Soluble Insoluble total sugar Total free phenols in the soft wood cuttings of *Lagerstroemia speciosa*

	Sugars (mgg^{-1})			Total free phenols
Treatments	**Soluble**	**Insoluble**	**Total**	**($mgmL^{-1}$)**
SWC	0.8 ± 0.07ab	2.7 ± 0.04a	3.5 ± 0.12a	4.6 ± 0.3c
500 ppm SW	0.7 ± 0.02a	2.8 ± 0.02a	3.5 ± 0.05a	0.7 ± 0.2a
1000 ppm SW	0.9 ± 0.01b	2.8 ± 0.01a	3.7 ± 0.03a	0.6 ± 0.2a
1500 ppm SW	1.2 ± 0.04c	2.8 ± 0.04a	4.0 ± 0.04b	2.5 ± 0.1b
3000 ppm SW	0.9 ± 0.06b	2.7 ± 0.06a	3.6 ± 0.12a	1.0 ± 0.2a

All data represent the means (±S.E) of the three replicates. Means showing different letters are significantly different at $p<0.05$. (**SWC**) Softwood control and **(SW)** Softwood cuttings.

4.5 Effect of IBA treatments on Rooting Number roots per cutting Length of the longest root in the semi-hardwood cuttings of *Lagerstroemia speciosa*

Results from this experiment show that IBA showed significant effect on rooting in the semi-hardwood cuttings. Cuttings that were treated with IBA showed the highest percentage rooting compared to those with zero IBA, but the IBA treatments were not significantly different from one other. In this experiment 1500 ppm treatment gave a significantly ($p<0.05$) higher number of roots per cutting (2.1) when compared with the 4500 ppm treatment and the control but not significantly different from 3000 ppm treatment of IBA (Table 4.3).

Table 4.3: Effect of IBA treatments on Rooting Number of roots/cutting Length of the longest root in the semi-hardwood cuttings of *Lagerstroemia speciosa*

Treatment (IBA)	% Rooting	NRPC	LLR (cm)
Control	0.0 ± 0.0a	0.7 ± 0.0a	0.0 ± 0.0a
1500 ppm	14.8 ± 1.8b	2.1 ± 0.2b	6.6 ± 0.9b
3000 ppm	14.8 ± 1.8b	1.5 ± 0.1bc	6.3 ± 1.0b
4500 ppm	12.9 ± 1.2b	1.8 ± 0.2c	6.0 ± 1.5b

All data represent the means (±S.E) of three replicates. Means showing different letters are significantly different at $p<0.05$. **(NRPC)** Number of roots per cutting, **(LLR)** Length of the longest root.

4.6: Effect of IBA on the levels of Soluble Insoluble Total Sugar Total free phenols in the semi-hardwood cuttings of *Lagerstroemia speciosa*

Effect of IBA on the levels of soluble, insoluble and total sugars in semi-hardwood cuttings of *Lagerstroemia speciosa* was not significantly ($p<0.05$) different between treatments (Table 4.4). However, IBA had a significant ($p<0.05$) increase on total free phenols in the semi-hard cuttings treated with 1500 ppm (2.7 mg/mL), 3000 ppm (31 mg/mL) and semi-hardwood cuttings with no IBA treatment (3.6 mg/mL).

Table 4.4: Effect of IBA on the levels of Soluble Insoluble Total sugar Total free phenols in the semi-hardwood cuttings of *Lagerstroemia speciosa*

	Sugars (mgg^{-1})			Total free phenols
Treatments	Soluble	Insoluble	Total	($mgmL^{-1}$)
SHWC	0.4 ± 0.02a	2.8 ± 0.02a	3.2 ± 0.05a	3.6 ± 0.3b
1500 ppm SHW	0.5 ± 0.02a	2.8 ± 0.02a	3.3 ± 0.09a	2.7 ± 0.4ab
3000 ppm SHW	0.7 ± 0.04a	2.8 ± 0.02a	3.5 ± 0.02a	3.1 ± 0.1b
4500 ppm SHW	0.6 ± 0.0a	2.7 ± 0.0a	3.3 ± 0.15a	1.4 ± 0.1a

All data represent the means (±S.E) of three replicates. Means showing different letters are significantly different at $p<0.05$. **SHWC** – Semi-hardwood control and **SHW** – Semi-hardwood cuttings.

4.7 Effect of IBA treatments on Rooting Number of roots/cutting Length of the longest root in the hardwood cuttings of *Lagerstroemia speciosa*

Results from the experiment below show that IBA had a significant ($p<0.05$) effect on rooting in hardwood cuttings. Cuttings treated with IBA gave the highest rooting percentage than cuttings without IBA treatment. Percentage rooting in 1500 ppm and 4500 ppm treatments were significantly ($p<0.05$) higher than 3000 ppm treatment but not significantly different from the control. Number of roots per shoot in 1500 ppm treatment was significantly ($p<0.05$) different from the other treatments. There was no significant difference between treatments in the length of longest root (Table 4.5).

Table 4.5: Effect of IBA treatments on Rooting Number of roots/cutting Length of the longest root in the hardwood cuttings of *Lagerstroemia speciosa*

Treatment (IBA)	% Rooting	NRPC	LLR (cm)
Control	14.8 ± 1.8c	1.6 ± 0.2a	9.8 ± 3.9a
1500 ppm	16.6 ± 1.8c	2.5 ± 0.2c	10.6 ± 5.0a
3000 ppm	8.6 ± 4.3b	1.4 ± 0.4ab	4.0 ± 2.4a
4500 ppm	12.9 ± 1.3bc	1.7 ± 0.2b	8.0 ± 1.2a

All data represent the means (±S.E) of three replicates. Means showing different letters are significantly different at $p<0.05$. **(NRPC)** Number of roots per cutting, **(LLR)** Length of the longest root.

4.8: Effect of IBA on levels of Soluble Insoluble Total Sugar Total free phenols in the hard wood cuttings of *Lagerstroemia speciosa*

Effect of IBA on the levels of soluble, insoluble and total sugars in hardwood cuttings of *Lagerstroemia speciosa* was not significantly ($p<0.05$) different between treatments (Table 4.6). However, IBA had a significant ($p<0.05$) increase on total free phenols in the hardwood cuttings treated with 4500 ppm, being higher (7.3 mg/mL) than in hardwood cuttings with no IBA treatment (3.4 mg/mL) (Table 4.6).

Table 4.6: Effect of IBA on the levels of Soluble Insoluble Total Sugar Total free phenols in the hard wood cuttings of *Lagerstroemia speciosa*

	Sugars (mgg^{-1})			Total free phenols
Treatments	**Soluble**	**Insoluble**	**Total**	**($mgmL^{-1}$)**
HWC	0.6 ± 0.02a	2.8 ± 0.02a	3.4 ± 0.04a	3.4 ± 0.2a
1500 ppm HW	0.5 ± 0.03a	2.7 ± 0.03a	3.2 ± 0.07a	4.5 ± 0.0b
3000 ppm HW	0.7 ± 0.03a	2.8 ± 0.03a	3.5 ± 0.06a	5.4 ± 0.4b
4500 ppm HW	1.1 ± 0.03a	2.7 ± 0.03a	3.8 ± 0.25a	7.3 ± 0.0c

All data represent the means (±S.E) of three replicates. Means showing different letters are significantly different at $p<0.05$. (**HWC**) Hardwood control and (**HW**) Hardwood cuttings.

4.9 Effect of IBA treatments on Percentage rooting in semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa*

Results in Table 4.7 show that IBA treatments had a significant ($p<0.05$) effect on rooting percentage in both semi-hardwood and hardwood cuttings. Semi-hardwood cuttings treated with IBA gave the highest rooting percentage compared to the control. Percentage rooting in 1500 ppm and 3000 ppm treatments of IBA were significantly ($p<0.05$) higher than 4500 ppm treatment in semi-hardwood cuttings but not significantly different from each other. There was no significant ($p<0.05$) difference between IBA treatments and control in the hardwood cuttings. However, in hardwood cuttings, the percentage rooting in control, 1500 ppm and 4500 ppm treatments of IBA were significantly ($p<0.05$) higher than percentage rooting in 3000 ppm treatment of IBA. Also, the means of IBA treatments had a significant ($p<0.05$) effect on rooting in both hardwood and semi-hardwood cuttings as compared with the control (Table 4.7). Both semi-hardwood and hardwood cuttings treated with 1500 ppm and 4500 ppm of IBA were significantly ($p<0.05$) higher than the cuttings treated with 3000 ppm of IBA (Table 4.7).

