STEM CUTTING PROPAGATION PROTOCOL FOR ROSE-SCENTED GERANIUM (*PELARGONIUM GRAVEOLENS*)

By

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Submitted in partial fulfilment of the requirements for the degree of

MSc (Agric.) Horticulture

Department of Agronomy –Horticulture

In the Faculty of Science and Agriculture

University of Fort Hare

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October 2017

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N. MATAFENI

DECLARATION

I hereby declare that the thesis submitted for M.Sc (Agriculture): Horticulture, University of Fort Hare, is my original work and has not been previously submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged in my reference list.

Ntombekhaya Matafeni Date

DECLARATION OF PLAGIARISM

I **Matafeni Ntombekhaya,** student number **200704124**, hereby declare that the thesis submitted for Masters in Agriculture (Horticultural Science), University of Fort Hare, is my original work and has not been previously submitted to any other institution of higher education. I am fully aware of the University of Fort Hare Policy on Plagiarism and that I have taken every possible precaution to comply with the regulations pertaining to this policy furthermore, I declare that all sources cited or quoted are indicated and acknowledged in my reference list.

Matafeni Ntombekhaya Date

DECLARATION ON RESEARCH ETHICS CLEARANCE

I, **Matafeni Ntombekhaya**, student number **200704124**, hereby declare that I am fully aware of the University of Fort Hare Policy on Research Ethics and that I have taken every possible precaution to comply with the regulations pertaining to it. Ethical clearance for the present study which was done between year 2014 and 2015, was not performed. This was because the present study was focused on plants not on animals or human beings.

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LIST OF ABBREVATION

AP	After planting
ANOVA	Analysis of variance
ARF	Adventitious roots formation
cv.	Cultivars
DPP	Directorate plant production
hrs.	Hours
IAA	Indole-3-acetic acid
IBA	Indole-3-butric acid
LSD	Least significant difference
NAA	Naphthalene acetic acid
RCD	Randomised complete design
RCBD	Randomised complete block design
RFM	Root fresh mass
RH	Rooting hormone
RHA	Root holding ability
SAEOPS	South African Essential Oils Producers Association
SANDA	South African Nation Department of Agriculture
Spp.	Species
WAP	Weeks after planting
WESGRO	Western Cape Trade and Investment Promotion Agency
WHC	Water holding capacity
WHP	Wound healing period
WRC's	Wound related compounds
^{0}C	Degrees Celsius

ACKNOWLEDGMENTS

I wish to express my sincere gratitude and appreciation to the following people and institutions:

- Dr Bahlebi Kibreab Eiasu, my supervisor, for the support he gave me throughout this research. He was not just a supervisor to me but a mentor and a role model; he was supportive, available and resourceful.
- Dr Stephen Amoo, my co-supervisor, for the interest, all the inputs, guidance and support that he provided.
- Dr Patric Adebola (ARC VOPI), Mrs M. Maphaha and Mrs V. Bomali (UFH Horticulture Department.) for their academic input, support and for motivting me to do this work.
- Mr Mish Syden from Govan Mbeki Research and Development Centre (GMRDC) for his Statistical Support.
- Dr Chitsamatanga, Bellita Banda for her great work in editing my thesis and for her academic input.
- Agricultural Research Council (ARC) for funding this work through Personal Development Programme.
- The Essential Amatole Community Company for providing me with planting materials and labour for this study. I would also want to acknowledge Mr Vena the farm supervisor and all the technicians at Essential Amatole Nursery for their respective input in this work.
- The University of Fort Hare, Sustainable Agriculture and Food Security for the financial support through Research Niche Area.
- I am appreciative to my dear husband Mr Pampila Siyabulela, who has stood by me and provided unconditional encouragement, motivation, financial and moral support as a mentor and friend.

- ❖ My fellow Masters students Ms Dyafta Viwe and Mr Thamsanqa Fumba for their support and availability to help me at all times.
- ❖ I salute my mother, a very wise woman who as a single parent continued encouraging me to go to school and make sure that I obtain the education which she never got. My mother in-law and my sister deserve special thanks for their love and support.

My Lord and my saviour Jesus Christ for the strength and confidence He gave me throughout this work when I sometimes wanted to give up, I was always encourage by His powerful words; ‘‘For I know the plans I have for you, declares the Lord, plans to prosper you and not to harm you, plans to give hope and future’’ **Jeremiah 29:11**. To God be the Glory.

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ABSTRACT

Rose-scented geranium (*Pelargonium graveolens*), is a high value essential oil plant that is used in the perfumery, cosmetic, aromatherapy and food flavouring industries. The increasing demand for this plant, due to its economic importance necessitates the development of an efficient propagation protocol for quality seedling and its maximum production. The present study therefore, sought to develop effective stem cutting propagation protocol which could facilitate multiplication of rose-scented geranium stem cuttings. Three separate experiments were undertaken to determine factors influencing effective propagation of rose-scented geranium. These factors were: rooting media, rooting hormone, cutting length and wound healing period on rooting and development of rose-scented geranium stem cuttings. The cuttings were assessed based on root number, length and fresh mass, plant height, leaf number, and stem circumference. In terms of root measurements, the growing media were washed out from the root system of plantlets, their roots were separated from stem before data was recorded which comprised of root number, length and root fresh mass. Root holding ability (RHA) on rooting medium was determined by visual observation and rated on a 1-5 scale where 1 = very loose, not acceptable; 2 = loose, not acceptable; 3 = medium, marginally acceptable; 4 = tight, acceptable; 5 = very tight, acceptable. The experiments were carried out at Essential Amatole Nursery, at the University of Fort Hare Research Farm, Alice Campus (located at 32^0 47′3″S, 26^0 50′43″ E, with an altitude of 519 m.a.s.l). All the experiments were carried out under mist conditions on bottom-heated beds in a greenhouse (with polycarbonate roofing of about 40% shading effect) for the first three weeks after sticking the cuttings to the growing medium to facilitate root induction in relatively high

temperature and relative humidity. Thereafter, the plantlets were grown in a shade house with 70% light penetration until the termination of the experiment.

To optimize the technology for the propagation of this plant the present experiment was designed with the objective to determine the efficient growing medium and proper rooting hormone for successful rooting and development of quality seedlings of rose-scented geranium. The experiment was set up in a complete randomized design (CRD) and was replicated three times with two factors 7×4, seven different growing media i.e. (**1**) Mixture growing medium which serves as control (pine bark 8 bags + sand 2 bags + lime 4kg + coconut 10 blocks + talborne 6.25 kg + bone meal 2 kg); (**2)** River sand only; (**3)** Pine bark; (**4)** Hygrotex (commercial rooting media); (**5)** Pine bark + river sand (at1:1 ratio on volume basis); (**6)** Pine bark + hygrotex (at 1:1, ratio on volume basis), and; (**7)** Pine bark + river sand + hygrotex (at 1:1:1 ratio on volume basis) and four different IBA hormone levels (auxins, types of IBA) applied as treatment were (**1**) Dynaroot (1 – 1g/kg), (**2**) Dynaroot (2-3g/kg), (**3**) Dynaroot (3-8g/kg) and (**4**) Control (untreated with hormone). Hygrotex was identified as the best growth media for quicker regeneration giving the highest root number, length and fresh mass. While, hygrotex + pine bark (v/v 1:1) was efficient in producing more leaves, stem circumference and other aerial parameters. Dynaroot 3 was identified as the best rooting enhancer with maximum root number, length, fresh weight and plant height. Both Dynaroot 3 and Dynaroot 2 did not have major differences on giving highest leaf number. Control (untreated with hormone) was consistent in giving the greatest stem circumference than any other treatment. To maximize stem circumference, a combination of control (untreated with hormone) and hygrotex + pine bark (v/v 1:1) was identified as the best treatment. Based on the investigation for maximum production and quality seedlings of rose-scented geranium, hygrotex and Dynaroot 3 were identified as the best combination for successful rooting.

The ideal cutting stem length, rooting hormone and growing medium for quality seedlings of rose-scented geranium were also investigated. The experimental lay out was in randomized complete design (RCD) with a 4 × 4 × 2 factorial treatment combination. Treatments used were, four different cutting lengths viz. 10, 12, 14 and 16 cm long; four different

concentrations of IBA rooting hormone (Dynaroot 1, 2, 3 (powder form) and distilled water (control) and two types of growing medium (hygrotex and hygrotex + pine bark v/v 1:1) were used. Stem cuttings of 14 and 16 cm length gave the highest root number of 34, 38 and 35.13, and root length of 3.40 and 3.51cm respectively, with no significance. Cutting length of 10 cm favoured stem circumference (3.1 cm) as compared to other treatments. Whereas, cuttings treated with Dynaroot 3 showed a better root number (33.46 roots), root length (3.54 cm), root fresh mass (0.59 mg), leaf number of (11.08) as well as highest root holding ability (5). However, they showed no significance difference with Dynaroot 2 treated cuttings. In addition, cuttings treated with Control favoured shoot number (3.79) and stem circumference (3.05). Visually, hygrotex was observed to be better substrate though it was not significantly different from hygrotex + pine bark (1:1 v/v) on propagation of rose-scented geranium stem cuttings. Therefore, it is recommended that rose-scented geranium should be propagated through the combination of 14 cm cuttings length and treated with Dynaroot 2 IBA rooting hormone. Both hygrotex and hygrotex + pine bark (1:1 v/v) are the best growing media for root formation and growth of rose-scented geranium, though hygrotex alone is more economical.

Wound healing period (WHP) of stem cutting was evaluated using 4 × 4 factorial, cuttings were separated into four groups during the healing duration (intervals of 24 hrs: days 0, 1, 2 and 3). These four groups were further subdivided into four subgroups of rooting hormone viz. Dynaroot 1, 2, 3 of indole-3-butyric acid and control (water). Experiment was laid out in a randomised complete block design (RCBD) with three replicates. The results obtained from the study revealed that rose-scented geranium rooted easily when planted on Day 2 of the wound healing period such that root holding ability was at its highest. While, Day 0 cuttings showed good response for stem circumference and shoot number. The study recommends that rose-scented geranium be propagated using cuttings that have enough time to heal the wound that is, Day 2 cuttings. Dynaroot 3 (IBA hormone concentration) showed good response to rooting and other arial parameters except for stem circumference which was favoured by application of control. Based on the results of the study, it can be concluded that propagation of rose-scented geranium requires a wound healing period of about three days in room temperature and application of IBA hormone before sucking cuttings in growing medium. Instead, of Dynaroot 3 or 2, Dynaroot 1 can also be used because, it is less economical and they all have a similar effect on cuttings that have been healing for three days.

Keywords: cutting length; growing medium; IBA; propagation; rooting hormone; rose-scented geranium; stems cuttings; wound healing period.

CHAPTER 1
GENERAL INTRODUCTION

Rose-scented geranium (*Pelargonium graveolens*) is a perennial plant belonging to the Geraniaceae family (Rao et al., 1996; Eiasu et al., 2008). It is cultivated mainly for its high value essential oil and well known for its medicinal and fragrance properties (Rao, 2002). According to Eiasu et al. (2009), rose-scented geranium essential oil, widely known as 'geranium oil', is found mainly in fresh leaves with a small amount in tender stems and flowers. Geranium oil can be extracted by steam distillation and/or hydro-distillation techniques. Motsa et al. (2006) describes geranium oil as a fine rose with citrus and mint-like odours and is used in perfumery, aromatherapy, cosmetic, food and pharmaceutical industries (Singh, 1999; Lis-Balchin, 2002; Rao, 2002; Eiasu et al., 2008).

According to Bown (1995), this plant is originally from the Republic of South Africa in the Mediterranean climate of the Western Cape Province. Countries such as China, Egypt, Algeria and Morocco, have also grown it over the past years for perfumery and medicinal uses (Narayana, 1986; Rao, 2002). In addition, rose-scented geranium it has also been applied for the same purposes in Reunion Islands. This has led to its, current international demand and about 600 tonnes of geranium oil is being met largely by China, Egypt, Morocco, and the Reunion Islands (SANDA, 2006).

This perennial shrub has herbaceous to woody stem (depending on age), strongly rose-scented leaves, and pinkish-white hermaphroditic flowers (Weiss, 1997; Motsa, et al., 2006). Moreover, this herbaceous plant is often used in hedgerows with a height up to about 100 cm and with pointed leaves which are serrated at the edges (Bown, 1995). Rao et al. (1993), also states that the *P. graveolens* cultivars have a wide variety of smells, including rose, citrus, mint, coconut, nutmeg, as well as various fruit scents. However, the most commercially important varieties are those with rose scents (Lis-Balchin, 2002). The rose-scented essential oil from these varieties is an important ingredient in the perfume industry that is highly prized for its high aromatic value Rao et al. (1993).

The major natural components of geranium oil include citronellol, geraniol, linalool, iso-menthone, citronellyl formate, and geranoil formate (Miller, 2002; Lis-Bachinin, 2004; Shawl

et al., 2006; Eiasu, 2008; Verma et al., 2010). Earlier research studies also indicate that, traditional healers in the Southern Africa use this plant in traditional medicine (Narayana et al., 1986). For instance, the roots may be directly chewed or ground into powder and mixed with food (Latté and Kolodziej, 2004). It is also believed to chase evil spirits away (Bathheaven, 2007). Rose-scented geranium is also used commercially in the aromatherapy industry for facial steams, and is reputed for having anti-aging effects on the skin (Gilbert, 2011).

However Bhan et al. (2005) indicated that to date the South African geranium oil production business has not made a significant contribution to the world essential oil market. Only 3 tons of rose-scented geranium oil is produced in South Africa every year, and this is very small when compared to the total world production (WESTGRO, 2006; Eiasu, 2009). And therefore the contribution of South Africa is likely to increase with 47 ton production per year (WESGRO, 2006; Eiasu, 2009). At the present moment the demand for geranium oil worldwide is estimated to be around 600 tons per year (Shawl, 2006; Eiasu, 2009). About 20-25 tons of geranium oil is required to close the world's demand for essential oil (Demame, 2002; Eiasu, 2009).

Given its economic importance and its increasing demand, there is an urgent need to increase production of rose-scented geranium plant (DPP, 2009). Though, the propagation of this plant can be done by seed, the problem is, it is a hybrid therefore, it rarely produces viable seeds that may assist in producing seedlings (DPP, 2009). According to Hartmann and Kester (1997), this plant can also be propagated by means of tissue culture and cuttings. However, plant breeders and commercial propagators prefer vegetative propagation through cuttings (Hartmann and Kester, 1997; Hartmann et al., 2002).

However, research studies indicate that, commercial propagators and nurseries (including geranium growers) encounter huge losses during vegetative propagation. This has been attributed mainly to the poor quality seedlings due to poor rooting of cuttings (Leakey, 1990; Hartmann and Kester, 1997; Mamba and Wahome, 2010). This problem affects geranium growers, particularly those who raise their own seedlings (plantlets) (Hartmann and Kester, 1997; Anonymous, 2006). For example, the Essential Amatole (a community-company

partnership) essential oil production project in the Eastern Cape experienced challenges in quality seedling production which hampered expansion of the production area in the planned time frame (Wier, 2014: personal communication).

The drawbacks of vegetative propagation by cuttings include the need for large planting material and expensive labour costs particularly, in large-scale cultivation of the rose-scented geranium (DPP, 2009). On the other hand, the advantages of vegetative propagation far outweigh its disadvantages because it is economical, grow rapidly, requires less space and it is a simple and easy way to propagate plants (Hartmann and Kester, 1997; Hartmann et al., 2002). As such, appropriate vegetative propagation protocols of rose-scented geranium by stem cuttings could act as an alternative to counteract challenges posed by poor rooting of seedlings. It could also help meet the current and future quality seedling demands (Anonymous, 2006). Therefore, vegetative propagation through stem cuttings remains the best option for this plant (Hartmann and Kester, 1997).

Hartmann et al. (2002), states that, for raising successful seedlings through cutting, adventitious root formation is a prerequisite. Adventitious root formation (ARF) in stem cuttings is a crucial physiological process for propagation of many ornamental plant species. However, though intensive control of environmental factors in the modern propagation industry have been enforced, high economic losses still occur as a result of insufficient rooting (Sorin et al., 2006). This calls for different factors influencing the success rate of a vegetative propagation protocol, including growing medium, length of cuttings, rooting hormones and their concentrations, wound healing period. These, need to be optimised to improve propagation protocol of ornamental and medicinal plants species (Hartmann and Kester, 1997). According to Leakey (2004), adventitious root initiation cuttings of succulent stem can take about five weeks after planting and the cuttings may be ready for transplanting in six to seven weeks depending on cultivars and growing conditions. However, the extant literature shows that, the effect of growing medium, rooting hormone, cutting length and wound healing period of rose-scented geranium is still not clear for the purpose of optimising its vegetative propagation protocol.