Table 4.7: Effect of IBA treatments on percentage rooting in semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa*

Treatment	Semi-hardwood	Hardwood	Means
Control	0.0 ± 0.0a	14.8 ± 1.8c	7.4a
1500 ppm	14.8 ± 1.8c	16.6 ± 1.8c	15.7b
3000 ppm	14.8 ± 1.8c	8.6 ± 4.3b	11.7c
4500 ppm	12.9 ± 1.2bc	12.9 ± 1.3bc	12.9bc
Mean	10.6a	13.2a	

4.10 Effect of IBA treatments on number of roots in semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa*

Table 4.8 showed that IBA had a significant effect on number of roots in both semi-hardwood and hardwood cuttings. The number of roots of cuttings treated with 1500 ppm IBA in both wood-type was significantly ($p<0.05$) higher than the cuttings treated with 3000 ppm and 4500 ppm IBA (Table 4.8). The number of roots in semi-hardwood cuttings treated with 4000 ppm IBA was significantly ($p<0.05$) higher than hardwood cuttings (Table 4.8).

Table 4.8: Effect of IBA treatments on number of roots in semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa*

Treatment	Semi-hardwood	Hardwood	Mean
Control	0.7 ± 0.0a	1.6 ± 0.2a	1.1a
1500 ppm	2.1 ± 0.2b	2.5 ± 0.2b	2.3c
3000 ppm	1.5 ± 0.1ab	1.4 ± 0.4a	1.4ab
4500 ppm	1.8 ± 0.2b	1.7 ± 0.2a	1.7b
Mean	1.5a	1.8a	

4.11 Effect of IBA treatments on length of the longest roots in semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa*

IBA had no significant ($p<0.05$) effect on length of the longest roots in both semi-hardwood and hardwood cuttings (Table 4.9).

Table 4.9: Effect of IBA treatments on length of the longest roots in semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa*

Treatment	Semi-hardwood	Hardwood	Mean
Control	0.0 ± 0.0a	9.8 ± 3.9a	4.9a
1500 ppm	6.6 ± 0.9a	10.9 ± 5.0a	8.3a
3000 ppm	6.3 ± 1.0a	4.0 ± 2.4a	5.1a
4500 ppm	6.0 ± 1.5a	8.0 ± 1.2a	7.0a
Mean	4.7a	8.2a	

4.12 Soluble Insoluble Total Sugars Total free phenols in softwood, semi-hardwood and hard wood cuttings from the start and end of experiment

Table 4.10 compares the effect of IBA treatments on total free phenols, soluble, insoluble and total sugars and in hardwood, softwood and semi-hardwood cuttings of *Lagerstroemia speciosa*. Soluble sugars in softwood cuttings were significantly affected by 1500 ppm IBA. IBA treatments were found to have no significant ($p<0.05$) differences in the levels of insoluble sugars in hardwood, softwood and semi-hardwood cuttings. Total sugars and total free phenols were also significantly different among softwood, semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa* with hardwood having the highest total free phenols content (Table 4.10).

Table 4.10: Soluble Insoluble Total sugars and Total free phenols in softwood, semi-hardwood and hard wood cuttings of *Lagerstroemia speciosa*

Treatment	Sugars (mgg^{-1})			Total free phenol ($mgmL^{-1}$)
	Soluble	Insoluble	Total	
SWC	0.4 ± 0.07ab	2.7 ± 0.04a	3.1 ± 0.1bcd	4.6 ± 0.3de
500 ppm SW	0.5 ± 0.02a	2.8 ± 0.04a	3.3 ± 0.05bcd	0.7 ± 1.2a
1000 ppm SW	0.9 ± 0.01b	2.8 ± 0.01a	3.7 ± 0.03de	0.6 ± 0.2a
1500 ppm SW	1.2 ± 0.04c	2.8 ± 0.04a	4.0 ± 0.04f	2.5 ± 0.1bc
3000 ppm SW	0.9 ± 0.06b	2.7 ± 0.06a	3.6 ± 0.12de	1.0 ± 0.2a
SHWC	0.4 ± 0.02a	2.8 ± 0.02a	3.2 ± 0.05a	3.6 ± 0.3cd
1500 ppm SHW	0.5 ± 0.02a	2.8 ± 0.02a	3.3 ± 0.09abc	2.7 ± 0.4c
3000 ppm SHW	0.7 ± 0.04a	2.8 ± 0.02a	3.5 ± 0.02bcd	3.1 ± 0.1c
4500 ppm SHW	0.6 ± 0.0a	2.7 ± 0.0a	3.3 ±0.15abcd	1.4 ± 0.2ab
HWC	0.6 ± 0.02a	2.8 ± 0.02a	3.4 ± 0.04abcd	3.3 ± 0.2c
1500 ppm HW	0.5 ± 0.03a	2.7 ± 0.03a	3.2 ± 0.07ab	4.5 ± 0.0de
3000 ppm HW	0.7 ± 0.03a	2.8 ± 0.03a	3.5 ± 0.06bcd	5.4 ± 0.4e
4500 ppm HW	1.1 ± 0.03a	2.7 ± 0.03a	3.8 ± 0.25ef	7.1 ± 0.0f

All data represent the means (±S.E) of three replicates. Means showing different letters are significantly different at $p<0.05$. **SWC** – Softwood control and **SW** – Softwood cuttings, **SHWC** – Semi-hardwood control, **SHW** – Semi-hardwood cuttings, **HWC** – Hardwood control and **HW** – Hardwood cuttings

4.13 Effect of Hydrogen Peroxide, Bleach and Sulphuric acid on the Germination Percentage, the Speed of Germination and the Germination Mean Time of *Lagerstroemia speciosa* seeds

Table 4.11compares the effect of hydrogen peroxide, bleach and sulphuric acid on germination percentage, speed of germination and germination mean time of *Lagerstroemia speciosa* seeds. Hydrogen peroxide (5 % and 10 %), bleach (50 % and 75 %) and sulphuric acid (5 min., 10 min.,

15 min. and 20 min.) treatments had no significant ($p<0.05$) effect on germination percentage, speed of germination and germination mean time of *Lagerstroemia speciosa* seeds.

Table 4.11: Effect of Hydrogen Peroxide, Bleach and Sulphuric acid on the Germination Percentage, the Speed of Germination and the Germination Mean Time of *Lagerstroemia speciosa* seed

Treatment	G (%)	MT	SOG
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
5 % H_2O_2	2.6 ± 2.6a	0.0 ± 0.0a	0.01± 0.0a
10 % H_2O_2	2.6 ± 2.6a	0.0 ± 0.0a	0.01± 0.0a
50 % Bleach	0.0	0.0	0.0
75 % Bleach	0.0	0.0	0.0
5 min. H_2SO_4	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
10 min. H_2SO_4	2.6 ± 2.6a	0.0 ± 0.0a	0.0 ± 0.0a
15 min. H_2SO_4	3.7 ± 3.7a	1.3 ± 1.3a	0.0 ± 0.0a
20 min. H_2SO_4	3.7 ± 3.7a	1.3 ± 1.3a	0.0 ± 0.0a

Means of different letters are significantly different at $p<0.05$. **G –** Germination percentage, **MT –** Mean time germination and **SOG** – Speed of germination

4.14 Effect of Hydrogen Peroxide (H_2O_2) on the Germination Percentage, the Speed of Germination, the Coefficient of Germination and the Germination Mean Time of *Lagerstroemia speciosa* seed

Hydrogen Peroxide (H_2O_2**)** had no significant effect on germination percentage of *Lagerstroemia speciosa* seeds. There was no significant difference between the germination percentages in seeds treated with both 5% and 10% (H_2O_2**)** (Table 4.12).