The aim of this study, therefore, seeks to develop effective stem cutting propagation protocol which could facilitate multiplication of rose-scented geranium stem cuttings.

The objectives of this research were:

- to study the effect of propagation media and rooting hormone on the vegetative propagation of rose-scented geranium;

- to determine the effect of stem cutting length and rooting hormone on rooting and growth of rose-scented geranium stem cuttings during vegetative propagation

- to determine the effect of wound healing period (WHP) on rooting and development of rose-scented geranium stem cuttings

CHAPTER 2
LITERATURE REVIEW

2.1 INTRODUCTION

Pelargonium species were introduced to the western world when naturalists, plant collectors and ship surgeons sailing trade routes around the Cape of Good Hope took along the plants during their voyages in the early seventeenth century (Miller, 2002). The first *Pelargonium* species cultivated in Europe is believed to be *P. triste* and was taken to the botanic garden in Leiden around 1631 (Miller, 2002). Though, *Pelargonium* species have been captivating gardeners for centuries it is the 1800s that marked the height of *Pelargonium* species popularity in the whole world (Browner, 2003).

Even though *Pelargonium* species originated primarily from South Africa, there are some few species that occur naturally in Australia, Eastern Africa, New Zealand, the Middle East and the islands of Madagascar (Van der Walt and Vorster, 1988; Lis-Balchin, 2004). The genus *Pelargonium* has a diverse group of plants with a wide variety of growth habits and habitats (Miller, 1996; Lis-Balchin, 2002), which range from rocky slopes to grasslands, forests and along streams (Miller, 1996).

Some *Pelargonium* species grow erect while, others have a trailing habit, and some have tuberous roots (Van der Walt and Vorster, 1988; Miller, 1996; Lis-Balchin, 2002). *Pelargonium* species grow in areas with low rainfall and low humidity (Jim and Brawner, 1996). These species are evergreen perennials and are drought and heat-tolerant, and can only tolerate only minor frosts (Van der Walt and Vorster, 1988; Lis-Balchin, 2002; Lis-Balchin, 2004).

Research studies show that they are extremely popular garden plants, grown as bedding plants in temperate regions (Lis-Balchin, 2002). The *Pelargonium* species are among these popular garden plants and they are appreciated by herb enthusiasts for their fragrant leaves. However, not all plants in the genus are scented, and not all of the scents are pleasant (Miller, 1996). According to Van der Walt (1985) and Miller (1996), the genus *Pelargonium* is made

up of more than 270 species, most of which are aromatic. The four most important polargonium species in geranium oil production are *P. capitatum, P. odaratissimum, P. radens* and *P.* graveolens.

Pelargonium graveolens, a 'Rose' cultivar, is believed to be the core source of oil which gave rise to the commercial 'Bourbon geranium oil, Bourbon' (Demarne and Van der Walt, 1989). The main constituents of geranium oil include citronellol, geraniol, linalool, iso-menthone, citronellyl fomate and geraniol formate (Miller, 2002; Lis-Bachinin, 2004; Shawl et al., 2006; Eiasu, 2008; Verma et al., 2010).

2.2 ROSE-SCENTED GERANIUM (*PELARGONIUM GRAVEOLENS*)

Rose-scented geranium is a perennial herb that belongs to the Geraniaceae family (Rao et al. 1996; Eiasu et al., 2008). It is the largest family of flowering plants whose species are well known for its medicinal and fragrance properties (Miller, 2002). About 80% of those species occur in Southern Africa having the highest concentration in South West region (Van der Walt and Vorster, 1988). The hybrid species, *Pelargonium graveolens L.,* was developed from crossing *P. capitatum* × *P. raden* (Weiss, 1997; Miller, 2002), in England, in the eighteenth century. This was then taken to the South of France and the Reunion Island and later to China (Demarne and Van der Walt, 1989). Even though, it is indigenous to South Africa, the countries that produce *Pelargonium* for essential oil are India, Morocco, Egypt and Algeria (Motsa, 2006).

This herbaceous plant is known to grow above the ground level up to a height of 100 cm, depending on varieties and environmental conditions, although it can grow to a height of more than 1 meter in its native habitats (Weiss, 1997; Miller, 2002). The stems of rose-scented geranium are initially herbaceous but become woody as plant age advances whereas, its root system can grow as deeper as 30 cm (Lis-Bachin, 2004). The leaves are similar to ferns, rose-scented, covered with fine hairs with a velvety texture and deeply-cut blades (Weiss, 1997; Bown, 1995; Motsa, 2006).

The flowers of this plant species are hermaphroditic (Motsa, 2006) and are white or pinkish-white with purple, pink markings (Lis-Bachin, 2004). Although, they are often used in hedgerows landscape horticulture, the rose-scented geranium is widely used in the perfumery, aromatherapy, cosmetic, food and pharmaceutical industries (Singh, 1999; Lis-Balchin 2002 and Rao, 2002; Eiasu et al., 2008).

2.3 PRODUCTION LEVEL OF GERANIUM OIL

Previous reports indicate that China produces most of the world's supply of geranium oil (80 -110 tons), followed by Egypt (50-55 tons), Reunion Island (6 tons) and South Africa (approximately 3 tons) annually (Dermane, 2002; Bhan et al., 2006; Eiasu, 2009). However, South Africa has not made a significant mark in the production of geranium oil compared to the total world demand of about 600 tonnes (Weiss 1997; DPP, SAEOPA et al., 2009). This is despite the fact that South Africa is expected to increase production of essential oil by 47 ton annually (Sangwan et al. 2001; WESGRO, 2006; Eiasu et al., 2008).

As a result, a number of essential oil producing companies have been established in South Africa due to the commercial viability of rose-scented geranium (Bhan et al., 2005). Moreover, a number of provinces are involved in the essential oil production in the country however, the major production comes from Mpumalanga, KwaZulu-Natal, Western Cape, and with a small amount from Eastern Cape and Limpopo provinces (DPP, 2009).

2.4 ECONOMIC VALUE OF ROSE-SCENTED GERANIUM

The importance of cultivating rose-scented geranium plant is demonstrated by its high value essential oil (Rao et al., 1996; Eiasu et al., 2008). Its oil is obtained mainly from fresh leaves, and a small amount from tender stems and flowers. The oil is extracted by steam or hydro-distillation techniques (Eiasu et al., 2008). Geranium essential oil is highly used in

perfumery, cosmetic, pharmaceutical and food industries (Lis-Balchin, 1996), in addition to its use in traditional medicine (Narayana et al., 1986). In the aromatherapy industry, geranium oil is used for facial steams, as it is believed to have anti-aging effect on the skin (Gilbert, 2011). It is one of the best skincare oils because it is good in opening and cleaning oily complexions (Peterson et al., 2005).

In addition, is known as the "woman oil" in the pharmaceutical industry due to its effectiveness in treating menstrual and menopausal-related ailments (Narayana, 1986). Verma et al. (2010) support the above assertions and state that, geranium oil is used for the treatment of heavy menstrual flows and menopause problem. Earlier research by (Motsa 2006; Shawl et al., 2006) is also used for the treatment of dysentery, haemorrhoids, inflammation, wounds, skin disorders, cancer, diabetes, diarrhoea, gall bladder problems, gastric ulcers, jaundice and liver problems. Whereas, in the food industry, it is used for making sweet syrups added to candies or drinks and also used for food flavouring (Bio-Africa, 1999).

The leaves of rose-scented geranium are also used as a form of herbal tea to distress, fight anxiety, ease tension, improve blood circulation and cure tonsillitis (Peterson et al., 2005). According to Narayana et al. (1986), rose-scented geranium is used in folk medicine by traditional healers in Southern Africa . The roots of the plant may be directly chewed or powdered and then mixed with food as traditional medicine to treat a large number of illnesses (Latté and Kolodziej, 2004). The Sotho, Xhosa, Khoi-San and Zulu people use this plant as a traditional remedy to treat illnesses such as diarrhoea, colds and lung infection (Watt and Breyer-Brandwijk, 1962; Lis-Balchin, 1996). It is also used to control mite infestation (Jeon et al., 2008).

Rose-scented geranium oil was found to have antibacterial properties (DPP, 2009). A safety measure of geranium oil is that, it is non-toxic, non-irritant and generally non-sensitizing. Though, there is no indication for any side effects, sensitivity in some people has been reported. Reason being, it balances the hormonal system thus, it might not be a good idea to use in pregnancy (Watt and Breyer-Brandwijk, 1962).

Nonetheless, the use of this plant for commercial purposes has increased rapidly over the past few years due to its utilization in plant products by many industries throughout the world (WESGRO, 2006). Moreover, the trade in essential oils is projected to increase in the near future as a result of increasing demand (Sangwan et al., 2001).

2.5 PROPAGATION OF ROSE-SCENTED GERANIUM

Plant propagation is the process of creating new plants from a variety of sources such as seeds, cuttings, bulbs and other plant parts. The main aim of propagation is to retain a species, and to preserve its youthfulness (rejuvenation) (Hartman and Kester, 1983). Plant propagation can refer to both the artificial or natural dispersal of plants (Davies et al., 1994). It is classified in two broad categories namely asexual and sexual (Hartman and Kester, 1983; Leakey, 1990).

Sexual (seed) propagation involves the floral part of the plant which encourages union of pollen (male) with the egg (female) and takes advantage of meiosis (reductive cell division) and recombination of genetic material (Leakey, 1990). The offspring has genetic material from both the maternal and paternal parent (Sedibe, 2012). However, the focus of the current study is on asexual (vegetative) propagation. In this case, the plant vegetative parts (roots, stems, or leaves) regenerate into a new plant and the off-springs are genetically identical to the maternal parent only (Davies et al., 1994).

Propagators are familiar with both propagation methods however, effective plant propagation requires a scientific knowledge of plant growth, its development, and morphology and different possible methods by which certain plant species can be propagated (Hartman and Kester, 1983; Leakey, 1990). In general, a robust and efficient propagation protocol is required for large scale or commercial cultivation in order to meet market need. Unavailability of effective propagation protocol can be a major hindrance for large scale production of the crop.

2.5.1 Vegetative Propagation by stem cutting

In the ancient days, a large number of plants were propagated without the application of scientific knowledge as such, only the natural skills were used as their guidance. At a later stage, it was discovered that plants can be reproduced through the use of different vegetative propagation methods (Hartman and Kester, 1983). Grafting, budding, layering and cuttings were found to be the major methods of vegetative propagation. Cutting propagation technique is largely practiced as an economical means of vegetative propagation for a wide range of horticultural crops such as, ornamentals, fruits, nuts and vegetables (Hartman et al., 1990; Hartman et al., 2002).

Cutting propagation method involves rooting a severed piece of the parent plant while, layering involves rooting a part of the parent and then severing it. Budding and grafting involve joining two plant parts (rootstock and scion) from different varieties (Hartman and Kester, 1983). Although there seem to be no problem where propagation is done by means of root cuttings or by suckers however, there is a big difference in the establishment rate as compared to stem cuttings, which are usually quicker (Hartman and Kester, 1983).

Stem cutting propagation is advantageous in that it is the easiest and fastest way to propagate plants as it bypasses the juvenile characteristics of certain species (Hartman et al., 2002). Besides, it does not require special techniques as in grafting and budding (Mpati, 2006). However, rooting success of cuttings is dependent on several factors which include type of rooting media used, type of rooting hormone applied, length of the stem cutting (Hartman et al., 2002) and wound healing period of the cuttings (Cline and Neely, 1983; Takagaki et al., 2000).

The extant literature shows that influence of rooting hormone, cutting stem length and wound healing period (WHP) on rose-scented geranium have not been studied. Although, Mamba and Wahome (2010) studied the effect of propagation media on geranium, there is still a gap in the literature on potential interaction effect of growing media with rooting hormone of rose-scented geranium stem cuttings. Therefore, this study attempted to close that gap by evaluating how propagation media, rooting hormone, cutting stem length, and wound healing period influenced rooting of rose-scented geranium stem cuttings.

The results of the study are expected to assist in the establishment of cheap, fast, reliable and simple techniques of propagation for rose-scented geranium, which can be used by resource-poor farmers. The establishment of a rapid propagation technique will contribute greatly to the production and availability of this plant species for the geranium oil industry while meeting its ever-increasing demand for other purposes.

2.5.2 Propagation medium and rooting hormone

The effect of propagation media and rooting hormone has been documented in a number of studies (Mialoundama, 2002; Araya et al., 2007; Blythe, 2007; Moreira et al., 2009). The significant difference in rooting of cuttings is mostly recognised between the interaction of propagation media and growth regulators.

Propagation medium

Propagation medium is a basic need of any plant, where growth takes place (Hartmann et al., 1990). Its purpose is mainly to provide the necessities required by the plant throughout its life. Furthermore, it provides the cuttings with physical support, stores oxygen and water, and creates a dark condition around the rooting zone (Hartmann et al., 1990; Akwatulira et al., 2011). The commonly used media for rooting cuttings include sand, hygrotex (commercial rooting media), pine bark etc. Unfortunately, for propagators there cannot be a universal propagation medium for growing cuttings (Hartman and Kester, 1997; Araya, 2005). The choice of medium component used plays a significant role on rooting performance and growth of the plant (Hartmann and Kester, 1997; Araya et al., 2007).

Even though the choice of the propagation medium is important for propagators, rooting of cuttings does not only depend on the type of rooting media alone but, on other factors such as availability and cost of material, type of species used, part of plant used, time of planting and season. (Leakey, 1990; Mamba and Wahome, 2010). The rooting media provide enabling conditions for the rooting of cuttings and their further growth and development into a plant because they are a store house of water, air and mineral (Hartmann et al., 1990; Moreira et al., 2009).

According to Leakey (1990), the choice of growing medium can influence the rooting of cuttings during vegetative propagation. For example, Şekeroğlu et al. (2001) showed that the highest rooting rates were obtained from the sand and perlite media at *Thymbra spicata L*. In a study conducted on *Dacryodes edulis* with different types of media, the results showed that there was no significant difference in mean number of roots produced per cuttings (Mialoundama, 2002). In the case of *P. hortorum* stem cuttings, the highest number of roots was recorded when mixture of garden soil, compost and sand at the ratio of 1:1:1 (v/v) was used. While, the use of vermiculite gave the lowest number of roots per cutting (Mamba and Wahome, 2010).

Plant roots need air to respire, hence, good aeration and water holding capacity (WHC) of rooting media is very important. For instance, Mpati (2006) conducted research on fever tea and reported the highest root length with stem cuttings grown on pine bark. Similar results also were found when *Warburgia ugandensis* stem cuttings were grown on three different media (milled pine bark, top forest soil and sand). Stem cuttings that were propagated in milled pine bark had the highest number of root sand shoots, shoot length, and root length when compared to cuttings propagated in top soil and sandy soil rooting media (Akwatulira et al., 2011). The findings from both experiments were attributed to high aeration and water holding capacity in pine bark. Pine bark is loose in texture as compared to soil and sand hence, it allows good aeration and water flow.

The rooting media used in stem cuttings also has an effect on the number of leaves produced by cuttings (Khayyat, 2007). A study conducted by Kiran et al. (2007), on Dahlia (*Dahlia pinnata*), reported a high number of leaves obtained with cuttings grown on a medium which consists of leaf-mold and peat mixture compared to other six growing media that were tested. These included peat moss and sand mixture as well as coco peat and leaf-mold mixture.

Good water holding capacity (WHC) and the ability of a substrate to provide sufficient nutrients were proven to affect the rooting of cuttings (Hartmann and Kester, 1997). Akinyele (2010) confirmed this in his work on *Buchhholzia coriacea* stem cuttings, grown in sawdust and topsoil gave the highest root length (3.8 cm) as compared to river sand with the least root length (1.4 cm).

Rooting hormones

Different types of rooting hormones are used for the enhancement of rooting of cuttings. Amongst them, indole-3-butyric acid (IBA) is rated as the most reliable and effective rooting hormone, followed by naphthalene acetic acid (NAA), which has been shown to be more effective more than indole-3-acetic acid (IAA) (Hartmann et al., 1990). The IBA is widely used for a wide range of plant species due to its non-toxicity to most plants over a wide concentration range (Hartmann et al., 1990). Currently, IBA and NAA remain the most widely used auxins for promoting root formation on stem cuttings (Blythe et al., 2007).

The main aim of treating cuttings with auxin is to stimulate root formation, increase overall rooting percentage, and increase the number and quality of roots and uniformity of rooting (Araya et al., 2007; Blythe et al., 2007). Nonetheless, some plant species may root naturally because they can endogenously release sufficient auxin concentration to naturally enhance rooting. Consequently, the effectiveness of auxins in promoting root initiation has been shown in a variety of plant species to break root apical dominance induced by cytokinin (Cline, 2000). Even though, IBA is generally non-toxic, the effect of its concentration on rooting has been proven to differ with different plant species (Blythe et al., 2004).