Table 4.12: Effect of Hydrogen Peroxide (H_2O_2) on the Germination Percentage, the Speed of Germination, the Coefficient of Germination and the Germination Mean Time of *Lagerstroemia speciosa* seeds

Treatment (H_2O_2)	**G (%)**	**MT**	**CV**	**SOG**
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
5 %	2.6 ± 2.6a	0.0 ± 0.0a	0.0 ± 0.0a	0.01 ± 0.012a
10 %	2.6 ± 2.6a	0.0 ± 0.0a	0.0 ± 0.0a	0.01 ± 0.012a

All data represent the means (±S.E) of three replicates. Means showing different letters are significantly different at $p<0.05$. **G –** Germination percentage, **MT –** Mean time germination and **CV–** Coefficient of Variation of germinated seeds.

4.15 Effect of Bleach (Clorox) on Germination Percentage, Speed of Germination, Coefficient of Germination and Germination Mean Time of *Lagerstroemia speciosa* seeds

Results in the table below show that bleach had no significant effect on germination percentage of *Lagerstroemia speciosa* seeds. There was no significant difference between the germination percentages in seeds treated with both 50% and 75% bleach.

Table 4.13: Effect of Bleach (Clorox) on the Germination Percentage, the Speed of Germination, the Coefficient of Germination and the Germination Mean Time of *Lagerstroemia speciosa* seeds

Treatment (Clorox)	**G (%)**	**MT**	**CV**	**SOF**
Control	0.0	0.0	0.0	0.0
50 %	0.0	0.0	0.0	0.0
75 %	0.0	0.0	0.0	0.0

All data represent the means (±S.E) of three replicates. Means showing different letters are significantly different at $p<0.05$. **G –** Germination percentage, **MT –** Mean time germination and **CV–** Coefficient of Variation of germinated seeds.

4.16 Effect of Sulfuric acid (H_2SO_4) on the Germination Percentage, the Speed of Germination, the Coefficient of Germination and the Germination Mean Time of *Lagerstroemia speciosa* seeds

Results in the table below show that (H_2SO_4) had no significant effect on germination percentage of *Lagerstroemia speciosa* seeds. There was no significant difference between the germination percentages in seeds treated with (H_2SO_4) in 5 minutes, 10 minutes, 15 minutes and 20 minutes.

Table 4.14: Effect of Sulfuric acid (H_2SO_4) on the Germination Percentage, the Speed of Germination, the Coefficient of Germination and the Germination Mean Time of *Lagerstroemia speciosa* seeds

Treatment (H_2SO_4)	**G (%)**	**MT**	**CV**	**SOG**
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
5 minutes	2.6 ± 2.6a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
10 minutes	3.7 ± 3.7a	1.3 ± 1.3a	0.00203 ± 0.00203a	0.024 ± 0.024a
15 minutes	2.7 ± 2.6a	0.0 ± 0.0a	0.0 ± 0.0a	0.012 ± 0.0a
20 minutes	3.7 ± 3.7a	1.3 ± 1.3a	0.00203 ± 0.00203a	0.024 ± 0.024a

All data represent the means (±S.E) of three replicates. Means showing different letters are significantly different at $p<0.05$. **G –** Germination percentage, **MT –** Mean time germination and **CV–** Coefficient of Variation of germinated seeds.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of IBA treatments on Rooting Number of roots/cutting Length of the longest root in softwood cuttings of *Lagerstroemia speciosa*

The results of experiment 1 indicated that generally, the IBA treatments did not have significant effect on percent rooting in softwood cuttings. However, 1500ppm IBA had significant effect on percentage rooting. The control (no IBA) did not develop any roots which confirmed that rooting hormone was required to promote root formation in softwood cuttings of *Lagerstroemia speciosa*. Results from the current experiment are similar to findings by Yakandawala and Adhikari, (2014) that *Lagerstroemia speciosa* cuttings do not root readily unless they are treated with rooting hormone. IBA treatments showed significant effect on number of roots at the 1500 ppm IBA level. There was no significant effect on length of the longest roots. A comparison of the results of 500 ppm, 1000 ppm and 3000 ppm IBA levels with 1500 ppm IBA level of experiment one shows significant difference between the rooting performances of softwood cuttings. The differences in rooting percentage may be caused by sensitivity of the softwood tissues to 1500 ppm IBA penetration, as a result of reduced lignin formation in the stem of cuttings. It could be concluded that IBA application promoted rooting of softwood cuttings significantly.

Generally, it was observed that the high percentage rooting of the cuttings with auxin treatments contained higher contents of phenolic in softwood cutting indicating that *Lagerstroemia speciosa* cuttings materials with high total content of phenol had high content of orthodihydroxyphenols. This eventually reduced activity of IAA-oxidase and increased the IAA concentration endogenously IAA (Coll *et al*. 1992), thereby increasing the percentage rooting of cuttings. Also,

Jarvis and Shaheed (1986), confirmed that acidic phenol (diphenols which inhibit the IAA-oxidase) controls activity of IAA-oxidase activity.

In the current experiment, treatments that gave higher rooting percentages contained lower total sugar contents. The reducing sugars suggests that total sugars were essential in root formation process and converted to reducing sugars (Henrique *et al.* 2006).

5.2 Effect of IBA treatments on Rooting Number roots/cutting Length of the longest root in the semi-hardwood cuttings of *Lagerstroemia speciosa*

Cuttings not treated with IBA did not root. This finding supports results obtained from research done on importance of auxin application with regards to the promoting root formation in cuttings (Hartmann *et al.* 2002 and Yakandawala and Adhikari, 2014). Haissig (1974), stated that the use of rooting hormone enhanced rooting by increasing the transformation speed and movements of sugars to cuttings base which encourage rooting. IBA application also increased the level of endogenous auxins which is important in cell division and differentiation for rooting of cuttings (Hartmann *et al.* 2002).

It was noticed that the high percentage of rooting found in cuttings that which were treated with auxin had higher contents of phenol in semi-hardwood cuttings of *Lagerstroemia speciosa*. Also according to Hess (1962), the structural necessity of phenols is able to stimulate root initiation in *Hibiscus rosa-sinensis* and *Hedera helix* L. Leopold (1964), resolved that auxin had an important role to play on physiological procedures and development in cuttings, which required other substances such as rooting co-factors that may stimulate rooting, and was also a fact that was observed by Weaver (1982).

5.3 Effect of IBA treatments on Rooting Number of roots/cutting Length of the longest root in the hardwood cuttings of *Lagerstroemia speciosa*

The results of experiment 3 indicated that hardwood cuttings treated with IBA showed a significant effect on rooting in hardwood cuttings of *Lagerstroemia speciosa.* The hardwood cuttings with no IBA treatment (control) produced no roots which moreover confirmed that rooting hormone was required to promote root formation in stem cuttings of *Lagerstroemia speciosa.* Percentage rooting showed significant effects at 1500 ppm and 4500 ppm IBA levels. This was similar to findings by Yakandawala and Adhikari (2014) that *Lagerstroemia* cuttings rooted readily when treated with a rooting hormone (IBA) in *Lawsonia inermis.* However, this contradicted with results in experiment 1 where IBA did not increase percentage rooting. There was no significant difference in percentage rooting in softwood cuttings of *Lagerstroemia speciosa.*

A comparison of the results of this experiment with the results of experiment 2 showed a significant effect on rooting performance in semi-hardwood and hardwood cuttings. The average rooting percentages for the semi-hardwood cuttings was 14.8% while that of hardwood cuttings was 16.6%. The differences in the percentage rooting may be caused by a sensitivity of the stem tissues to the penetrations of IBA. Therefore, moderate to higher concentrations of rooting hormones are needed to enhance high rooting in both semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa.* It was also observed that high percentage rooting of the cuttings with auxin application contained higher phenolic contents in hardwood cuttings. The current results showed that carbohydrates may be needed for root formation and also agrees with studies by Bartolini *et al.* 2008, Aslmoshtaghi and Shahsavar, 2016, Henrique *et al.* (2006), Delrio *et al.* 1991 and Leakey, 2004.