Ofori et al. (1996) observed that IBA has no significant positive effect on the final rooting percentage of *Milicea excelsa* stem cuttings. On the other hand, the use of root initiation promoters, usually auxin-containing products is very important and has commercial benefits, especially in the case of difficult-to-root species (Blythe et al., 2007). Akwatulira et al. (2011) also posits that the use of 0.8% w/w IBA concentration was the most effective in improving rooting of *Warbugia ugandensis* stem cutting as compared to concentrations of 0.0, 0.3 and 0.6% (w/w) of IBA. Meanwhile, the *Dovyali caffra* stem cutting, a woody plant, rooted significantly higher when treated with "dip'n root", this was followed by Dynaroot 3, 2, 1, and zero hormone (water only). This effect was attributed to rooting hormone concentration (Hae and Funnah, 2011). Similarly, Stafanini (2004), witnessed the effect of different rooting hormone dose on *Aloysia triphylla (L' Hérit)*, his results indicated that Britton performed significantly better when treated with 250 $mg.L^{-1}$ of IBA+ Boric acid compared to other treatment (water; 150 mg L^{-1} of IBA; 150 $mg.L^{-1}$ of IBA+ Boric acid; 250 $mg.L^{-1}$ of IBA).

Consequently, determining the optimal dosage of rooting hormones is essential for effective rooting of different types of plant. According to Grossmann (2000), increasing concentration, auxins can produce a variety of growth abnormalities within 24 hours after treatment, including leaf epinasty (downward curvature), stem curvature, intensified green leaf pigmentation, and growth inhibition. Reductions in stomatal aperture, transpiration, and carbon assimilation can also occur. A high level of auxin stimulates biosynthesis of ethylene, which in turn triggers abscisic acid (ABA) production. The ABA is translocated through the plant, triggering stomatal closure and, together with ethylene, promotes leaf senescence and ultimately, death (Grossmann, 2000). For instance, rooting of tomato stem cuttings did not respond effectively when the cuttings were treated with auxin concentrations in comparison to cuttings treated with no rooting hormone (Khan et al., 2011).

Interactions between rooting medium and rooting hormones applied plays a decisive role in the establishment of seedlings raised through vegetative propagation to stimulate root growth. Rooting performance is not only influenced by the type of medium used for propagation, but also the interaction of rooting hormone as well (Hartmann et al., 1990). For instance, no significant differences were observed among three media (river sand, hygrotex and manure-amended soil 50:50) used on rooting performance of *Dovyalis caffra* (Kei apple) cuttings. However, when rooting hormone (Dip'n root) was applied on hardwood cuttings planted on sand significant difference were observed on percent rooting and root number was greatly increased by combination of hygrotex and Dynaroot 2 at ($P < 0.05$) (Hae and Funnah, 2011). The results from Usman and Akinyele (2015) on *Massularia acuminate*, shows that river sand + Sawdust had the highest mean shoot length while river sand alone had the least.

2.5.3 Effect of stem cutting length

Cutting length is one of the important physical factors that influence rooting capacity in stem cuttings. There is no universal internode length recommended for propagating different species as the length is often dependent on the type of species involved (Hartmann et al., 2002). However, according to the author, the stem lengths of 12 cm and below with less than four nodes are recorded as short cuttings, while, 14 cm stem length and above with more than

four nodes are considered to be long cuttings. This has led to the peculations that the long stem cuttings are most likely to develop roots faster and give a more vigorous plant than the shorter ones (Welch- Keesey and Lerner, 2002). This speculation might be due to the increased diameter and number of nodes in the long stem cuttings (Awan et al., 2012).

According to Tchoundjeu and Leakey (1996), African mahogany (*Khaya ivorensis*) long stem (39 mm) cutting gave higher root number and length, and percentage of rooted cuttings than short stem cuttings (19 mm). In addition, Leakey (1983) and (Leakey and Mohammed, 1985; Araya, 2005) mention that cuttings length have more control on the number of roots per rooted cutting than rooting percentage of cutting. Cuttings length of 12 cm rooted better than 6 cm stem cuttings length of fourwing saltbush (*Atriplex canescens)* (Richardson et al., 1979). Similarly, Kowalczyk and Kobryn (1999) observed that long intermodal length (10 cm) gave the highest root length and root number than short (5 cm) intermodal stem cuttings of *Solanum muricatum*. Thus, Hartmann et al. (1990) concluded in their study that a build-up of carbohydrates at the base of the cutting qualified effective rooting of cuttings with long basal than short ones.

Meanwhile, rooting performance of short cuttings (10 cm) of *Larix kaempferi* was significantly improved when compared to the long cuttings (15 and 20 cm lengths) (Wang et al., 1997). Awan et al. (2012) reported similar results in a study conducted on an olive plant cultivar (*Azerbaijan*). The authors observed an increase in root length and survival rate of 20 cm long stem cuttings in comparison to cuttings that were 25, 30 or 35 cm long. According to the authors, these short stem cuttings (15 cm) showed improved sprouting and maximum number of roots when compared to other cutting lengths of 20, 25, 30 and 35cm.

Therefore, it may be inferred that the good performance that is experienced with some short stem cuttings may be species-specific (Leakey 1983; Leakey and Mohammed, 1985; Araya, 2005), or related to the physiological state of the mother plant from which the cuttings were obtained (Leakey and Mohammed, 1985; Wilson, 1993; Mitchell et al., 2004; Naidu and Jones, 2009). Poor performance of short stem cuttings may be due to immaturity, inadequate maintenance of food reserves (Hegde, 1988) and insufficient transfer of nutrients (Good and Tukey, 1966) for production of roots and shoots.

2.5.4 Wound healing period (WHP)

A wound is a breach in the normal tissue continuum, resulting in a variety of cellular and molecular effect. Wound causes the plant to secrete auxins, which promote root formation (Pop et al., 2011). Wound-induced roots are the major type of adventitious root formation in stem cuttings. Once the stem (or shoot) is cut off from the plant (wounding), a series of wound responses occur and *de novo* adventitious root regeneration proceeds (Hartmann et al., 2002). Wounding of stem cuttings occurs when the material is first collected from the stock plant. But, additional basal stem wounding can be beneficial to rooting success with certain species (Macdonald, 1986; Hartmann et al., 2002).

The wound healing period (WHP) is governed by an array of endogenous physiological factors (Hartmann et al., 2002). For instance, cuttings must survive physiological stress after severance from the stock plant. However, if they receive little water or nutrient uptake, they can be affected by the usually reduced stomata conductance, until the development of the roots (Pop et al., 2011). Cuttings are commonly put (stuck) into the rooting medium as soon as they are detached from the mother plant to avoid water loss which can lead to the death of cutting, disease infection and other damages that can directly or indirectly inhibit rooting rate (Hartmann et al., 2002; Leakey, 2002).

When a plant is wounded, auxins collect briefly around the wound and alter the nature of cell division in the cambium so that it begins to form embryonic root tissues (Hartmann et al., 2002). Nolte (2003) states that, at the wounded sites, protection from desiccation and pathogen occurs by the production of suberized, protective cells. Therefore, wound healing is accomplished by a series of distinct processes. These processes can take several days, weeks, or months depending on the type of species (Muralidhar et al., 2013).

On the other hand, for potato tuber, wound healing period may take three to five days at room temperature or under good conditions. But, it can take over two weeks under 10^0C and several weeks at even colder temperatures (Chase, 1989). It is clear that temperature and relative humidity are major factors that affect the speed of wound healing (Widdington, 1974; Sabba and Lulai, 2002). It is important to note that, as the temperature increases, there is also a promotion of microbial growth and that if relative humidity is too high (>97%), cell

proliferation at the surface can occur as accompanied by growth of anaerobic bacteria that cause soft rot (Widdington, 1974).

Wound healing period (WHP) and developmental stages of adventitious roots formation (ARF) in an apple were summarized by De Klerk et al. (1999). The first phase [0-24 hr. after indole-3-butyric acid (IBA) treatment] involves dedifferentiation of shoot tissues, and was marked by the accumulation of starch grains and wound-related compounds (WRCs). The second phase, induction period (24-96 hrs.), was marked by the initiation of cell division, of about 48 hours. The degradation of starch grains; at the end of this phase, meristemoids of about 30 cells were observed. In Petunia, the first macroscopic anatomical events associated with adventitious root formation were observed 72 hours after treatment with IBA (Ahkami et al., 2009). The third phase was root outgrowth within the stem lasting between 96-120 hours. By this time, auxin was no longer required and can even become inhibitory. In the final phase, 120 hours after wounding, new roots have grown through the epidermis of the stem.

If the wound is permitted to heal for hours or even weeks, the callus dries and becomes hard. When it comes in contact with moist soil, the callus remains somewhat soft, and the burgeoning roots beneath it can emerge (Ahkami et al., 2009). To optimize propagation of pineapple, the crowns are set aside for few days to allow the wound to heal and dry before planting (De Klerk et al., 1999; Trail, 2001). On the other hand, in a fig tree, the wound healing process delayed planting of cuttings for a few weeks and the number, length and dry weight of roots was improved compared to the cuttings that were immediately planted (Takagaki et al., 2000).

Most succulent cuttings root quicker and efficiently when left to heal at room temperature or in open air until the surface dries (Fox et al., 1971). Soft cuttings can dry out quickly and are often stored in a plastic bag to prevent them drying up until they can be placed in a room temperature (Douglas, 2008). According to Kelly (2009), it is essential that all cacti species (Cylindropuntia (Cholla) and Opuntia (Prickly pear), be allowed to heal at the cut end surface or air dried until the soft inner tissue calluses over, this processes will take weeks to a month depending on the temperature.

Wound healing can also occur in well-watered soils in warm temperatures (Hartmann et al., 2002). The callus formed will protect the plant from most of the soil-borne diseases and will promote rooting once the cutting is placed in the rooting medium. Puri and Thompson (2003) believed that soil moisture has a major effect on rooting. Whereas, water-stressed cuttings took a longer time to root and had fewer roots. Pre-soaking of cuttings stimulated rooting, particularly under the drier soil moisture conditions. Initially the water potential of cuttings decreased with time and with the formation of roots it stabilized in all the pre-treatments. The reduction in water potential of cuttings, after planting, was related to an increase in resistance to water flow in the xylem vessels (Runkle, 2006).

The use of auxin during adventitious rooting enhances the formation of callus in addition to inducing the formation of roots. Cells in the vicinity of the vascular cambium and phloem (near the source of hormones and carbohydrates) begin to divide and initiate adventitious roots (Hartmann et al., 2002). In the case of agaves, the wounded endings of the cutting were dusted with sulphur and rooting hormones and rooting was promoted. These were allowed to heal or air dry until the cut was callused over to prevent the cutting from root rot (Kelly, 2009). In other words, if this step was not taken, the cutting would rot and have to be discarded.

CHAPTER 3
ROOTING OF ROSE-SENTED GERANIUM CUTTINGS AS AFFECTED BY GROWING MEDIA AND ROOTING HORMONE

3.1 ABSTRACT

Growing media and rooting hormones play an important role in rooting of stem cuttings. This can either be through their direct effect on the cuttings or through their interaction. To maximize rose-scented geranium production, the effect of growing medium and rooting hormone on successful rooting and development of rose-scented geranium was investigated. The experiment was laid out in a randomized complete design (RCD) and was replicated three times with two factors 7×4, seven different growing media i.e. (**1**) Mixture which serves as control (pine bark 8 bags + sand 2bags + lime 4kg + coconut 10 blocks + talborne 6.25 kg + bone meal 2 kg); (**2)** River sand only; (**3)** Pine bark; (**4)** Hygrotex (commercial rooting media); (**5)** Pine bark + river sand (at1:1 ratio on volume basis); (**6)** Pine bark + hygrotex (at 1:1, ratio on volume basis), and; (**7)** Pine bark + river sand + hygrotex (at 1:1:1 ratio on volume basis) and four different IBA rooting hormone concentrations (auxins, types of IBA) applied as treatment were (**1**) Dynaroot (1 – 1g/kg), (**2**) Dynaroot (2- 3g/kg), (**3**) Dynaroot (3-8g/kg) and (**4**) Control (untreated with hormone). Cuttings propagated in hygrotex recorded the maximum mean number of roots, highest root length, maximum fresh mass, and highest shoot number, while hygrotex + pine bark (v/v 1:1) was efficient in producing more leaves, plant height and other aerial parameters. The highest rooting and root length were obtained where cuttings were treated with Dynaroot 3. Dynaroot 2 and pine bark + hygrotex (at 1:1 v/v) interacted better and promoted root holding ability (RHA). In addition, a combination of control and hygrotex interacted significantly well and recorded the greatest stem circumference than any other treatment. Vegetative propagation of rose-scented geranium through stem cuttings can be appropriately achieved by treating the cuttings with Dynaroot 3 IBA hormone using hygrotex as a growth medium for successful rooting, good seedling quality and early transplanting of seedlings of rose-scented geranium stem cuttings.

Keywords: Auxin; Dynaroot; Indole-3-butyric acid (IBA); propagation medium; rooting hormone and rose-scented geranium

3.2 INTRODUCTION

Propagation medium is a basic need of any plant, where growth takes place (Hartmann et al., 1990). Its purpose is mainly to provide the necessities required by the plant throughout its life (Altman and Freudenberg, 1983). Furthermore, it provides the cuttings with physical support, stores oxygen and water, and creates a dark condition around the rooting zone (Hartmann et al., 1990; Akwatulira et al., 2011). During propagation by stem cuttings, root and shoot development are simultaneous prerequisites for successful development of independent plants (Joyner, 2002; Jooste, 2004). Even though the choice of the propagation medium is important for propagators, rooting of cuttings does not only depend on the type of rooting media alone but, on other factors such as availability and cost of material, type of species used, part of plant used, time of planting and season. (Leakey, 1990; Mamba and Wahome, 2010).

At commercial level, geranium plant is reproduced through stem cutting propagation technique and commercial propagators or nurseries often encounter failure during vegetative propagation, which are mainly associated with poor rooting of cuttings (Hartmann and Kester, 1997). Usually, such losses affect geranium growers particularly those who raise their own seedlings (plantlets). The losses are as a result of production of poor-rooted seedlings or delayed rooting (Hartmann and Kester, 1997; Anonymous, 2006). According to Hartmann et al. (2002), root formation is a prerequisite for raising successful seedlings through cutting propagation. Unfortunately, for propagators, there is no universal propagation medium and a standard rooting hormone for growing cuttings (Hartman and Kester, 1997; Araya, 2005). This is despite the fact that, the choice of both propagating medium and rooting hormone plays a significant role on rooting performance and growth of the plant (Hartmann and Kester, 1997; Hartmann et al., 2002).

Even though the choice of the propagation medium is important for propagators, rooting of cuttings does not depend on the type of rooting medium only. There are other factors that need to be taken into consideration such as, the availability and cost of planting material, type of species, part of plant used, time of planting, etc. (Leakey, 1990; Mamba and Wahome,

2010). Being a storehouse of water, air and mineral supply, rooting media aid rooting of cuttings and their further growth and development into whole plants (Hartmann et al., 1990).

Auxin treatments are also used in commercial plant propagation to increase the overall rooting percentages, hasten root initiation, increase the number and quality of roots, and encourage uniformity of rooting (Hartmann and Kester, 1997; Hartmann et al., 2002). This is despite the fact that some plant species may root naturally because they can endogenously release sufficient auxin concentration to naturally enhance rooting. Consequently, the effectiveness of auxins in promoting root initiation has been shown in a variety of plant species to break root apical dominance induced by cytokinin (Cline, 2000). According to Blythe et al., (2007), optimal rooting response by cuttings typically occurs when auxin is supplied soon after cutting preparation. In examining the response of stem cuttings of mung bean [*Vigna radiata (L.) R. Wilcz. (syn. Phaseolus aureus Roxb.*)] to IBA, Jarvis et al. (1983) reported that auxin should be supplied immediately after the cutting is prepared to obtain the maximum effect of auxin.

Meanwhile, the extant literature indicates that information is limited, if any, on the effect of rooting media and rooting hormones on rose-scented geranium stem cuttings. This shows that, identifying appropriate growing media and rooting hormone would save planting materials and minimize production cost associated with raising seedlings. Therefore, the objective of the current study was to evaluate rooting performance and early seedling growth of rose-scented stem cuttings raised in different growing media and treated with different IBA hormone concentrations.

3.3 MATERIALS AND METHODS

3.3.1 Experimental site and facilities

The study was carried out at the Essential Amatole Nursery, at the University of Fort Hare Research Farm, Alice Campus (located at 32^0 47′3″S, 26^0 50′43″ E, and an altitude of 519 m.a.s.l) from September 2014 to November 2014. The experiment was carried out in misting conditions on bottom-heated beds in a greenhouse (with polycarbonate roofing of about 40%

shading effect) for the first three weeks. This was done after sticking the cuttings to the growing medium to facilitate root induction in relatively high temperature and relative humidity. Thereafter, the plantlets were grown in a shade-cloth house with 70% light penetration until the termination of the experiment.

3.3.2 Treatments

A 7 x 4 factorial experiment (7 growing media combinations and 4 different IBA hormone levels, respectively) was set up. The following seven types (combinations) of rooting medium were applied as treatment:

1. Mixture rooting medium [pine bark (200kg + sand (100 kg + lime (4 kg) + coconut (25 kg) + talborne (6.25 kg) + bone meal (2 kg)]: (control),
2. River sand only,
3. Pine bark,
4. Commercial rooting media - Hygrotex,
5. Pine bark + river sand (at 1:1 v/v),
6. Pine bark + hygrotex (at 1:1 v/v) and
7. Pine bark + river sand+ hygrotex (at 1:1:1 v/v/v).

The rooting hormone treatments were (1) Dynaroot 1, (2) Dynaroot 2, (3) Dynaroot 3, and (4) Control (untreated with hormone). The compositions of Dynaroot treatments that were used are presented in Table 3.1.

Table 3.1: Active ingredients in IBA rooting hormone used

Commercial name	Composition
Control	4-indole-3-butyric acid: 0g/kg
Dynaroot 1	4-indole-3-butyric acid: 1g/kg
Dynaroot 2	4-indole-3-butyric acid: 3g/kg
Dynaroot 3	4-indole-3-butyric acid: 8g/kg

Source: Efekto Trading (2006)

3.3.3 Experimental layout

The experiment was laid out in a completely randomised design (CRD) and each treatment combination was replicated three times. During the planting process, cuttings were grouped into 28 groups, each group treated with one of the 7 x 4 (growing media-hormone) treatment combinations. The basal part (lower 1 cm) of the stem cuttings were dipped in distilled water and thereafter into the rooting hormone to pick the powder (Dynaroot 1, 2 or 3). Excess rooting powder was tapped away before planting in order to avoid yellowing of the powder in planting (Hartmann and Kister, 1983; Araya, 2005). The cuttings were then directly planted (stuck) in pre-wetted growing media combination in seedling trays, using a planting depth of 2 cm. The control plants (without hormone treatment) were planted on the growing media after dipping in water.

3.3.4 Source of plant materials and cutting preparation

Stem cuttings were collected from mature, healthy rose-scented geranium plants grown at Essential Amatole Farms (around the Hogsback area of the Nkonkobe Municipality). Shoots of about 30-40 cm long were cut from the stock plants in the early hours of the day (between 05:00 and 06:30). These were immediately placed in plastic bags in order to minimize water loss until they were taken to the shade condition at the working area. At Essential Amathole, stem cuttings of 9-11 cm length with 3-5 nodes and a stem circumference of 2-2.3 cm were used as propagation materials. On the cutting preparation area, stem cuttings of 10 cm length with 4-5 nodes and stem circumference of 2-2.3 cm were prepared as planting material. All the open leaves stem cuttings were removed to minimize water loss through evapotranspiration and to avoid crowding of plantlets (Hartmann and Kister, 1983).

3.3.5 Plant culturing process

After the planting of the cuttings into the growing media, the cuttings were grown under greenhouse conditions, placed on a bottom-heating bed under misting condition to hasten root initiation for the first three weeks. After three weeks, the plantlets were moved from the greenhouse into a shade-house for another five weeks for subsequent growth and for hardening purpose under near prevailing field weather condition. In the shade house, the seedling trays were placed on 1-meter high metallic parallel bars to prevent fungal infection and facilitate drainage. Throughout the experimental period (September to November 2014), the temperature of the shade house was measured using a thermograph. At the experimental site, the mean minimum and maximum weakly temperature ranged between 20 °C to 29 °C and average relative humidity was 81% during the period of the experiment. Air temperature and relative humidity were measured with a portable thermo-hygrometer throughout the duration of the experiment. Watering was done twice a day with overhead spray lines. A complete nutrition fertilizer was applied once a week for the duration of the experiment; and 150 ppm nitrogen of a balance fertilizer (20-10-12) was used.

3.3.6 Data collection and statistical analysis

Data collection started one week after planting (WAP) and continued on a weekly basis until the Eighth WAP, when the experiment was terminated. Destructive data collection method was used; samples of four cuttings were harvested from each replication of all treatments and data were recorded. Cutting growth and rooting parameters collected included root number, length and fresh weight, plant height, leaf number, and stem circumference. For root measurements, the growing media were washed out from the root system of plantlets and their roots were separated from stem before data such as root number, length and root fresh weight were recorded. Root holing ability (RHA) on rooting medium was determined by visual observation and rated on a 1-5 scale, where 1: very loose, not acceptable; 2: loose, not acceptable; 3: medium, marginally acceptable; 4: tight, acceptable; and 5: very tight, acceptable. This scale was used following the rating scale used by Rajapakse et al. (1996) on *Chrysanthemum* species cuttings. The data collected were subjected to analysis of variance

(ANOVA), using the JMP® Release 11.0.0 statistical software package (SAS, 2010). Where significant differences were established, treatment means were separated using the least significant difference (LSD) test at α level of 0.05.

3.4 RESULTS AND DISCUSSION

3.4.1 Effect of growing media on root production of rose-scented geranium cuttings

The results of this study were presented from Week three of the experiment due to inadequate results (high variability) which showed on Week 1 and 2. A clear distinction was observed from Week 3 in all the parameters tested. Interaction effect between propagation media and rooting hormone was reported on root number, root length and stem circumference only (Table 3.4), while, other parameters showed no significance effect on interaction between propagation media and rooting hormone. Even though, the interaction of propagation media and rooting hormone on root holding ability was not statistically analysed, visual analysis showed differences (Table 3.5). The main factors, factor A (growing media) and factor B (rooting hormone) were therefore discussed separately. The effect of factor A is presented on table 3.2 and factor B (rooting hormone) on table 3.3 respectively.

Root number

The analysis of variance showed that the type of growth medium has a significant effect on root production based on the number of roots produced per cutting as indicated in Table 3.2. The maximum number of roots (39.08 roots) was recorded with the use of hygrotex, followed by pine bark + hygrotex (1:1 v/v) (33.58 roots) and mixture rooting medium (35.33 roots), though there was no significant difference between the latter two growing media. The use of river sand as a growing medium gave the lowest number of roots. This observation indicates that sand is a poor substrate for promoting root production on stem cuttings. This could be attributed to low nutrient content on river sand due to high rate of leaching , its poor water holding capacity as result of high proportion of macro pores that result in a weak capillary force and prone to leaching of applied fertilizer (Leakey, 1990; Berhe and Negash, 1998).

An ideal propagation medium should provide sufficient porosity to allow good aeration for the developing root system (Hartmann and Kester, 1997). The main reason for maximum

number of roots could be the availability of essential nutrients in the hygrotex medium, and the internal high temperature of the medium which favoured quick and maximum rooting of the cuttings (Hartmann and Kester, 1997). The differential root production by cuttings as affected by growing medium was observed on *Milicia excelsa* (Ofori et al., 1996) and on *Ficus Binnendijkii* (Amastel Queen) cuttings (Shah et al., 2006).

Root length

The mean root length per cutting as affected by different growing media is represented in Table 3.2. Growing media used in the experiment significantly influenced the root lengths of cuttings (at $p < 0.05$). The highest root length (51.08 cm) was observed in hygrotex, followed by pine bark + hygrotex (1:1 v/v) medium (42.92 cm). While pine bark + sand (1:1v/v) resulted in the lowest root length (30 cm). It may be inferred that the study's findings might be due high organic matter content and to the high water-holding capacity of hygrotex growing medium in comparison to the poor water retention of bark + sand (1:1v/v).

Table 3.2: Effect of growing media on rooting and growth parameters of rose-scented geranium stem cuttings at week eight after planting (AP)

Growing medium	root number	root length (cm)	root fresh mass (mg)	Leaf number	Stem circumference (cm)	Shoot number	plant height (cm)
Control	35.33 B	37.00 C	365.8 C	8.250 B	3.125 C	3.083 A	13.24 A
River sand	23.92 E	34.58 D	300.0 D	4.000 G	3.100 C	1.833 C	13.02 B
Pinebark	33.58 C	33.33 D	356.7 C	7.167 D	3.242 A	3.000 A	12.76 C
Hygrotex	39.08 A	51.08 A	452.5 A	7.750 C	3.208 AB	3.167 A	12.97 B
Pinebark + sand	30.17 D	30.08 E	367.5 C	5.167 F	3.158 BC	2.583 B	12.73 C
Pinebark + hygrotex	35.67 B	42.92 B	395.0 B	9.167 A	3.208 AB	3.083 A	13.02 B
Pinebark+sand +hygrotex	32.50 C	38.00 C	368.3 C	6.750 E	3.133 C	2.250 B	12.80 C
Grand mean	**32.893**	**38.143**	**372.262**	**6.893**	**3.168**	**2.714**	**12.933**
CV (%)	**5.93**	**4.23**	**6.04**	**11.30**	**2.82**	**15.57**	**0.80**
LSD (P<0.05)	**1.596**	**1.320**	**18.37**	**0.3460**	**0.07315**	**0.3460**	**0.08577**

Mean value in the same column bearing the same letter are not significantly different at p<0.05

Other researchers have observed similar influence of growing medium on length of root produced on stem cuttings during vegetative propagation. For example, Al-Saqri and Alderson (1996) observed that root lengths of *Rosa centifolia* cuttings were significantly higher when grown in perlite as compared to vermiculite. Mpati et al. (2006) also reported the production of significantly longer roots in fever tea cuttings when grown in pine bark as compared to sand as growing medium.

Root fresh mass

In general, there was an increasing trend in root fresh mass with an exponential increase between the fourth and sixth week after planting, after which there was a clearly observable difference in root development performance among the growing media (results not published). Statistical analysis of the data collected at the end of the experiment showed that different propagation media significantly influenced the fresh mass of roots produced by the cuttings (Table 3.2). The highest root fresh weight (452.5 mg) was obtained with the use of hygrotex as growing medium, followed by pine bark + hygrotex (1:1 v/v) (395 mg), whereas the river sand medium gave the lowest root fresh mass (300 mg).

A possible reason for the performance of hygrotex as a growing medium is in its high organic matter content, which increases the water and nutrient holding capacity of the medium. Another possible reason for the best performance of hygrotex over sand is the downward moment of water and nutrients because the roots had to absorb enough water and nutrients to increase their length. Cuttings grown on this medium also produced the maximum number of roots and highest root lengths. Perhaps, this was due to a relatively high absorption rate of available nutrients, which in turn results in maximum root fresh mass. Mpati (2006) found similar results in pine bark when compared to sand for fever tea cuttings.

3.4.2 Effect of growing media on leaf production of rose-scented geranium cuttings

Similar to root development parameters, noticeable leaf number variations among plantlets raised in different growing media started in Week 6. Based on data collected at the end of the experiment, growth media significantly affected leaf number of rose-scented geranium

plantlets raised from stem cuttings (Table 3.2). Cuttings propagated in pine bark + hygrotex gave a significantly high number of leaves (9.167), followed by cuttings propagated in mixture growing medium (8.250) and hygrotex (7.167). It is worth mentioning that all these media that significantly promoted leaf production were all hygrotex containing media. One of the reasons was because hygrotex has high organic matter content and this increases the water and nutrient holding capacity of the medium (Hartmann et and Kester, 1997). The medium also has a high N content which plays vital role in the vegetative growth of the plants. Joiner and Nell (1982) found similar results in a peat + perlite mixture and commercial rooting media for *Aglaonema and Dieffenbachia*.

The use of sand as a growing medium resulted in the lowest number of leaves (4.00). The poor performance of sand as a growing medium can be attributed to its poor water holding capacity and low nutrient retention as well as poor aeration (Ofori et al., 1996). The above findings are echoed by a study by Yeboah and Amoah (2009) on *Vitellaria paradoxa* plantlets indicated that poor aeration of rooting media may reduce metabolic activities and collapse plant development.

3.4.3 Effect of growing media on stem circumference of rose-scented geranium cuttings

Stem circumference was significantly affected by different growing medium used, such that cuttings rooted in pine bark had the thickest stem (3.242 cm) than all other treatments used. These were not significantly different from hygrotex (3.208 cm) and hygrotex + pine bark (1:1) (3.208 cm). When visually evaluated, pine bark tends to have better stem circumference as compared to the latter two media. On the other hand, the use of sand and sand containing medium gave plantlets with low stem circumference (Table 3.2). Akwatulira et al. (2011) also reported the highest rooting and stem development with *Warburgia ugandensis* stem cuttings grown on pine bark. The findings from the current study could be attributed to high aeration and water holding capacity (WHC) in pine bark as well as hygrotex. Pine bark is loose in texture compared to sand hence, it allows for good aeration and water flow.

3.4.4 Effect of growing media on shoot production from rose-scented geranium cuttings

Shoot number

The number of shoot initiated and developed from rose-scented geranium stem cuttings established in different growing media varied significantly (Table 3.2). All growing media that contained sand [river sand (1.33 shoots); pine bark + hygrotex + sand (at a ratio of 1:1:1) (2.250 shoots); and pine bark + sand (at a ratio of 1:1) (2.583 shoots)] gave the lowest number of shoots. Although there was no significant difference in shoot number among cuttings propagated in hygrotex (3.167), hygrotex + pine bark (1:1) (3.083) and mixture rooting media (3.083), cuttings planted in hygrotex alone tended to produce a relatively higher number of shoots as compared to cuttings propagated in other media.

The possible reason for better performance of the hygrotex-containing media could be the internal high temperature of the medium which could have created conducive conditions for sprouting. The plants in these media also have maximum number of roots, which indicate high absorption rate of available nutrients, which in turn results in large number of shoots. Similar findings were reported by Olabunde and Fawusi, (2003) and Puri and Thompson (2003), who worked on Queen of Philippines (*Mussaenda philippia Rich.)* and *Dalbergia sisso Roxb* cuttings respectively, as well as Shah et al. (2006), who studied Ficus Binnenduijki (*Amstel queen*) stem cuttings propagation. In many species, the presence of good roots system on cuttings exerts a strong stimulatory influence on promoting areal parts of the plant (Hartmann et al., 1990). This reflects the role of roots as a source of both auxins and carbohydrates. The main reason for the poor performance of sand could be attributed to the number of roots and root length produced Akwatulira et al. (2011).

Plant height

In Week 3 to Week 5 after planting, growing media showed a slight difference on plant height. The difference became remarkable starting from Week 6 onwards. The effect of growing media on plantlet height was established and grown on different growing media at Week 8 is depicted in Table 3.2. The highest plant height that was recorded for the cuttings planted mixture rooting medium was (13.24 cm) and the pine bark recorded the lowest plant height (12.76 cm). The reason for highest plant height on mixture rooting medium may be attributed to its fairly loose texture, which might have led to better aeration, drainage and

moisture retention (Olabunde and Fawusi, 2003; Puri and Thompson, 2003). On the other hand, poor performance of pine bark on plant height may be attributed to poor aeration, drainage and moisture retention. A study of rooting performance on *Vitellaria paradoxa*, by Yeboah and Amoah (2009), showed that poor aeration in rooting media is responsible for demoting metabolic activities and decreases root initiation.

3.4.5 Effect of rooting hormone on rooting performance of rose-scented geranium cuttings

Root number

The results of the current research showed that different rooting hormone concentrations resulted in significant differences in root number of cuttings (Table 3.3). Root number increased as the concentration of active ingredient (IBA hormone) rooting agent increased. Thus, the highest root number was recorded on cuttings treated with Dynaroot 3 (34.67), which was significantly different (at $p < 0.05$) from the other treatments. Even though there was no significant difference between untreated cuttings (control) and those treated with Dynaroot1, the root number was slightly higher in Dynaroot 1 (32.00) than in control (31.57).

Table 3.3: Effect of different rooting hormone concentrations on rooting and growth parameters of rose-scented geranium stem cuttings at week eight AP

rooting hormone	root number	root length (cm)	root fresh mass(mg)	leaf number	Stem circumference	plant height (cm)
No-hormone (control)	31.57 C	35.33 D	345.2D	6.381 B	3.324 A	12.73 D
Dynaroot 1	32.00 C	37.00 C	362.9C	6.762 AB	3.162 B	12.86 C
Dynaroot 2	33.33 B	39.14 B	381.4B	7.238 A	3.110 BC	13.00 B
Dynaroot 3	34.67 A	41.10 A	399.5A	7.190 A	3.076 C	13.15 A
Grand mean	32.893	38.143	372.3	6.893	3.168	12.933
CV (%)	5.93	4.23	6.04	11.30	2.82	0.80
LSD (P<0.05)	1.207	0.4817	13.89	0.4817	0.0553	0.0648

Mean value in the same column bearing the same letter are not significantly different at p<0.05 using Tukey's pairwise comparison (LSD).