5.4 Effect of Hydrogen Peroxide (H_2O_2) on the Germination Percentage, the Speed of Germination, the Coefficient of Germination and the Germination Mean Time of *Lagerstroemia speciosa* seeds

Among the three treatments of *Lagerstroemia* seeds, both the 5% and 10% H_2O_2 showed the same germination (2.6%) and there was no significant effect of Hydrogen Peroxide on germination. The results were similar to the findings by Laude (2017), that hydrogen peroxide does not result in faster emergence and in greater number of seedlings of *Piptatherum miliaceum* (Smilo grass). The results however contradict earlier works on mechanism for promoting the germination of *Zinnia elegans* seeds by Ogawa *et al.* (2001) who concluded that hydrogen peroxide promotes germination as a respiratory inhibitor. The poor germination in this experiment may be due to the poor imbibition which was caused by the impermeable seed coat and mycropylar plug of it Lagerstroemia seeds Kannan *et al.* (2016). This may be due to the light weight of seeds that make them float on water. Liu *et al.*, (2012) also suggested that seeds may be characterised by poor germination as a result of a low plasma membrane H^+-ATPase activity. This enzyme activity might partially clarify poor germination, root elongation and a low post-germination seedling growth (Sveinsdottir *et al*. 2009). Liu *et al.* (2012) also concluded that ATP in seeds is produced from decomposed form of glucose which includes glycolysis and oxidative phosphorylation in cellular respiration chain. Under hypoxic conditions, 1 mole of glucose can only generate 2 moles of ATP through glycolysis. However, ethanol as a by-product from fermentation of glucose is toxic to embryo which can be formed and collected and this may hinder seed germination.

5.5 Effect of Bleach (Chlorox) on the Germination Percentage, the Speed of Germination, the Coefficient of Germination and the Germination Mean Time of *Lagerstroemia speciosa* seeds

There was no seed germination in this experiment. This may be due to the higher concentrations of bleach (50 % and 75 %) used. Work done by Laude (2017), in Smilo grass, 30 % bleach was used to treat seeds and 31.8 % germiantion was obtained. No germination occurrence may also be due to seed damage. Studies done by Banik (1992), on morphological characteristics of the *Lagerstroemia speciosa* confirmed that it was difficult to separate the seeds from the fruit without damaging them. This is because the pericarp of the seeds is tightly attached to the seed base but thicker and fibrous towards the apex. According to Harper (1977), seeds may fail to germinate due to innate dormancy. The paper however described innate dormancy as the incapability of a seed to germinate even though in the presence of suitable conditions such as oxygen, temperature and moisture. The innate dormancy is usually an adaptation by seeds that delays germination until the most favourable growth season (Harper, 1977).

5.6 Effect of Sulfuric acid (H_2SO_4) on the Germination Percentage, the Speed of Germination, the Coefficient of Germination and the Germination Mean Time of *Lagerstroemia speciosa* seeds

Among the three treatments of *Lagerstroemia* seeds, sulphuric acid (H_2SO_4) showed the highest germination (3.7 %). This may be because of the softening ability of the coat of seeds by H_2SO_4 which was better than Hydrogen Peroxide and household bleach (Azad *et al.* 2010). There was no significant effect of H_2SO_4 on seed germination. Aref *et al.* (2011) confirms that increasing the time (15 min) of immersion in acid (98 % H_2SO_4) decreases germination percentage in seeds.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

Rooting of stem cuttings of *Lagerstroemia speciosa* was realised using IBA. From the stem cutting propagation experiment, it was generally observed that 1500 ppm IBA showed a significant effect on rooting performance of softwood, semi-hardwood as well as hardwood cuttings of *Lagerstroemia speciosa.* Hence, 1500 ppm IBA was the best for rooting and root growth in *Lagerstroemia speciosa*. Hardwood cuttings were better propagules for *Lagerstroemia speciosa* than both softwood and semi-hardwood cuttings. IBA also contributed significantly by increasing the total sugar levels and total free phenols in the softwood cuttings but these did not enhance rooting. The effects of total free phenols and total sugars were not consistent.

Germination of *Lagerstroemia speciosa* seeds was generally very poor. However, some germination was possible using hydrogen peroxide and sulphuric acid.

There was no germination when household bleach (chlorox) was used to treat *Lagerstroemia* seeds. *Lagerstroemia speciosa* seeds treated with 90% sulphuric acid (H_2SO_4) for 10 minutes and 20 minutes produced the highest germination percentage.

Based on the results of this research it is recommended that:

1. The highest rooting of 16.6 % obtained in the study should be improved upon by studying the:
   (i) Use of lower concentrations of IBA on hardwood cuttings, since the 1500 ppm IBA was better than 3000 ppm and 4000 ppm.
   (ii) Effect of varying concentrations of 1-Naphthaleneacetic acid (NAA) and combinations of NAA and IBA on rooting of *Lagerstroemia speciosa* cuttings.

(iii) Effect of mineral nutrition such as N, P and K of mother plants of *Lagerstroemia speciosa* cuttings.

2. The influence of plant juvenility conducted on the propagation of cuttings of *Lagerstroemia speciosa.*
3. Air layering as a propagation method could be explored in *Lagerstroemia speciosa.*
4. Other seed pre-germination treatments, such as Potassium nitrate (KNO_3), GA_3, Kinetin and Cytokinin should be investigated for the germination of *Lagerstroemia speciosa* seeds.

REFERENCES

Amissah, N., Akakpo, B., Yeboah, J., and Blay, E. (2013). Asexual propagation of sheanut tree (*Vitellaria paradoxa* CF Gaertn.) using a container layering technique. *American Journal of Plant Sciences*, *4*(09), 1758.

Amri, E., Lyaruu, H. V. M., Nyomora, A. S., and Kanyeka, Z. L. (2010). Vegetative propagation of African Blackwood (*Dalbergia melanoxylon* Guill. & Perr.): Effects of age of donor plant, IBA treatment and cutting position on rooting ability of stem cuttings. *New Forests*, *39*(2), 183–194. doi:10.1007/s11056-009-9163-6

Anim-Tawiah, M., Larbie, C., Appiah-Oppong, R., Tuffour, I., Owusu, K. B. A., and Aning, A. (2016). Phytochemical, Antioxidant and Cytotoxity of Hydroethanolic Extracts of *Crotolaria retusa* L. *World Journal of Pharmaceutical Research*.Vol. 5. Issue 2. 162-179.

Arancon, N. Q., Edwards, C. A., Babenko, A., Cannon, J., Galvis, P., and Metzger, J. D. (2008). Influences of vermicomposts, produced by earthworms and microorganisms from cattle manure, food waste and paper waste, on the germination, growth and flowering of petunias in the greenhouse. *Applied Soil Ecology*, *39*(1), 91–99. doi:10.1016/j.apsoil.2007.11.010.

Arauz, L. F., and Sutton, T. B. (1989). Influence of Temperature and Moisture on Germination of Ascospores and Conidia of *Botryosphaeria obtusa*. *Phytopathology* 79:667-674.

Aref, I. M., Ali, H., Atta, E., Shahrani, T. Al, and Ismail, A. (2011). Effects of seed pretreatment and seed source on germination of five *Acacia spp*. *African Journal of Biotechnology*, *10*(71), 15901–15910. doi:10.5897/AJB11.1763.

Aslmoshtaghi, E., & Shahsavar, A. R. (2016). Biochemical changes involved in self-incompatibility in two cultivars of olive (*Olea europaea* L.) during flower development. *The Journal of Horticultural Science and Biotechnology*, *91*(2), 189-195.

Azad, S., Paul, N. K., and Matin, A. (2010). Do Pre-Sowing Treatments Affect Seed Germination in *Albizia richardiana* and *Lagerstroemia speciosa*? *Frontiers of Agriculture in China*, *4*(2), 181–184. doi:10.1007/s11703-010-0100-4

Bandurski, A. K. and R. S. (2017). The One Hundred-Year Period for Dr . Beal ' s Seed Viability Experiment Author (s): A . Kivilaan and Robert S . Bandurski Source : *American Journal of Botany* , Vol . 68 , No . 9 (Oct ., 1981), pp .