These results are in reference to the composition of rooting enhancers used in this experiment, it is clear that the only difference between Dynaroot 1, Dynaroot 2 and Dynaroot 3 is the concentration of IBA (Table 3.1). Results obtained in this study align with a study done by Hae and Funnah (2011). In addition, these results agree with those of Blythe et al. (2004) who obtained similar outcome where stem cuttings of *Ficus benjamina* and *Gardenia augusta* were used in percent rooting, root number and root length.

Root length

The emerging results also indicated that different IBA rooting concentrations affected root length significantly (at $p < 0.05$), such that there was an increase in root length with an increase in rooting hormone concentration (Figure 3.3). The findings of the study further showed that cuttings rooted best when treated with Dynaroot 3 (41.10 cm) as compared to all the other growth regulator concentrations applied. The second best root length was observed in the cutting treated with Dynaroot 2 (39.14 cm). On the other hand, the lowest rooting length was produced by control (35.33 cm) although it was not significantly lower (at $p < 0.05$) than Dynaroot 1 (37.00 cm).

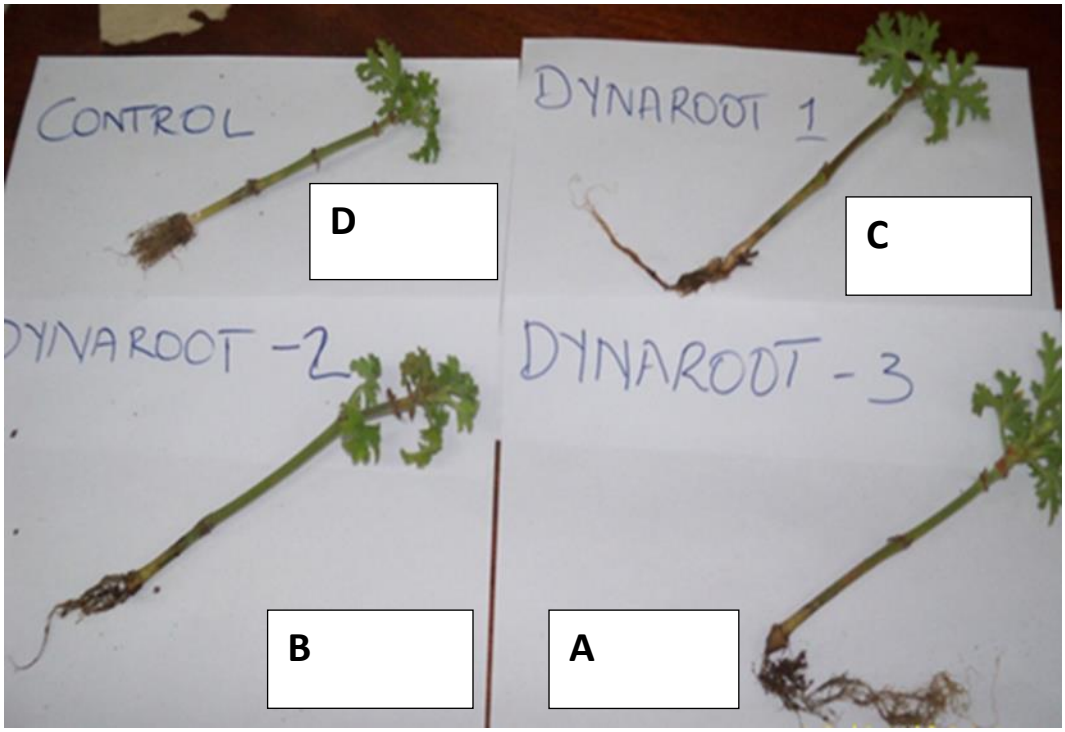

Figure 3.1: Effect of different IBA hormone concentration on roots. ***Where A- Dyanaroot 3; B- Dynaroot 2; C- Dynaroot 1 and D- distilled water (control)***

The results of the present study align with the findings of Davis and Hassing (1990), who observed that IBA application is known to trigger the innate genetic potential of plants for rhizogenesis, thereby improving root formation and elongation. In figure 3.1, comparisons were made between IBA rooting hormone concentration and control. Results show that, there is evidence of an increase in root length with an increasing concentration of IBA, such that Dynaroot 3 gave the maximum root length and control gave the minimum root lenght. Similarly, Blythe et al. (2004) found that IBA enhanced root length in *Ficus benjamina* and *Gardenia augusta* stem cuttings.

Root fresh mass

In the current research, root fresh mass was higher when Dynaroot 3 (399.5 mg) was used as compared to all other rooting hormone treatments (Table 3.3). Cuttings that were treated with no IBA rooting hormone (control) (345.2 mg) gave the lowest root fresh mass. Auxins are known to promote root development of stem cuttings through their ability to promote the initiation of lateral root primordial and to enhance transport of carbohydrates to the cutting base (Leakey et al., 1982; Hartmann et al., 1990). Thus, the main reason involved in the for efficient performance of Dynaroot 3 on promoting root fresh mass could be the effect of

auxins that has root enhancing effect through the shifting the sink strength towards root, making more carbohydrates and other nutrients translocate to the rooting zone (Middleton et al., 1980).

3.4.6 Response of stem circumference to rooting hormone application on rose-scented geranium cuttings

Stem circumference was significantly affected (at $p < 0.05$) by the different IBA concentrations applied as treatments (Table 3.3.). In general, the control treatment produced superior stem circumference (3.076 cm). Stem circumference decreased with an increase in rooting hormone concentration, there was a slight but significant difference among the three other treatments (Dynaroot 1, Dynaroot 2 and Dynaroot 3). The increase in IBA hormone concentration was accompanied by decreases in stem circumference, suggesting that IBA hormone concentrations were phototoxic for stem circumference formation process. These results response suggest that when promoting stem circumference of rose-scented-geranium through vegetative propagation the growth regulator used should not contain IBA. This was consistent with the observation in the previous sections that IBA shifts sink strength towards the root system. Similar results were reported by Ofori et al. (1996) on *Millisa excels*.

3.4.7 Plant height as affected by rooting hormone on rose-scented geranium cuttings

The present investigation reveals that auxins significantly ($p< 0.05$) affected plant height of rose-scented geranium. Plantlet height progressively increased as the rooting hormone concentration increased (Table 3.3). The highest concentration used (Dynaroot3) resulted in the highest plant height (13.15 cm) and the least (12.73 cm) was observed in cuttings that did not receive rooting hormone (control). A comparison of this data (plantlet height) with the data on stem circumference highlights that IBA encourages unidirectional expansion of cells (cell length rather than diameter). It has been widely documented that the rooting hormones such as auxins promote adventitious roots, uniformity in rooting of cuttings, increasing cell elongation as well as development of stem cuttings (Moon et. al. 1991, Henry et al. 1992, Leakey 1992, Kwon et al. 1997, Wang et al. 1997, Kowalczyk and Kobryn 1999). This

indicates that treating stem cuttings with auxins before planting is essential for cell elongation. Nicola et al. (2005) determined that the use of auxins rooting products have a positive effect on cell elongation, both in terms of the longest root length and stem length. Therefore, it may be implied that the increase in plant height in cuttings treated with rooting hormone may be due to the accumulation of metabolites at the site of application of auxins, cell enlargement, enhanced hydrolysis of carbohydrates, synthesis of new proteins, and cell division (Hartman and Kester 1983).

3.4.8 Interaction effect of growing medium × rooting hormone

Root number

There was an interaction effect between growing medium and rooting hormone treatments on root production as indicated by root number per cutting (Table 3.4**)** at Week 8 after planting. The results of the study indicate that there were increases in root number as response to the rooting hormone concentration (Dynaroot 3, 2, 1 and control) where hygrotex was used as a medium. The highest mean root number (41.33) was recorded in Dynaroot 3 × hygrotex and in Dynaroot 2 × hygrotex (40.00) and the lowest in Dynaroot 1 × hygrotex (38.00) and control × hygrotex (37.00), respectively. These results support the findings of Ofori et al. (1996), who reported that the mean number of roots per rooted cuttings was high when the cuttings of *Milisia excelsa* were treated with IBA and sawdust. Similar results were also reported by Hae and Funnah (2011) on Kie apple stem cuttings. Thus, the performance of Dip ‘n root was exceptionally high irrispective of the growing media [River sand= SND, Commercial rooting media- hygrotex (CRM) and manure-ammended soil 50:50 v/v (M+S)] used. The use of Dynaroot 3 resulted in similar rooting percent in all three rooting media treatment. Root number was at its highest where cuttings were treated with Dip and root and Dynaroot 3 and rooted in SND and CRM. On the other hand, Dynaroot 2 outperformed Dynaroot 3 where M+S and SND were used.

However, where sand and IBA hormone was used, number of roots showed an increasing trend with a decrease in IBA hormone concentration (Table 3.4). Such that cuttings grown in sand and treated with control (no-hormone) were found to have significantly the highest root

number (27.67). While, IBA rooting hormone and sand treated cuttings recorded the lowest root length and they had no significance amongst each other (Dynaroot 1 (23.33), Dynaroot 2 (22.67) and Dynaroot 3 (22.00) respectively). The poor performance of river sand as a growing medium on rooting can be attributed to its poor water holding capacity and low nutrient retention (Hartmann and Kester, 1997). Root number was at its highest where cuttings were treated with Dynaroot 3 and rooted in hygrotex while the Dynaroot 3 × sand treatment combination gave the lowest root number. In general, the performance of Dynaroot 3 was remarkably high in all of the growing media used, except in sand.

Root length

Results of interaction effect of growing media and rooting hormone treatment combination on root length are presented in Table 3.4. The data indicates that effect of rooting hormone on root length varied with growing media. For instance, cuttings grown in hygrotex had their highest root length (56.33cm) when treated with Dynaroot 3; whereas, cuttings grown in sand gave the lowest root length (32.33cm) when sand was treated with Dynaroot 3. Results also showed that when sand was treated with control (no-hormone), it gave the highest root length (37.33) as compared to IBA hormone-treated cuttings, although they were significant different from each other. The results of the study align with the statement from Coutessa and Valentini (2011) that treating stem cuttings with different hormone concentrations before planting in a suitable rooting medium is required for effective rooting. Similarly, Hartmann et al. (1990) reported that rooting performance is not only influenced by the type of medium used for propagation, but also the interaction of rooting hormone as well. Furthermore, Hae and Funnah (2011) on Kie apple stem cuttings reported that the longest roots were obtained with the use of hygrotex growing medium in all growth regulators used (Dynaroot 1, Dynaroot 2, Dynaroot 3, and Dip'n root). However, when manure-ammended soil (50:50 v/v) was used Dip and root gave the poorest root length. These results are in agreement with the findings of Davis and Hassing (1990), who observed that IBA application is known to trigger the innate genetic potential of plants for rhizogenesis, thereby improving root formation and elongation.

Stem circumference

The results presented in Table 3.4 shows that the stem circumference was affected by the interaction of propagation medium and rooting hormone on Week 8 after establishment. Cuttings rooted in pine bark × zero hormone had a thicker stem than cuttings rooted in pine bark× IBA hormone. In contrast, cuttings rooted in sand × zero hormone had a thinner stem circumference than cuttings established in sand with IBA rooting hormone (Table 3.4). The decline in stem circumference on cuttings grown in pine bark containing media and treated with IBA hormone suggests that IBA hormone inhibits stem thickness of rose-scented geranium, except when cuttings are rooted in sand. The inhibition of stem thickening in rose-scented geranium cuttings as a response to increase in IBA concentrations has also been recorded in other species, such as *Millisa excelsa* and *Cordia alliodora*, as reported by Ofori et al. (1996) and (Mesen, 1993), respectively, such phenomenon may arise as a result of IBA-induced basipetal transport of assimilates, with progressively enhancing of sink strength of the root with increases in IBA concentration (Hartmann et al., 1990).

Table 3.4: Interaction effect of growing media and rooting hormone on rooting and growth parameters of rose-scented geranium stem cuttings at week eight AP

Growth medium	Rooting hormone	Root number	Root length (cm)	Stem circumference (cm)
Mixture	Control	33.67 FGH	32.33 LM	3.333 ABCD
	Dynaroot 1	33.67 FGH	34.67 J	3.133 FGH
	Dynaroot 2	35.67 CDEF	38.67 H	3.067 GHI
	Dynaroot 3	38.33 ABC	42.33 F	2.967 I
Sand	Control	27.67 L	37.33 I	3.033 HI
	Dynaroot 1	23.33 M	34.67 J	3.033 HI
	Dynaroot 2	22.67 M	34.00 JK	3.133 FGH
	Dynaroot 3	22.00 M	32.33 LM	3.200 DEFG
Pine bark	Control	31.67 HIJ	30.00 NO	3.367 ABC
	Dynaroot 1	33.33 FGHI	32.00 M	3.267 CDEF
	Dynaroot 2	33.67 FGH	34.67 J	3.133 FGH
	Dynaroot 3	35.67 CDEF	36.67 I	3.100 GHI
Hygrotex	Control	37.00 BCDE	45.67 D	3.433 AB
	Dynaroot 1	38.00 BCD	48.67 C	3.200 DEFG
	Dynaroot 2	40.00 AB	53.67 B	3.133 FGH
	Dynaroot 3	41.33 A	56.33 A	3.067 GHI
Pine bark + Sand (v/v, 1:1)	Control	28.33 KL	27.00 P	3.333 ABCD
	Dynaroot 1	30.00 JKL	29.33 O	3.167 EFGH
	Dynaroot 2	30.33 IJKL	30.67 N	3.100 GHI
	Dynaroot 3	32.00 GHIJ	33.33 KL	3.033 HI
Hygrotex + Pine bark (v/v,1:1)	Control	32.67 FGHIJ	40.00 G	3.467 A
	Dynaroot 1	34.33 EFGH	42.33 F	3.200 DEFG
	Dynaroot 2	37.33 BCDE	43.67 E	3.133 FGH
	Dynaroot 3	38.33 ABC	45.67 D	3.133 FGH
Hygrotex + Pine bark + Sand (v/v/v, 1:1:1)	Control	30.00 JKL	35.00 J	3.300 BCDE
	Dynaroot 1	31.33 HIJK	37.33 I	3.133 FGH
	Dynaroot 2	33.67 FGH	38.67 H	3.067 GHI
	Dynaroot 3	35.00 DEFG	41.00 G	3.033 HI
Grand mean		32.893	38.143	3.168
CV (%)		5.93	4.23	2.82
LSD (P < 0.05)		3.193	1.274	0.1463
Growth medium (G)		*	*	*
Rooting hormone (R)		*	*	*
G x R		*	*	*

Mean value in the same column bearing the same letter are not significantly different at p<0.05 using Tukey's pairwise comparison (LSD). Ns= Not significant, * = P ≤ 0.05.

3.4.9 Interaction effect of growing medium and rooting hormone on root holding capacity of rose-scented geranium

Table 3.5 shows that root holding capacity was influenced by interaction effect of growing medium and rooting hormone used. At Week 7 and Week 8, the highest root holding ability was obtained from cuttings grown in mixture growing medium and pine bark and treated with control and Dynaroot 1 [4 (tight, acceptable); 5 (very tight, acceptable)]. While, for other media treatments, when the same rooting hormones (control and Dynaroot 1) were used, lowest root holding ability ware observed. This effect was then attributed to the fact that mixture growing medium and pine bark acted optimal at a lower IBA hormone concentration and poor at higher concentration of IBA hormone. The nutrient supplements of the entire rooting medium contain enough nutrients for effective ability of roots on holing medium (Olabunde and Fawusi, 2003; Puri and Thompson, 2003). The good root holding ability observed on mixture growing medium and pine bark when treated with control and Dynaroot1 is partially due to highest root number on these cuttings.

On the other hand, except for the mixture growing medium and pine bark, the growing media had the highest root holding ability (RHA) when cuttings were treated with Dynaroot 2 followed by Dynaroot 3 in all media used. Whereas, hygrotex rooted cuttings observations recorded that RHA was very tight when Dynaroot 2 and Dynaroot 3 were used. In addition, when cuttings were rooted in hygrotex + pine bark (1:1 v/v) and treated with Dynaroot 2, gave as high roots holding ability as that of cutting in hygrotex. The possible reason for the best performance of hygrotex was the availability of essential nutrient in the medium, and that Dynaroot 2 could enhance absorption of available nutrients and increased root holding ability.