Baskin, C. C., and Baskin J. M. (2009). Seeds: Ecology, Biogeogrphy, and Evolution of Dormancy and Germination. Jamestown Road, LondonAcademic Press Harcourt Place 32 NWI 7BY, UK.

Bassuk, N. L., & Howard, B. H. (1981). A positive correlation between endogenous root-inducing cofactor activity in vacuum-extracted sap and seasonal changes in rooting of M. 26 winter apple cuttings. *Journal of Horticultural Science*, *56*(4), 301-312.

Beal, S. K. (n.d). The Importance of Water in Seeds Germination. https://www.hunker.com/.../the-importance-of-water-in-seeds-germination (10/07/2017).

Benvenuti, S., Macchia, M., and Miele, S. (2000). Light , Temperature and Burial Depth Effects on *Rumex obtusifolius* Seed Germination and Emergence. *Weed Research* 41, 177-186.

Bartolini, G., Petrucceli, R., and Pestelli, P. (2008). Preliminary Study on in Vivo Rooting of Two *Olea europaea* L. genotypes. *Acta Hort* 791: 191-195.

Badawy, S. S., Issa, Y. M., and Mutair, A. A. (2005). Ion-selective electrodes for potentiometric determination of ranitidine hydrochloride, applying batch and flow injection analysis techniques. *Analytical sciences*, *21*(12), 1443-1448.

Banik, R. L. (1995). Diversities, reproductive biology and strategies for germplasm conservation of bamboos. *Bamboo and Rattan: Genetic Resources and Use. International Plant Genetic Resources Institute, Singapore*, 1-22.

Benvenuti, F., Carlini, C., Patrono, P., Galletti, A. M. R., Sbrana, G., Massucci, M. A., and Galli, P. (2000). Heterogeneous zirconium and titanium catalysts for the selective synthesis of 5-hydroxymethyl-2-furaldehyde from carbohydrates. *Applied Catalysis A: General*, *193*(1), 147-153.

Bewley, J. D. (1997). Seed Germination and Dormancy. *The Plant Cell*, Vol. 9, 1055-1066.

Black, A. M., and Wareing, P. F. (2017). The Role of Germination Inhibitors and Oxygen in the Dormancy of the Light-sensitive Seed of *Betula spp.* *Journal of Experimental Botany* 10(28), 134–145.

Blythe, E. K., Sibley, J. L., Tilt, K. M., and Ruter, J. M. (2007). Methods of auxin application in cutting propagation: A review of 70 years of scientific discovery and commercial practice. *Journal of Environmental Horticulture*, *25*(3), 166.

Çetinbaş, M., and Koyuncu, F. (2006). Improving Germination of *Prunus avium* L . Seeds by Gibberellic acid , Potassium nitrate and Thiourea. *Hort. Sci.* (Prague), 33, 119–123.

Chachalis, D., and Reddy, K. N. (2017). Factors Affecting *Campsis radicans* Seed Germination and Seedling Emergence, *Weed Science*, Vol. 48, No. 2 (Mar. - Apr., 2000), pp. 212-216

Chun, S., Schneider, R. W., Cohn, M. A., (1997). Sodium Hypochlorite : Effect of Solution pH on Rice Seed Disinfestation and Its Direct Effect on Seedling Growth. *Plant Dis.* 81:821-824.

Coll, J. B., Rodrigo, G. N., García, B. S. and Tamés, R.S. (1992). Ácido abscísico y otros inhibidores. In- *Fisiología vegetal*, ed. J.B. Coll, G.N. Rodrigo, B.S. García, R.S. Tamés. Madrid: Pirámide. pp. 369-379.

Delrio, C., Rallo, L., and Caballero, J. M. (1991). Effects of Carbohydrate Content on the Seasonal Rooting of Vegetative and Reproductive Cuttings of Olive. J. *Horticultural. Sci* 66(3): 301-309.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). Colorimetric Methods for Determination of Sugars and Related Substances. *Anal. Chem.*, 28: 350-356.

Ellis, R. H. (2004). Germination: Factors influencing the germination of myrtle (Lagerstroemia speciosa (L.) Pers. and L. floribunda L.) seeds. Department of Agriculture, The University of Reading, Earley Gate, Page 35-41

Eshed, Y., Riov, J., and Atzmon, N. (1996). Rooting Oak Cuttings from Gibberellin-Treated Stock Plants. *HortScience*, *31*(5), 872–873.

Fadl, M. S., and Hartmann, H. T. (1967). Isolation, purification, and characterization of an endogenous root-promoting factor obtained from basal sections of pear hardwood cuttings. *Plant Physiology*, *42*(4), 541-549.

Ghasemi, K., Ghasemi, Y., and Ebrahimzadeh, M. A. (2009). Antioxidant Activity, Phenol and Flavonoid Contents of 13 Citrus Species Peels and Tissues. Pak. J. *Pharm. Sci*., Vol. 22, pp. 277-281.

Hackett, W. P. (1985). Juvenility, maturation, and rejuvenation in woody plants. *Horticultural Reviews*, Vol. 7, 109-155.

Hansche, P. E., and Beres, W. (1980). Genetic Remodeling of Fruit and Nut Trees to Facailitate Cultivar Improvement. *Hortscience* Vol. 15 No. 6 1pp. 710-715 ref. 50.

Harper, J. L., & Harper, J. L. (1977). *Population biology of plants* (Vol. 892). London: Academic press.

Hartmann, H. T., Kester, D. E., Davies, F. T. Jr., and Geneve, R. L. (2002). "Plant Propagation: Principles and Practices." Prentice Hall, New Jersey.

Henrique, A., Campinhos, E. N., Ono, E. O., & Pinho, S. Z. D. (2006). Effect of plant growth regulators in the rooting of Pinus cuttings. *Brazilian Archives of Biology and Technology*, *49*(2), 189-196.

Heywood, V. H. (1999). *Use and potential of wild plants in farm households* (No. 15). Food & Agriculture Org.

Haissig, B. E. (1984). Carbohydrate accumulation and partitioning in *Pinus banksiana* seedlings and seedling cuttings. *Physiologia plantarum*, *61*(1), 13-19.

Haissig, B. E. (1974). Influences of auxins and Auxin Synergists on Adventitious Root Primordium Initiation and Development. *New Zealand Journal of Forestry Science*, 4(2), 311–323.

Hilhorst, H. W. M. (2008). A Critical Update on Seed Dormancy. I. Primary Dormancy. *Seed Science Research*, 5(02), 61–73. doi:10.1017/S0960258500002634.

Hess, C. E. (1962). Characterization of Rooting Co-factors Extracted from *Hedera helix* L. and *Hibiscus rosa-sinensis*. In: International Horticultural Congress, 16. Toronto. *Proceedings*… Toronto, Canada. pp. 382-388.

Hossain, M. A., & Ishimine, Y. (2007). Effects of farmyard manure on growth and yield of turmeric (Curcuma longa L.) cultivated in dark-red soil, red soil and gray soil in Okinawa, Japan. *Plant production science*, *10*(1), 146-150.

Howard, E. (1973). DNA content of rodent brains during maturation and aging, and autoradiography of postnatal DNA synthesis in monkey brain. *Progress in brain research*, *40*, 91-114.

Husen, A., and Pal, M. (2006). Variation in Shoot Anatomy and Rooting Behaviour of Stem Cuttings in Relation to Age of Donor Plants in Teak (*Tectona grandis* Linn. f.). *New Forests*, *31*(1), 57–73. doi:10.1007/s11056-004-6794-5

Iqbal, M., and Ashraf, M. (2005). Presowing Seed Treatment with Cytokinins and Its Effect on Growth, Photosynthetic Rate, Ionic Levels and Yield of Two Wheat Cultivars Differing in Salt Tolerance. *Journal of Integrative Plant Biology*, *47*(11), 1315–1325. doi:10.1111/j.1744-7909.2005.00163.x

Ingram, D. L., Henley, R. W., and Yeager, T. H. (1993). *Growth media for container grown ornamental plants*. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

Jarvis, A. B. C., Frankland, B., and Cherry, J. H. (2017). Increased Nucleic-Acid Synthesis in Relation to the Breaking of Dormancy of Hazel Seed by Gibberellic Acid Published by : Springer Stable URL : http://www.jstor.org/stable/23367513 Increased Nucleic-Acid Synthesis in Relation, (1968), 257–266.