Table 3.5: Interaction effect of growing media and rooting hormone on root ability to hold medium

	*** Root holding ability on media**						
		Weeks after planting					
Hormone	**Growing medium**	**3**	**4**	**5**	**6**	**7**	**8**
Control (no-hormone)	Mixture growing medium	1	2	3	3	4	5
	River sand	1	2	2	2	3	3
	Pine bark	1	2	2	3	4	5
	Hygrotex	1	2	3	3	3	4
	Pine bark + river sand (at 1:1 v/v)	1	2	2	3	3	4
	Pine bark + hygrotex (at 1:1 v/v)	1	2	2	3	3	4
	Pine bark + river sand+ hygrotex (at 1:1:1 v/v/v)	1	2	2	3	3	4
Dynaroot 1	Mixture growing medium	1	2	3	3	4	5
	River sand	1	2	2	3	3	3
	Pine bark	1	2	2	3	4	5
	Hygrotex	1	2	3	3	3	4
	Pine bark + river sand (at 1:1 v/v)	1	2	2	3	3	4
	Pine bark + hygrotex (at 1:1 v/v)	1	2	3	3	3	4
	Pine bark + river sand+ hygrotex (at 1:1:1 v/v/v)	1	2	3	3	3	4
Dynaroot 2	Mixture growing medium	1	2	2	3	3	4
	River sand	1	2	2	2	3	4
	Pine bark	1	2	2	3	3	4
	Hygrotex	2	2	3	4	5	5
	Pine bark + river sand (at 1:1 v/v)	1	2	2	3	3	4
	Pine bark + hygrotex (at 1:1 v/v)	1	2	3	4	5	5
	Pine bark + river sand+ hygrotex (at 1:1:1 v/v/v)	1	2	3	3	4	4
Dynaroot 3	Mixture growing medium	1	2	2	3	3	4
	River sand	1	2	2	3	3	4
	Pine bark	1	2	2	3	4	4
	Hygrotex	1	2	3	4	5	5
	Pine bark + river sand (at 1:1 v/v)	1	2	2	3	3	4
	Pine bark + hygrotex (at 1:1 v/v)	1	2	3	3	4	4
	Pine bark + river sand+ hygrotex (at 1:1:1 v/v/v)	1	2	3	3	3	4

*** root ability to hold medium [determined by visual observation and rated at 1-5 scale: 1 (very loose, not acceptable); 2 (loose, not acceptable); 3 (medium, marginally acceptable); 4 (tight, acceptable); 5 (very tight, acceptable).**

3.5 CONCLUSIONS AND RECOMMENDATIONS

The results of the current research show hygrotex as the best rooting medium for propagation of rose-scented geranium stem cutting. The use of hygrotex favoured a significant increase in the production of roots and root holding capacity. While, the mixture of hygrotex + pine bark (at 1:1 v/v) was efficient in producing more leaves, plant height and other aerial parameters. Although, there are various materials that can be used for propagation of rose-scented geranium stem cuttings, it is recommended that this plant should be propagated through commercial rooting media – hygrotex. However, due to economic constraints hygrotex + pine bark (v/v 1:1) could be an alternative.

On the basis of results obtained, the study further concludes that propagation of rose scented geranium through stem cuttings is not easy without the use of rooting hormones. The choice of a good rooting enhancer is very important especially where there is a wide array of rooting hormones in the market. In addition, IBA rooting hormone, Dynaroot 3 was found to be the best rooting enhancer and best promoter of quality seedling followed by Dynaroot 2. Although, there are various materials that can be used for rooting stem cuttings, a combination of Dynaroot 3 (IBA rooting hormone) and hygrotex appears to be most appropriate for rooting rose-scented geranium stem cuttings and should be promoted for mass propagation of this herbaceous plant. Whereas, Dynaroot 2 and pine bark + hygrotex (at 1:1 v/v) may be a suitable alternative especially in situations where hygrotex is in short supply or due economic constraints.

Moreover, the use of auxin rooting products has been necessary to guarantee the rooting of cuttings for earlier transplanting of rose-scented geranium. The results of this research can also be used as a basis for propagation by stem cutting even in other herbaceous species to meet future seedling demands.

CHAPTER 4
EFFECT OF STEM LENGTH, ROOTING HORMONE AND GROWING MEDIA ON ROOTING RESPONSE OF ROSE-SCENTED GERANIUM CUTTINGS

4.1 ABSTRACT

The increasing demand for rose-scented geranium due to its economic importance necessitates the development of an efficient propagation protocol for quality seedling production. To optimize the propagation protocol for this important plant, an investigation was under taken to determine the effect of cutting stem length, the application of different types of IBA rooting hormone and ideal growing media on rooting and development of quality seedlings of rose-scented geranium stem cuttings. The experiment was set up in a complete randomized design (CRD) with a 4 × 4 × 2 factorial treatment combination. Treatments used were, four different cutting lengths viz. 10, 12, 14 and 16 cm long; four different concentrations of IBA rooting hormone [(Dynaroot 1, Dynaroot 2, Dynaroot 3) in a powder form and distilled water (control)] and two types of growing medium (hygrotex and hygrotex + pine bark 1:1) were used. The maximum number of shoots (4.00), leaf number (11.12) were recorded by stem cuttings of 16 cm length. The 14 cm cutting length produced the highest root mass (0.48 mg). Stem cuttings of 14 and 16 cm length gave the highest root number (34, 38 and 35.13) and root length (3.40 and 3.51cm) respectively and they had no significant difference amongst each other. While, stem cuttings of 10 cm length gave significantly greater stem circumference (3.1 cm). Cuttings treated with Dynaroot 3 showed better root number (33.46 roots), root length (3.54 cm), root fresh mass (0.59 mg), leaf number of (11.08) as well as highest root holding ability (5) but they showed no significance difference with Dynaroot 2 treated cuttings. Cuttings treated with Control favoured shoot number (3.79) and stem diameter (3.05). Hygrotex was visually observed to better substrate though it was not significantly different from hygrotex + pine bark (1:1 v/v) on propagation of rose-scented geranium stem cuttings. It is recommended that rose-scented geranium should be propagated through the combination of 14 cm cuttings length and treated with Dynaroot 2 IBA rooting hormone. Both hygrotex and hygrotex + pine bark (1:1 v/v) are the best growing

media for root formation and growth of rose-scented geranium, though hygrotex alone is more economical.

Keywords: Auxin; cutting length; Dynaroot; Indole-3-butyric acid (IBA); rooting hormone; rooting medium; stem length

4.2 INTRODUCTION

According to DPP (2009), for stability of essential oil yield and quality, rose-scented geranium production is commonly established through stem cutting propagation technique as it rarely produces viable seeds. Hartmann and Kester (1997), further asserts that, this plant can also be propagated by both tissue culture and cuttings. Nevertheless, plant breeders and commercial propagators prefer vegetative propagation through cuttings because of availability of plant material for propagation and for the genetic stability of the crop (Hartmann and Kester, 1997; Hartmann et al., 2002). Another advantage of stem cutting is that it is the easiest and fastest way of propagation (Hartman et al., 2002). Stem cutting also results in quick growth and development because it bypasses the juvenile characteristics of certain species, reaching production stage early (Hartmann and Kester, 1997; Hartmann et al., 2002).

During propagation by stem cuttings, adventitious root production is a prerequisite for survival of the cutting and successful production of an independent plant (Altman and Freudenberg, 1983). Rose-scented geranium propagation materials are among the few easy-to-root cuttings without any manipulation or treatment. However, the stem cutting propagation technique, as demonstrated in many plant species, is proven to perform better when the cuttings are treated with rooting hormones (auxins) so as to hasten root initiation (Khan et al., 2011). Thus, the development of effective vegetative propagation method can facilitate the cultivation and management of rose-scented geranium (Hartman et al., 2002).

Cutting length is one of the important physical factors that influence rooting efficiency in stem cuttings. So far, research indicates that, there is no universal internode length or the number of nodes recommended for stem cuttings to be used in propagating different plant

species (Hartmann et al., 2002; Leakey et al., 2004). There is speculation that, the longer the intermodal length of the cuttings, the more likely they are to develop roots faster and the more vigorous would be the results of the plantlets (Welch- Keesey & Lerner, 2002). In addition, rooting success of cuttings depends on several factors, including growing media and rooting hormone, but, some studies indicated that some herbaceous plants species can root without the use of rooting enhancers (Leakey et al., 2004).

At present, there is a dearth of literature on the effect of all the aforementioned factors (cutting length, rooting hormone and propagation media) in the production of rose-scented geranium seedlings. Linked with the foregoing assertions, the aim of this study was to evaluate the effect of cutting length, rooting hormone and growing medium in order to obtain maximum rooting and subsequent growth of rose-scented geranium.

4.3 MATERIALS AND METHODS

4.3.1 Experimental site and facilities

The study was carried out at the Essential Amatole Nursery, at the University of Fort Hare Research Farm, Alice Campus (located at 32^0 47′3″S, 26^0 50′43″ E, and an altitude of 519 m.a.s.l). Throughout the experimental period, the temperatures of the experimental sites were measured using a thermograph. The mean minimum and maximum weakly temperatures ranged from 30 °C to 37 °C and the average relative humidity of 86 % was recorded during the experimental period (November 2014 to January 2015). For the first three weeks, the experiment was carried out in misting condition on bottom-heated beds in a greenhouse (with polycarbonate roofing of about 40% shading effect) to facilitate root induction. For the rest of the experimental period, the plants were grown in shade-cloth houses, with 70% light penetration.

4.3.2 Source of plant materials and cutting preparation

Stem cuttings were collected from mature, healthy rose-scented geranium plants grown at Essential Amatole Farms (around the Hogsback area of the Nkonkobe Municipality). Shoots of 30-40 cm long were cut from the stock plants in the early hours of the day (between 05:00 and 06:30) and were immediately placed in plastic bags in order to minimize water loss until they were taken to the working area. At the working area, under shade conditions, the braches were prepared into 10, 12, 14 and 16 cm long cuttings, and these were more or less of the same stem circumference (2 – 2.5 cm). All open leaves on the cuttings, except the ones at the topmost, were removed to reduce transpiration water loss and crowding (Hartmann et al., 2002).

4.3.3 Experimental layout and treatments

The experiment was laid out in a completely randomised design (CRD), each treatment combination replicated three times. During the planting process, the cuttings were grouped into 32 groups. A 4 × 4 × 2 factorial experiment (involving four stem lengths, four rooting hormones and two growing media as treatment combination) was set up. The stem lengths used were 10 cm (4 nodes), 12 cm (5 nodes), 14 cm (6 nodes) and 16 cm (7 nodes). At Essential Amathole farms, a stem cutting of 9-11 cm length, 3-5 nodes with stem circumference of 2-2.3 cm are used as propagation materials. Therefore, in this experiment a cutting length of 10 cm with 4-5 nodes and stem circumference of 2-2.3 cm were prepared as planting material which served as a control. For the purpose of this study, stem cutting length of less than 14 cm are considered as short cuttings and long cuttings would be stem cuttings from 14 cm up.

The rooting treatments comprised of (1) Dynaroot 1, (2) Dynaroot 2, (3) Dynaroot 3, and (4) Control (untreated with any hormone). The compositions of Dynaroot treatments are presented in Table 4.1. The basal (lower) 1cm of the stem cuttings were dipped in water and then into rooting hormone powder (Dynaroot 1, 2 or 3). The excess rooting powder was

tapped before planting in order to avoid yellowing of the powder in the plantlet (Hartmann and Kister, 1983; Araya, 2005). Then cuttings were then directly stack to (planted in) pre-wetted growing media combination filled in seedling trays, to a depth of 2 cm.

Table 4.1: Active ingredients in IBA rooting hormone used

Commercial name	Composition
Control	4-indole-3-butyric acid: 0g/kg
Dynaroot 1	4-indole-3-butyric acid: 1g/kg
Dynaroot 2	4-indole-3-butyric acid: 3g/kg
Dynaroot 3	4-indole-3-butyric acid: 8g/kg

Source: Efekto Trading (2006)

The two rooting media (combinations) used as were as follows:

1. Commercial rooting media- hygrotex,
2. Pine bark + hygrotex (1:1 v/v)

4.3.4 Plant culturing process

After planting the cuttings into the growing media, the cuttings were grown under greenhouse condition, placed on a bottom-heating bed under misting condition to hasten root initiation for the first three weeks. After three weeks, they were moved into a shade house for another five weeks for subsequent growth and hardening purpose under near prevailing field weather condition. In the shade house, the seedling trays were placed on 1-meter high metallic parallel bars to prevent fungal infection and facilitate drainage. Air temperature and relative humidity were measured with a portable thermo-hygrometer throughout the durations of the experiments. The plants were watered twice a day with overhead spray lines. A complete nutrition fertilizer was applied once a week for the duration of the experiment, where 150 ppm nitrogen of a balance fertilizer (20-10-12) was used.

4.3.5 Data collection and statistical analysis

Destructive data collection method was used. Starting from Week 3 after planting (AP), four cuttings were harvested from each replication of each treatment combination on a weekly basis until Week 8 AP, when the experiment was terminated (final sampling was done). At each sampling event, the following parameters were measured: root length, plant height, root and leaf number, and stem circumference. Thus, for root measurements, the growing media were washed out from the root system of plantlets, and the roots were separated from stem before data was recorded. These included, root number, length and root fresh mass. The root ability to hold rooting medium was determined by visual observation and rated on a 1-5 scale, representing **1**: very loose, not acceptable; **2**: loose, not acceptable; **3**: medium, marginally acceptable; **4**: tight, acceptable; and **5**: very tight, acceptable. The same procedure used by Rajapakse et al. (1996) on *Chrysanthemum spp.* cuttings was adopted. Where applicable, data collected were subjected to analysis of variance (ANOVA), using the JMP® Release 11.0.0 statistical software package (SAS, 2010). Where significant differences were established, treatment means were separated using the least significant difference (LSD) test at α level of 0.05.

4.4 RESULTS AND DISCUSSION

In the current research, analysis of variance showed that there was no significant interaction effect between factor A (stem cutting length) by factor B (rooting hormone) by factor C (growth media), as well as between factor A by factor B. Even though, statistically the was no significant effect on interaction between factor A (stem cutting length) and factor B (rooting hormone) on root holding ability (RHA). When data was determined by visual observation, interaction was observed and rated at 1-5 scale (Table 4.2). Therefore, the discussion will focus on the impact of the main factors. The effect of the main factors is presented from figure 4.1 to 4.12. Statistical analysis of data indicated that there is no significant difference amongst propagation media treatments (results not presented). Even though noticeable differences were observed among treatments, statistically, there were no significant differences detected due to propagation medium. Hygrotex was observed to perform slightly better than hygrotex + pine bark (1:1 v/v) on a number of roots, root length, leaf and shoots number. On the other hand, root fresh mass and stem circumference were relatively higher when hygrotex + pine bark (1:1 v/v) was used compared to hygrotex alone (results not published).

4.4.1 Effect of stem length on root production of rose-scented geranium cuttings

Root number

The results from the study indicated that there was an increasing trend in the mean root number with increase in cutting stem length throughout the experiment (Figure 4.1).The root number significantly improved with an increase in cutting length. However, the results also showed that there was no significance ($p > 0.05$) between root number of 14 cm and 16 cm cutting length (34.38 and 35.13) respectively. Whereas, the 16 cm cutting length had the highset number of roots as compared to other treatments. Janick (1986) reported that an important component of the capacity for a stem cuttings to root is the nutritional status of the

plant. It is therefore speculated that, the better performance of longer stems (14 and 16 cm) is due to an increase of stored carbohydrate with the maturity of the stem cuttings.

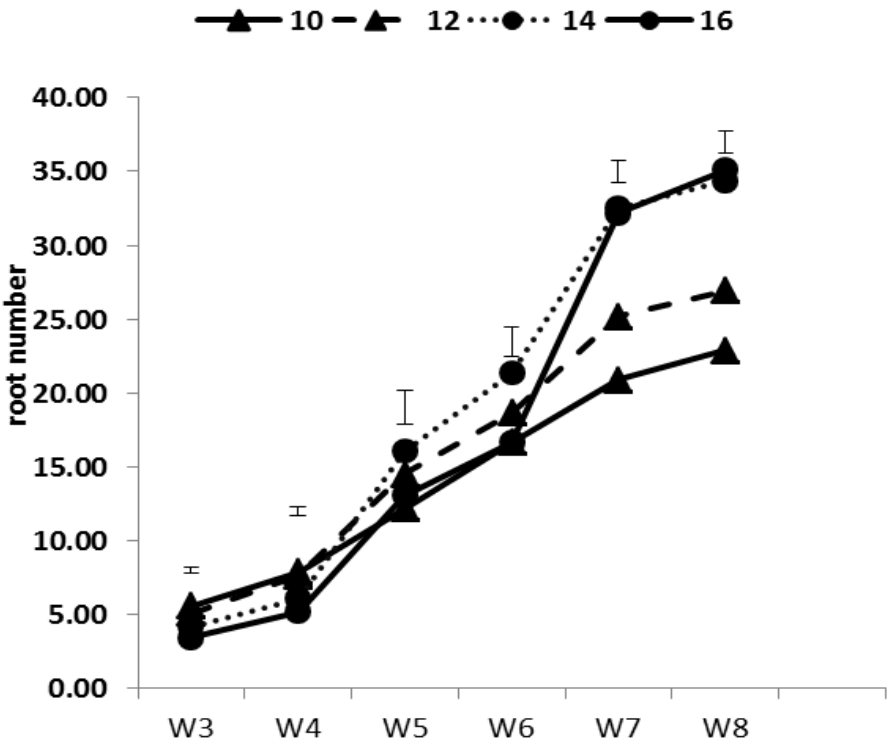

Figure 4.1: Effect of stem length on root number of rose-scented geranium cuttings. The vertical bars are LSD at α = 0.05

The results further showed that, the cutting length of 12 cm and 10 cm achieved the the lowest number of roots (26.92 and 22.88), even though 12 cm cutings length had signicantly ($p < 0.05$) higher root number than 10 cm cutting lenght. These results could be attributed to the lack of storage reserves and/or low photosythesis rate to produce sufficient roots. Contrary, the results of the present study are not in line with Wilson (1993) who investigated the effect of rooting on *Eucalyptus* globules Labill. spp. *Globules* stem cutting and it was found that shorter cutting stem length favoured rooting due to variation in stem size thickness. Similaly, Awan et al. (2012), reported that 15 cm cutting length had maximum number roots, followed by 20 cm cutting length, while 35 cm cutting length resulted in

minimum number of roots. However, the results of the current study are aligned with Ahkami et al. (2009), who found that, low carbohydrate levels in cuttings at the beginning of rooting limit the speed or intensity of subsequent adventitious root formation (ARF). According to Hegde (1988), the poor rooting of small size cutting was due to low stored carbohydrates in younger and immature stems.

Root length

Figure 4.2 shows that the root length was significantly affected by cutting stem length (at $p < 0.05$). It was observed that an increase in the stem cutting length resulted in an increase in root length of the plantlets.

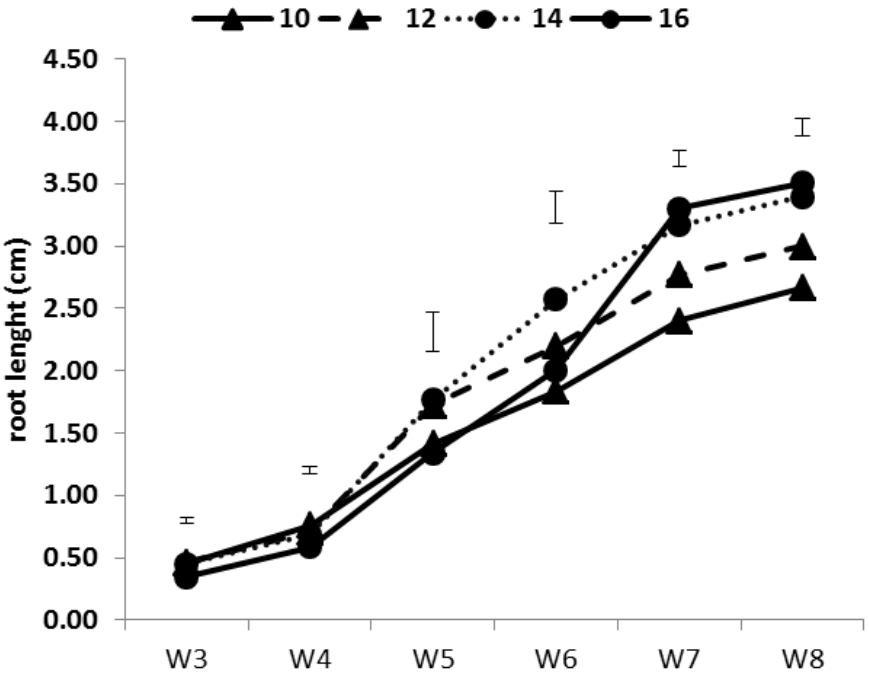

Figure 4.2: Effect of stem length on root length of rose-scented geranium cuttings. The vertical bars are LSD at α = 0.05.

Among the different lengths, the 16 cm and 14 cm long stem cuttings produced the longest roots (3.51 cm and 3.40 cm, respectively). However, visual observations showed that, the 16 cm cutting length had the longest root length as compared to 14 cm cutting length. Though, statistically there was no significance amongst these two cutting length treatments. The current results support the findings of Reinhard (2003) and Gopale et al. (2010) who reported that the more mature the plant stem, the easier it is to root, and the more easier it is to extends its root system in the growing media and effectively absorb nutrients and water.

The findings of the study highlighted that the length of roots per rooted cutting differed among the stem cutting lengths used. The 10 cm long stem cuttings produced the shortest root length (2.66 cm), and the second shortest was the 12 cm long cuttings (3.0 cm). Similar to the current results, Gopale et al. (2010) observed that reductions in cutting length resulted in reductions of roots of the plantlets. According to Good and Tukey (1966), poor performance of shorter stem cuttings is due to inadequate supply of nutrients accompanied by leaching of nutrients. In most studies on other species, root length was influence by cutting length of roots (Leakey 1992; Wang et al., 1997; Naidu et al., 2009).

Root fresh mass

Data regarding root fresh weight is presented (Figure 4.3). Fresh mass was affected by cutting lengths (10, 12, 14 and 16cm) applied as treatments used. The 14 cm cutting length produced the highest root weight (0.48 mg) which was significantly higher ($P<0.05$) than that of 16 cm and 12 cm length (0.46 mg and 0.46 mg), which then showed no significance difference amongst each other. On the other hand, cuttings of 10 cm length (0.40 mg) produced significantly lower root fresh mass.

According to Reinhard (2003), the rooting performance of stem cuttings varies with age, genotypes and physiological status of mother plant. Such characteristics are also amongst the reasons for good performance of the medium sized stem cuttings (14 cm) which gave the highest root fresh mass. The reason for producing high root fresh mass might be due to the fact that both 14 cm and 16 cm cutting lengths had contributed significantly better in most of

the root growth parameters such as, the number of roots and roots length. The vigorous rooting of the 14 cm and 16 cm stem length might have enabled the cuttings to absorb more nutrients and produce more and longer roots (Reuveni and Raviv, 1981; Leakey, 1982).

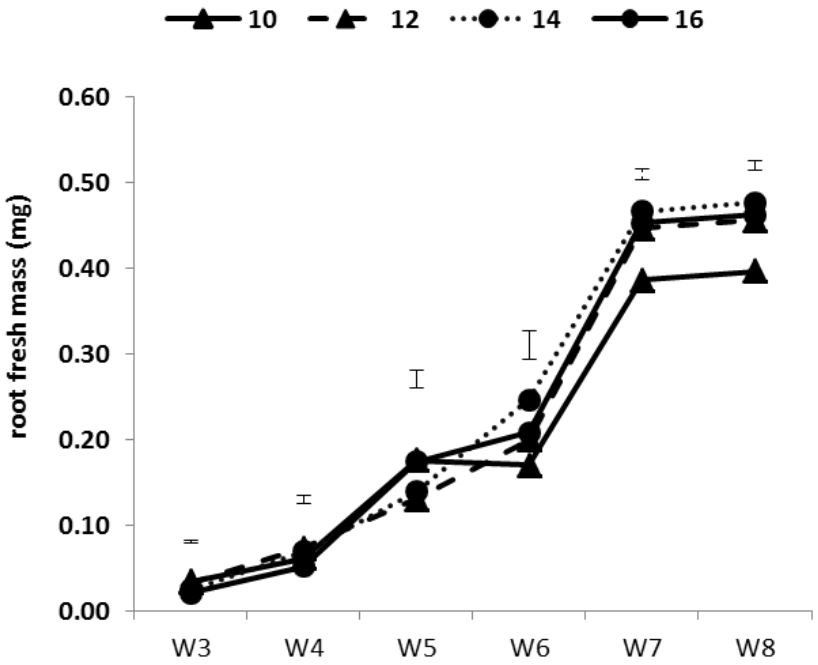

Figure 4.3: Effect of stem length on root fresh mass of rose-scented geranium cuttings. The vertical bars are LSD at $\alpha = 0.05$.

Stem circumference

Statistical analysis of the data of the conducted research indicated that there was a significant difference among cutting length, irrespective of the sampling date. Reductions in stem circumference were observed with reduction in cutting length (Figure 4.4). The thickest stem (3.1 cm) was found on cutting length measured 10 cm. This was followed by the 12 cm long cuttings (2.9 cm). However, the 14 cm and 16 cm stem lengths showed the smallest stem circumferences of 2.8 cm and 2.8 cm respectively. Even though, 14 cm and 16 cm cutting length had a greater area than 10 cm and 12 cm, but, these lengths could not maintain themselves due to higher transpiration. This may be attributed to the fact that juvenile tissues of certain plants tend to have higher phenols than their mature forms (Reuveni and Raviv, 1981; Leakey, 1982).

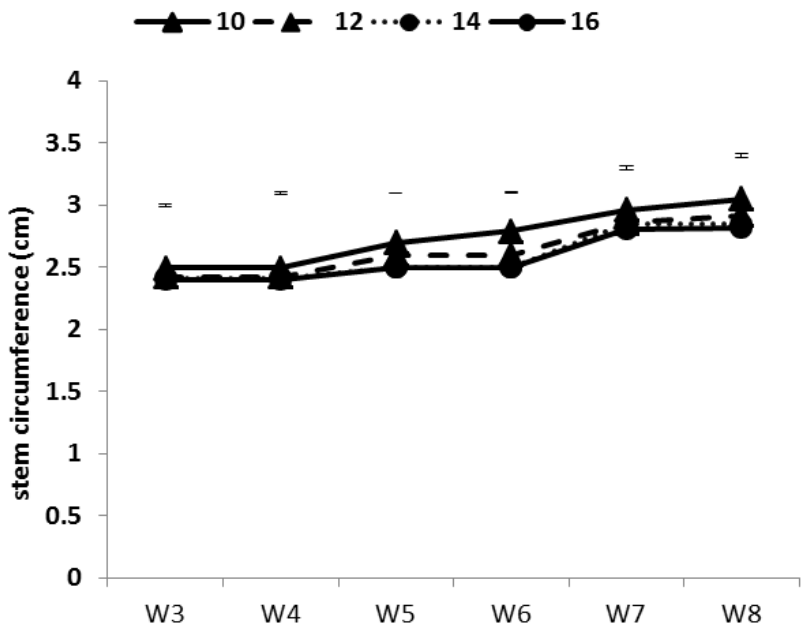

Figure 4.4: Effect of stem length on stem circumference of rose-scented geranium cuttings. The vertical bars are LSD at α = 0.05

According to Dole and Wilkins (1999) the success of stem cuttings in the majority of the ornamental plants depends on the physiological stage of the mother plant. The shorter cuttings are believed to have greater circumference when compared to longer cuttings. However, underperformance of large sized cuttings may be attributed to advanced age of the longer cuttings which could have led to stem lignification. As a result, the lignified stems stop (arrest) further expansion, resulting in thinner circumference (Kochhar et al., 2005). These observations are supported by Khan et al. (2006) who posit that the less mature a stem cutting, the easier it is to grow. This increase in stem circumference might be attributed to the fact that shorter length had less stress caused by higher transpiration from relatively smaller surface area. The author ascribed the better performance of thicker stem to an increase of stored carbohydrate with the diameter of the stem cuttings.

Shoot number

In case of cutting length, the collected data indicated a positive relationship between stem cutting length and shoot number (Figure 4.5). The maximum shoot number (4.00) was observed in 16cm cutting length followed by 14 cm cuttings (3.42) significantly (P< 0.05), whilst the lowest number of shoots (2.9) were recorded in 10 cm cutting length. Observations were made by the researcher that, with an increase in the cutting length there was also an increase in shoot number.

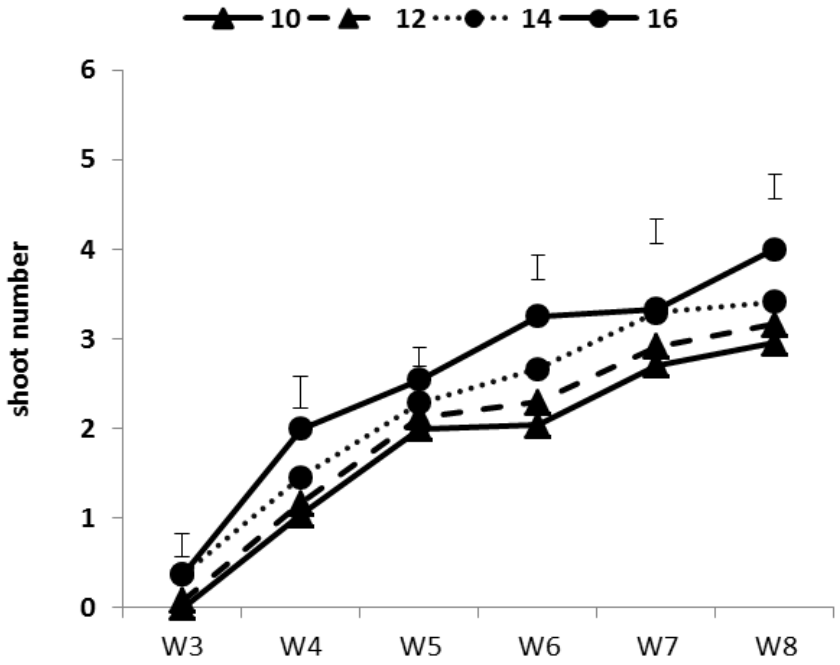

Figure 4.5: Effect of stem length on shoot number of rose-scented geranium cuttings. The vertical bars are LSD at α = 0.05.

This result may be due to the fact that, there was more area for shooting in 16 cm lengths as compared to 10 cm length. According to Hegde (1988), the poor performance of small size cutting in producing shoots, was because the cuttings were still under maturity and may be devoid of sufficient food material for induction of roots and shoots. Leakey and Mohammed (1985) on the other hand, reported that longer cuttings gave the best rooting and shoots as compared to shorter, which might be attributed to higher amount of stored food in large

(longer) cuttings. Similar results were reported by Awan et al. (2012), stating that there was the highest shoot number in 20 cm length as compared to 15 cm length on Olive cutting length.

Leaf number

Leaf number was significantly affected by differences in stem cutting length (Figure 4.6). Such that, the longer the cutting the more leaves were produced. Cutting length of 16 cm produced significantly higher number of leaves (11, 21), while the 12 cm stem length and 10 cm gave the lowest number of leaves (9.04 and 8.08, respectively).

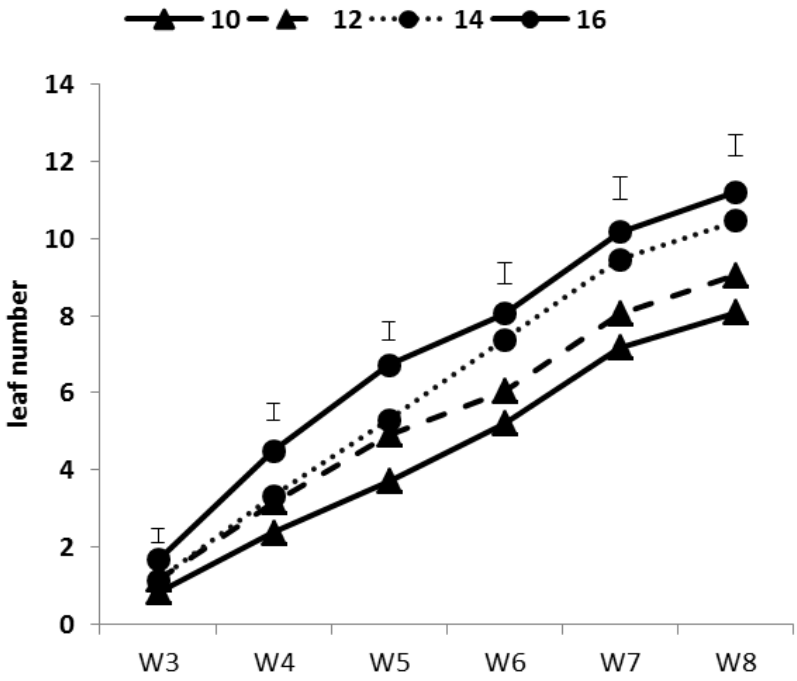

Figure 4.6: Effect of stem length on leaf number of root length of rose-scented geranium cuttings. The vertical bars are LSD at α = 0.05.