Jarvis, B. C. and Shaheed, A. I. (1986). Adventitious Root Formation in Relation to the Uptake and Distribution of Supplied Auxin. *New Phycologist*, 103, 23-31.

Kannan, C. S., Sudhakara, K., Augustine, A., and Ashokan, P. K. (1996). Seed dormancy and pre-treatments to enhance germination in selected Albizia species. *Journal of Tropical Forest Science*, 369-380.

Keys, R. D., Smith, O. E., Kumamoto, J., and Lyon, J. L. (1975). Effect of gibberellic acid, kinetin, and ethylene plus carbon dioxide on the thermodormancy of lettuce seed (*Lactuca sativa* L. cv. Mesa 659). *Plant Physiology*, *56*(6), 826-829.

Kibbler, H., Johnston, M. E., and Williams, R. R. (2004). Adventitious Root Formation in Cuttings of *Backhousia citriodora* F. Muell: 1. Plant Genotype, Juvenility and Characteristics of Cuttings. *Scientia Horticulturae*, *102*(1), 133–143. doi:10.1016/j.scienta.2003.12.012.

Kramer, P. J., and Kozlowski, T. T. (1960). Physiology of trees. *Physiology of trees.*

Kucera, B., Cohn, M. A., and Leubner-Metzger, G. (2005). Plant hormone interactions during seed dormancy release and germination. *Seed Science Research*, *15*(4), 281-307.

Laude, H. M. (2017). Treatments to improve the emergence and stand of *Piptatherum miliaceum* (smilo grass). *Journal of Range Management*, *4*(2), 88-92.

Lichtenhan, J. D., Vu, N. Q., Carter, J. A., Gilman, J. W., and Feher, F. J. (1993). Silsesquioxane-siloxane copolymers from polyhedral silsesquioxanes. *Macromolecules*, *26*(8), 2141-2142.

Leakey, R. R., Mesen, J. F. T., Tchoundjeu, Z., Longman, K. A., Dick, J. M., Newton, A., ... and Muthoka, P. N. (1990). Low-technology techniques for the vegetative propagation of tropical trees. *The Commonwealth Forestry Review*, 247-257.

Leakey, R. R. (2004). Physiology of vegetative reproduction. Academic Press.

Leakey, R. R. B., and Storeton-West, R. (1992). The rooting ability of *Triplochiton scleroxylon* cuttings: the interactions between stock plant irradiance, light quality and nutrients. *Forest Ecology and Management*, *49*(1-2), 133-150.

Leopold, A. C. (1964). Plant growth and development. *Plant growth and development.*

Liu, G., Porterfield, D. M., Li, Y., & Klassen, W. (2012). Increased oxygen bioavailability improved vigor and germination of aged vegetable seeds. *HortScience*, *47*(12), 1714-1721.

Loreti, F., and Pisani, P. L. P. (1982). Physiological and technical factors affecting rooting in woody species. In *Proceedings of the 21st Horticultural Congress, Hamburg* (pp. 294-309).

Marsh, S., Webb, A. D. P., Staden, J. V. A. N., and Wareing, P. F. (2017). Seed Dormancy in Acer : Changes in Endogenous Cytokinins , Gibberellins and Germination Inhibitors During the Breaking of Dormancy,*Acer Seed Dormancy in Acer*, *24*(78), 105–116.

Medina, E., Paredes, C., Pérez-Murcia, M. D., Bustamante, M. A, and Moral, R. (2009). Spent Mushroom Substrates as Component of Growing Media for Germination and Growth of Horticultural Plants. *Bioresource Technology*, *100*(18), 4227–32. doi:10.1016/j.biortech.2009.03.055.

Merhaut, D. J., Blythe, E. K., Newman, J. P., and Albano, J. P. (2006). Nutrient release from controlled-release fertilizers in acid substrate in a greenhouse environment: I. Leachate electrical conductivity, pH, and nitrogen, phosphorus, and potassium concentrations. *HortScience*, *41*(3), 780-787.

Mesen, F., Newton, A. C., and Leakey R. R. B. (1997). The Effects of Propagation Environment and Foliar Area on the Rooting Physiology of *Cordia alliodora* (Ruiz & Pavon) Oken cuttings, 404–411.

Million, J. B., Ritchie, J. T., Yeager, T. H., Larsen, C. A., Warner, C. D., and Albano, J. P. (2011). CCROP—Simulation model for container-grown nursery plant production. *Scientia horticulturae*, *130*(4), 874-886.

Missanjo, E., Chioza, A., & Kulapani, C. (2014). Effects of different pretreatments to the seed on seedling emergence and growth of *Acacia polyacantha*. *International Journal of Forestry Research*, *2014*.

Morinaga, C., Mandai, M., Watanabe, A., Kurimoto, Y., Hirami, Y., Daimon, T. and Terada, M. (2017). Autologous induced stem-cell–derived retinal cells for macular degeneration. *New England Journal of Medicine*, *376*(11), 1038-1046.

Newton, A. C. (2001). The Influence of Stockplant Environment on Morphology, Physiology and Rooting of Leafy Stem Cuttings of *Albizia guachapele*, 213–227.

Ofori-Gyamfi, E. (1998). Investigation into some Factors affecting Vegetative Propagation of Coffee (*Coffea canephora var Robusta Pierre*). M. PhiL thesis. University of Cape Coast, Cape Coast, Ghana p173.

Ogawa, K., and Iwabuchi, M. (2001). A Mechanism for Promoting the Germination of *Zinnia elegans* Seeds by Hydrogen Peroxide, *Research Institute for Biological Sciences Okayama* (RIBS), 7549-1 Yoshikawa, Kayou-cho, Okayama 716-1241, Japan, *42*(3), 286–291.

Orwa, C., Mutua, A., Kindt, R., Jamnaadass, R., and Simon, A. (2009). Agroforestry Database: A Tree Species Reference and Selection Guide Version 4.0.

Pijut, P. M., Woeste, K. E., and Michler, C. H. (2011). 6 Promotion of Adventitious Root Formation of Difficult-to-Root Hardwood Tree Species. *Horticultural reviews*, *38*, 213.

Paul, D. K. (1972). A Handbook of Nursery Practice for *Pinus caribaea var. hondurensis* and other Conifers in West Malaysia. Working Paper No. 19, FO: SF/MAL 12, UNDP/FAO, Kuala Lumpur.

Ranal, M. A., Santana, D. G. D., Ferreira, W. R., and Mendes-Rodrigues, C. (2009). Calculating germination measurements and organizing spreadsheets. *Brazilian Journal of Botany*, *32*(4), 849-855.

Reza-Shahsavar, A. (2010). Endogenous soluble sugars, starch contents and phenolic compounds in easy-and difficult-to-root olive cuttings. *Journal of Biological & Environmental Sciences*, *4*(11), 83-86.

Rinaldi, M., Di Paolo, E., Richter, G. M., and Payne, R. W. (2005). Modelling the Effect of Soil Moisture on Germination and Emergence of Wheat and Sugar Beet with the Minimum Number of Parameters. *Annals of Applied Biology*, *147*(1), 69–80. doi:10.1111/j.1744-7348.2005.00018.xRogers, A., Medlyn, B. E., Dukes, J. S., Bonan, G., Caemmerer, S., Dietze, M. C., ... and Prentice, I. C. (2017). A roadmap for improving the representation of photosynthesis in Earth system models. *New Phytologist*, *213*(1), 22-42.

Santapau, H. (1966). *Common trees* (p. 16). National Book Trust, India.

Sebastiani, L., and Tognetti, R. (2004). Growing season and hydrogen peroxide effects on root induction and development in *Olea europaea* L. (cvs "Frantoio" and "Gentile di Larino") cuttings. *Scientia Horticulturae*, *100*(1-4), 75–82. doi:10.1016/j.scienta.2003.08.008

Sveinsdóttir, H., Yan, F., Zhu, Y., Peiter-Volk, T., and Schubert, S. (2009). Seed ageing-induced inhibition of germination and post-germination root growth is related to lower activity of plasma membrane H+-ATPase in maize roots. *Journal of plant physiology*, *166*(2), 128-135.

Van Staden, J., and Wareing, P. F. (1972). The effect of light on endogenous cytokinin levels in seeds of *Rumex obtusifolius*. *Planta*, *104*(2), 126-133.

Vieira, D. L. M., de Lima, V. V., Sevilha, A. C., and Scariot, A. (2008). Consequences of dry-season seed dispersal on seedling establishment of dry forest trees: Should we store seeds until the rains?. *Forest Ecology and Management*, *256*(3), 471-481.

Vujanovic, V., St-Arnaud, M., Barabé, D., and Thibeault, G. (2000). Viability testing of orchid seed and the promotion of colouration and germination. *Annals of Botany*, *86*(1), 79-86.

Santapau, H. (1966). *Common trees* (p. 16). National Book Trust, India.

Toole, E. H., Toole, V. K., Borthwick, H. A., and Hendricks, S. B. (1955). Interaction of temperature and light in germination of seeds. *Plant Physiology*, *30*(5), 473.

Usenik, V., Solar, A., Colaric, M., and Stamper, F. (2006). Seasonal Variations of Selected Flavonoids, Phenolic Acids and Quinones in Annual Shoots of Common Walnut. *Plant Science* Vol. 170 Issue 3.

Vieira, D. L. M., De-Lima, V. V., Sevilha, A. C. and Scariot, A. (2008). Consequences of Dry-Season Seed Dispersal on Seedling Establishment of Dry Forest Trees: Should We Store Seeds Until the Rains? *Forest Ecology and Management* 471–481.

Vujanovic, V., St-Arnaud, M., Barabé, D., and Thibeault, G. (2000). Viability testing of orchid seed and the promotion of colouration and germination. *Annals of Botany*, *86*(1), 79-86.

Weaver, R. J. (1982). *Reguladores del Crescimento emla Agricultura*. Barcelona: *Trillas.*

Wendling, I., Trueman, S. J., and Xavier, A. (2014). Maturation and Related Aspects in Clonal Forestry-Part II: Reinvigoration, Rejuvenation and Juvenility Maintenance. *New Forests*, *45*(4), 473–486. doi:10.1007/s11056-014-9415-y

Woolley, J. T., and Stoller, E. W. (1978). Light Penetration and Light-induced Seed Germination in Soil. *Plant Physiology*, *61*(4), 597–600. doi:10.1104/pp.61.4.597

Yahiro, M., and Oryoji, Y. (1980). Effects of gibberellin and cytokinin treatments on the promotion of germination in papaya, *Carica papaya* L., seeds. *Memoirs of the Faculty of Agriculture, Kagoshima University, 1*6, 45-51.

Yakandawala, K., and Adhikari, A. (2014). *Lawsonia inermis* (Lythraceae): From the Wild to the Garden. *Journal of Environmental Professionals Sri Lanka*, *3*(2). doi:10.4038/jepsl.v3i2.7846

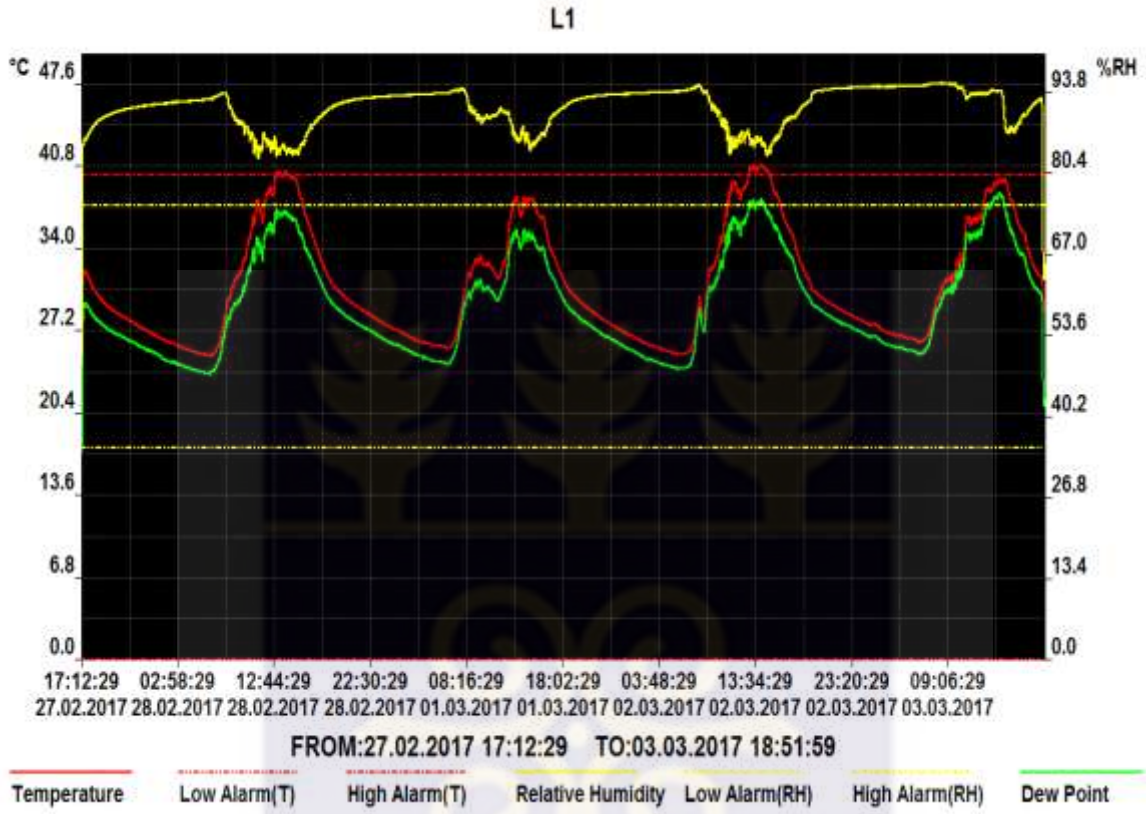
Yeboah, J., Lowor, S. T., Amoah, F. M., and Owusu-Ansah, F. (2011). Propagating structures and some factors that affect the rooting performance of shea (*Vitellaria paradoxa* Gaertn.) stem cuttings. *Agric. Biol. JN Am*, *2*, 258-269.

García-Rubies, A., Zabala, I. and Limousin, M. (1990). Effects of total fishing prohibition on the rocky fish assemblages of Medes Islands marine reserve (NW Mediterranean). *Scientia Marina, 1990, vol. 54, num. 4, p. 317-328*.

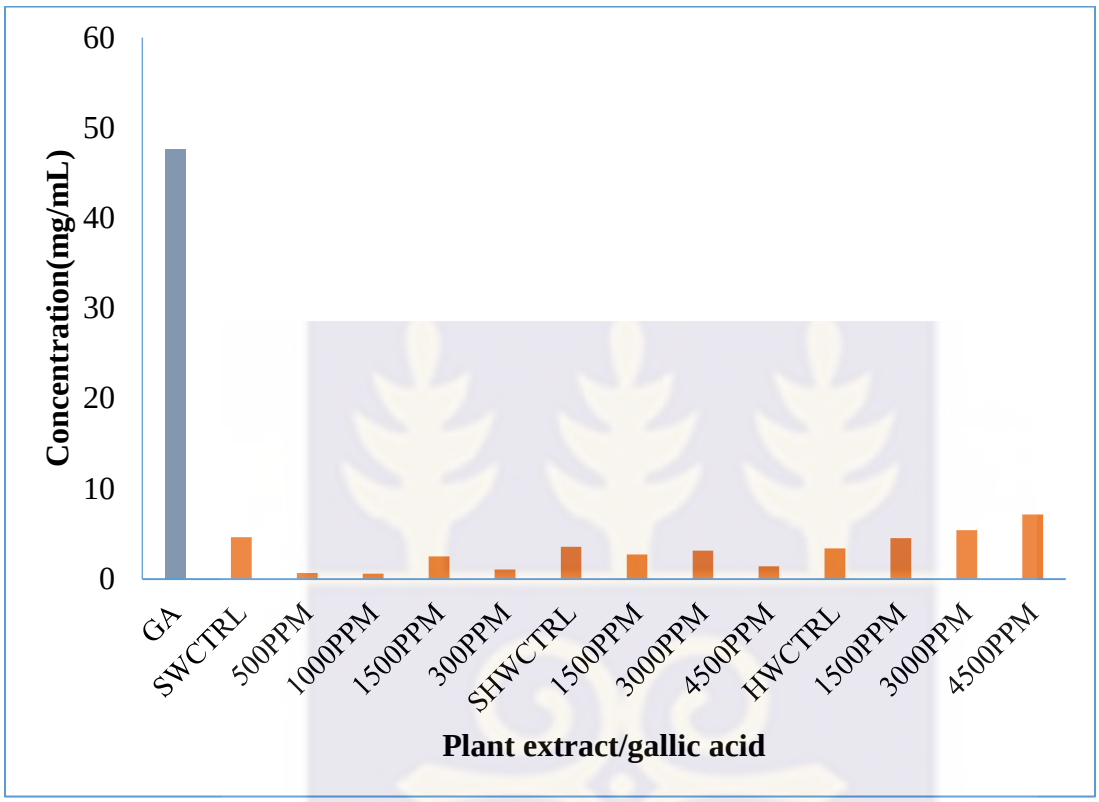
Zhou, J., Deckard, E. L., and Ahrens, W. H. (2017). Factors Affecting Germination of Hairy Nightshade (*Solanum sarrachoides*) Seeds, *Weed Science*, Vol. 53, No. 1 (Jan. - Feb., 2005), pp. 41-45

APPENDICES

Appendix 1: Graph showing minimum, maximum temperatures and relative humidity



Appendix 2: Graph of phenol contents in softwood, semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa*



SWCTRL – Softwood control; **SHWCTRL**- Semi Hardwood control; **HWCTRL** – Hardwood control; and **GA**- Gallic acid (standard)

Appendix 3: ANOVA TABLES

ANOVA table of mean time germination of *Lagerstroemia speciosa* seeds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	8	13.144	1.643	0.88	0.546
Residual	36	67.600	1.878		
Total	44	80.744			

ANOVA table of coefficient of variation in germination of *Lagerstroemia speciosa* seeds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	8	3.199E-05	3.999E-06	0.87	0.546
Residual	36	1.645E-04	4.570E-06		
Total	44	1.965E-04			

ANOVA table of percentage germination of *Lagerstroemia speciosa* seeds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	8	95.24	11.90	0.40	0.915
Residual	36	1078.00	29.94		
Total	44	1173.24			

ANOVA table of speed of germination of *Lagerstroemia speciosa* seeds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	8	0.0040000	0.0005000	0.57	0.797
Residual	36	0.0316800	0.0008800		
Total	44	0.0356800			

ANOVA table of the effect of IBA treatments on percentage rooting in softwood cuttings of *Lagerstroemia speciosa.*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
IBA	4	360.73	90.18	2.55	0.105
Residual	10	354.17	35.42		
Total	14	714.90			

ANOVA table of the effect of IBA treatments on number of roots per cutting in softwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
IBA	4	3.7266	0.9316	3.50	0.049
Residual	10	2.6654	0.2665		
Total	14	6.3920			

ANOVA table of the effect of IBA treatments on the length of longest root in softwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
IBA	4	63.309	15.827	2.86	0.081
Residual	10	55.295	5.530		
Total	14	118.604			

ANOVA table of the effect of IBA treatments on percentage rooting in semi-hardwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
IBA	3	457.021	152.340	30.06	<.001
Residual	8	40.539	5.067		
Total	11	497.560			

ANOVA table of the effect of IBA treatments on the number of roots in semi-hardwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
IBA	3	3.16382	1.05461	14.39	0.001
Residual	8	0.58628	0.07329		
Total	11	3.75010			

ANOVA table of the effect of IBA treatments on the length of longest root in semi-hardwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
IBA	3	89.622	29.874	10.10	0.004
Residual	8	23.660	2.958		
Total	11	113.282			

ANOVA table of the effect of IBA treatments on percentage rooting in semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Woodtype	1	40.97	40.97	3.41	0.084
IBA	3	214.77	71.59	5.95	0.006
Woodtype.IBA	3	347.48	115.83	9.63	<.001
Residual	16	192.38	12.02		
Total	23	795.60			

ANOVA table of the effect of IBA treatments on number of roots in semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Woodtype	1	0.4265	0.4265	2.83	0.112
IBA	3	4.6137	1.5379	10.19	<.001
Woodtype.IBA	3	0.9767	0.3256	2.16	0.133
Residual	16	2.4139	0.1509		
Total	23	8.4309			

ANOVA table of the effect of IBA treatments on the length of longest root in semi-hardwood and hardwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Woodtype	1	71.07	71.07	3.67	0.073
IBA	3	57.78	19.26	1.00	0.420
Woodtype.IBA	3	114.61	38.20	1.97	0.159
Residual	16	309.68	19.36		
Total	23	553.15			

ANOVA table of the effect of IBA on total free phenols in soft wood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Phenols	5	5.266E-05	1.053E-05	7037.81	<.001
Residual	12	1.796E-08	1.496E-09		
Total	17	5.268E-05			

ANOVA table of the effect of IBA on total free phenols in semi-hard wood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Phenols	4	4.851E-05	1.213E-05	1523.31	<.001
Residual	10	7.961E-08	7.961E-09		
Total	14	4.859E-05			

ANOVA table of the effect of IBA on total free phenols in hard wood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
IBA	4	4.360E-05	1.090E-05	3604.11	<.001
Residual	10	3.024E-08	3.024E-09		
Total	14	4.363E-05			

ANOVA table of the effect of IBA on the levels of soluble sugars in soft wood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Soluble Sugars	4	0.295547	0.073887	38.43	<.001
Residual	5	0.009613	0.001923		
Total	9	0.305160			

ANOVA table of the effect of IBA on the levels of insoluble sugars in soft wood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insoluble Sugars	4	0.016840	0.004210	0.94	0.511
Residual	5	0.022450	0.004490		
Total	9	0.039290			

ANOVA table of the effect of IBA on the levels of soluble sugars in semi-hardwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Soluble Sugars	3	0.11848	0.03949	2.47	0.201
Residual	4	0.06397	0.01599		
Total	7	0.18246			

ANOVA table of the effect of IBA on the levels of insoluble sugars in semi-hardwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insoluble Sugars	3	0.001600	0.000533	0.44	0.734
Residual	4	0.004800	0.001200		
Total	7	0.006400			

ANOVA table of the effect of IBA on the levels of soluble sugars in hardwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Soluble Sugars	3	0.44738	0.14913	3.65	0.122
Residual	4	0.16356	0.04089		
Total	7	0.61094			

ANOVA table of the effect of IBA on the levels of insoluble sugars in hardwood cuttings of *Lagerstroemia speciosa*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insoluble Sugars	3	0.000550	0.000183	0.12	0.945
Residual	4	0.006200	0.001550		
Total	7	0.006750			

ANOVA table of the effect of total free phenols in softwood, semi-hardwood and hard wood cuttings from the start and end of experiment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Phenols	13	5.653E-05	4.349E-06	999.62	<.001
Residual	28	1.218E-07	4.350E-09		
Total	41	5.666E-05			

ANOVA table of the soluble in softwood, semi-hardwood and hard wood cuttings from the start and end of experiment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Soluble Sugars	12	1.31646	0.10970	6.01	0.001
Residual	13	0.23714	0.01824		
Total	25	1.55360			

ANOVA table of the insoluble sugars in softwood, semi-hardwood and hard wood cuttings from the start and end of experiment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	12	0.021415	0.001785	0.69	0.733
Residual	13	0.033450	0.002573		
Total	25	0.054865			