Accordingly, Hegde (1988) ascribed the higher performance of mature cuttings to maturity of the cuttings. Reason being, the more mature stems could have the advantage of having sufficient stored food material and vice versa. The author observed that longer cuttings had more leaf buds as compared to shorter ones. Geneve and Kester (1991) and Awan et al.

(2012), are in line with the author, that the presence of buds is also known to be powerful sources of auxins, which promote root and number of leaf initials. As a result, in the previous section observations were made that cutting length of 16 cm had the highest number of roots and longest root length which might have complemented the better vegetative growth in these cutting lengths (14 cm and 16 cm).

4.4.2 Effect of rooting hormone on root production of rose-scented geranium cuttings

Root number

Effect of rooting hormone on number of roots produced on rose-scented geranium is presented on Figure 4.7. There were significant differences ($P < 0.05$) between the performances of different rooting hormones in root number. Cuttings rooted better (33.46) when Dynaroot 3 was used as compared to all other treatments. Dynaroot 2 ranked second highest in root number (31.04), followed by the Dynaroot 1 (28.33). The lowest root number was recorded for the control (26.4).

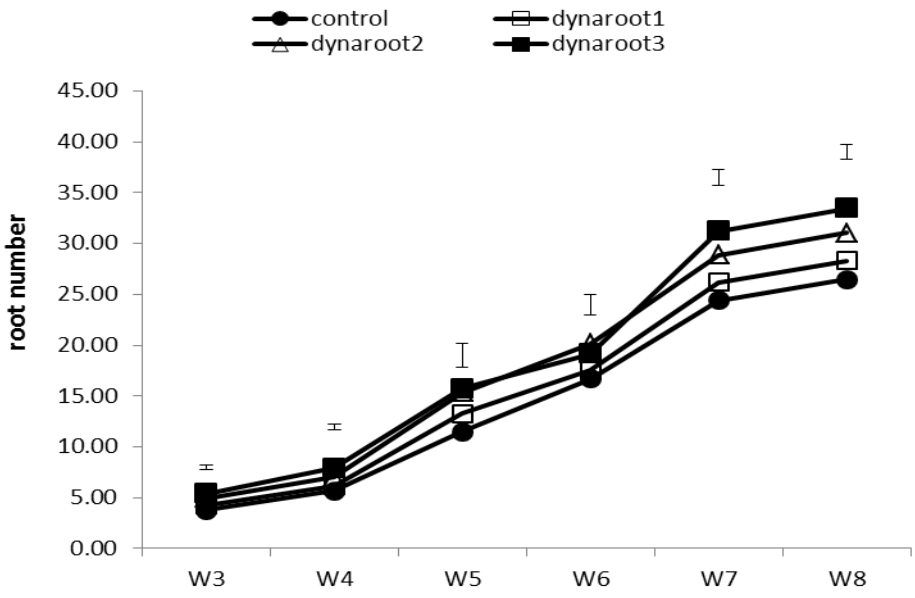

Figure 4.7: Effect of rooting hormone on root number of rose-scented geranium cuttings. The vertical bars are LSD at $\alpha = 0.05$.

The difference between treatments showed a slow increase with number of weeks after planting, especially between Week 3 and Week 6 as compared to those in Week 7 and Week 8. According to Blythe et al. (2007), the main aim of treating cuttings with auxin is to stimulate root formation, increase overall rooting percentage, and increase the number, quality and uniformity of rooting. Positive effects of hormones (IBA) were also reported on *Aloysia triphylla* (L' Hérit) (Stafanini, 2004), *Dovyali caffra* (Kei apple) (Hae and Funnah, 2011) and *Warbugia ugandensis*, (Akwatulira et al., 2011).

Root length as affected by rooting hormone

Cuttings treated with Dynaroot 3 produced the longest roots (3.54 cm), followed by Dynaroot 2 (3.24 cm) (Figure 4.8), while control recorded the shortest roots (2.78 cm). These results are in line with findings of Hae and Funnah (2011) in which application of Dynaroot 3 produced the highest rooting length on stem cuttings of *Dovyalis caffra*. This could be due to the effect of auxins that have been reported to enhance rooting through the translocation of carbohydrates and other nutrients to the rooting zone (Druege et al., 2004). On the contrary, Ofori et al (1996) and Khan, et al., (2011) observed no significant effect when cuttings were treated with IBA rooting hormone on *Milicea excels* and on tomato cuttings, respectively. With reference to the composition of rooting enhancers used in this experiment, it is clear that the only difference between control, Dynaroot 1, Dynaroot 2 and Dynaroot 3 is the concentration of IBA (Table 4.1). When comparisons are made between Dynaroot 1, Dynaroot 2 and Dynaroot 3, there was evidence of expansion in root length with an increasing concentration of IBA.

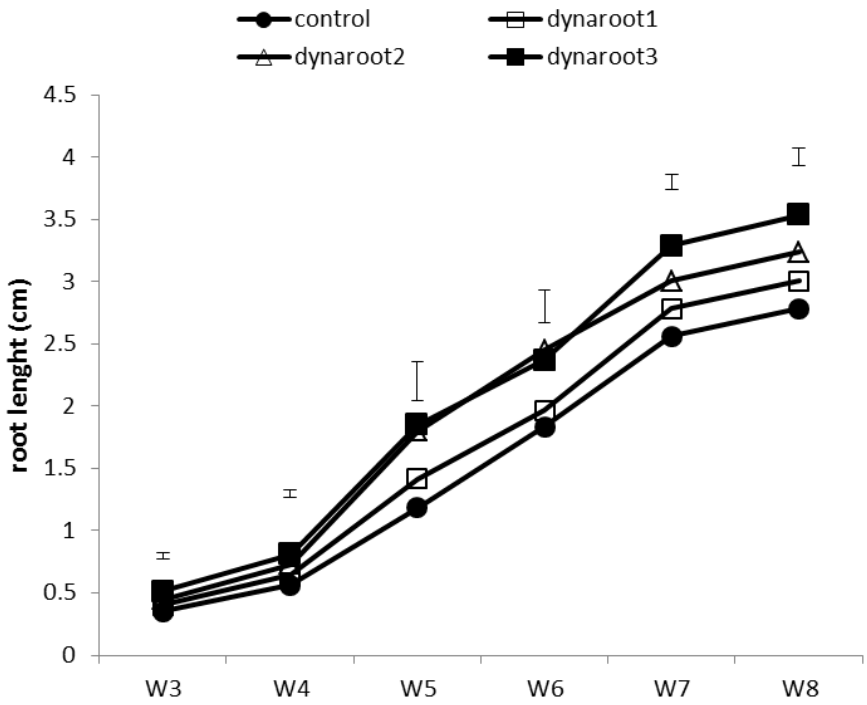

Figure 4.8: Effect of rooting hormone on root length of rose-scented geranium cuttings. The vertical bars are LSD at α = 0.05.

Root fresh mass as affected by rooting hormone

In general, there was positive relationship between root fresh weight and IBA concentration (Figure 4.9). The highest root fresh mass was recorded on Dynaroot 3 (0.59 mg) treated cuttings. There were no significant differences ($P<0.05$) between cuttings treated with Dynaroot 1 (0.42 mg), Dynaroot 2 (0.48 mg) in root fresh weight. The lowest root weight was observed in the zero IBA concentration (control) treatment. The maximum root fresh mass on cuttings treated with Dynaroot 3 was because both the root number and root length were high when cuttings were treated with Dynaroot 3 as compared to all other treatments. It may be inferred therefore, that, this may have attributed to the high root mass. Similar results were also reported by Hae and Funnah (2011) and Akwatulira et al. (2011). Contradictory results were reported by Khan et al., (2011) on their study of tomato cuttings (*Solanum esculentus* L.), which indicated that the maximum root fresh mass was obtained when control was used rather than any rooting enhancer used.

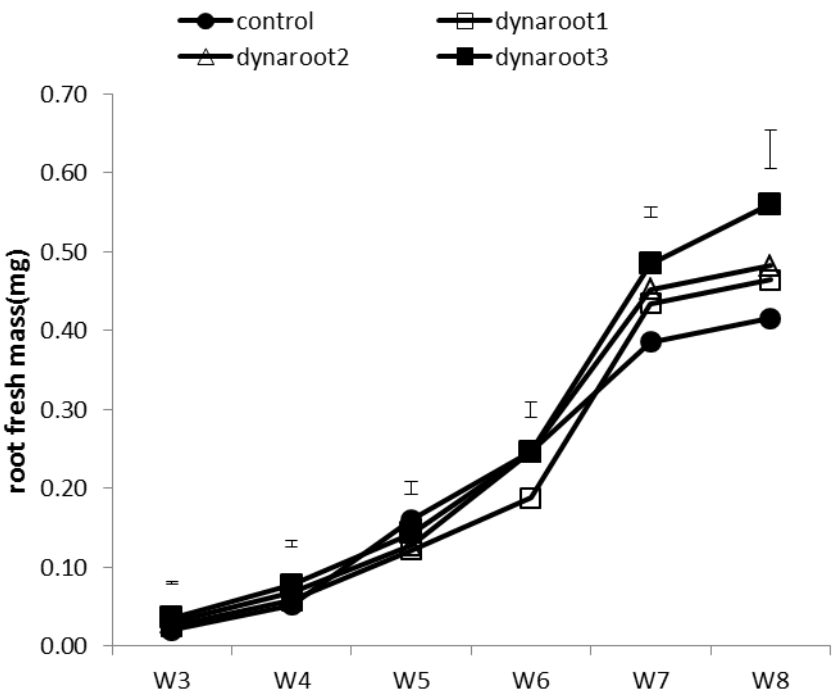

Figure 4.9: Effect of rooting hormone on root fresh mass of rose-scented geranium cuttings. The vertical bars are LSD at α = 0.05.

Leaf number as affected by rooting hormone

The results of the study showed that the leaf number positively responded to IBA concentrations. Significant differences ($P<0.05$) were observed amongst IBA hormone concentrations on leaf number (Figure 4.10). Mean leaf number of cuttings treated with Dynaroot 3 (11.08), Dynaroot 2 (10.04), Dynaroot 1 (9.25) and the control (8.42) showed consistent trend. This trend suggests that when rooting enhancers are used in vegetative propagation of rose-scented geranium, to promote leaf number, Dynaroot 3, IBA hormone concentration should be used. Results obtained in this study also support findings of a study reported by Mensèn et al. (1997). Blythe et al. (2004) also obtained similar results on stem cuttings of *Ficus benjamina* and *Gardenia augusta*. Thus, better leaf formation as a result of auxin treatment may be due to accumulation of metabolites at the site of application, synthesis of new protein, callus formation, cell division and cell enlargement.

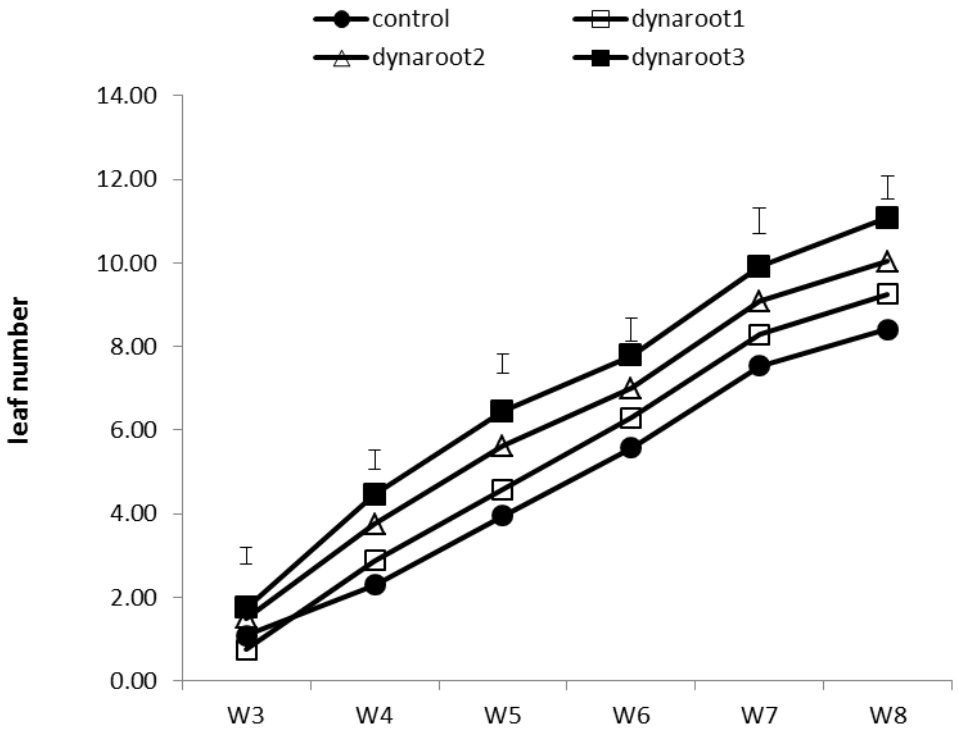

Figure 4.10: Effect of rooting hormone on leaf number of rose-scented geranium cuttings. The vertical bars are LSD at $\alpha = 0.05$.

Stem circumference as affected by rooting hormone

Statistical analysis of the data revealed that the stem circumference was significantly affected by different IBA hormone concentration used, even though significant differences were clearly observed on Week 7 and Week 8 of the experiment (Figure 4.11). Increases in stem thickness were observed with reduction in rooting hormone concentration. Control gave significantly the thickest stem (3.008) followed by the Dynaroot 1 (2.925) which was significantly lower than control, while Dynaroot 2 (2.854) and Dynaroot 3 (2.842) recorded the smallest stem circumference and there was no significance amongst IBA rooting treatments. The decline in stem circumference with increases IBA hormone concentration suggests that IBA hormone inhibits stem thickness of rose-scented geranium. The inhibition of stem thickening in rose-scented geranium cuttings as a response to increase in IBA concentrations has also been recorded in other species, such as *Millisa excelsa* and *Cordia*

alliodora, as reported by Ofori et al. (1996) and (Mesen, 1993), respectively. Such phenomenon may arise as a result of IBA-induced basipetal transport of assimilates, with progressively enhancing of sink strength of the root with increases in IBA concentration (Hartmann et al., 1990). Furthermore, the author observed that in order to maximize stem circumference during vegetative propagation of rose-scented geranium, cuttings should be treated with no-IBA hormone (control).

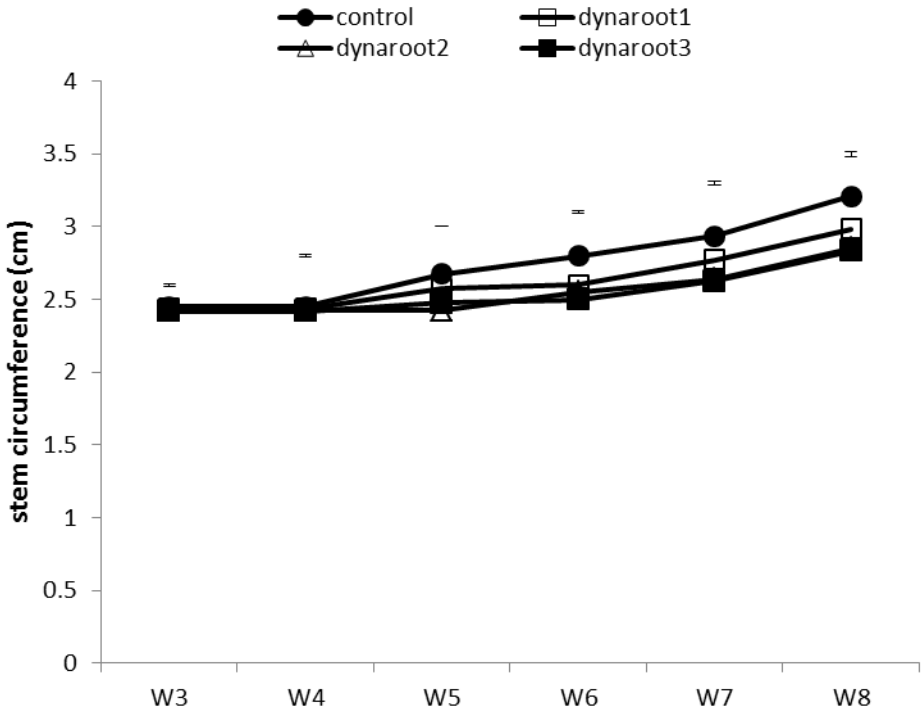

Figure 4.11: Effect of rooting hormone on stem circumference of rose-scented geranium cuttings. The vertical bars are LSD at α = 0.05.

Shoot number as affected by rooting hormone

The rooting hormone had a significant effect ($P < 0.05$) on number of shoots per rooted cutting (Figure 4.12). With an increase in IBA concentration, the number of shoots tended to decrease on rose-scented geranium cuttings. In general, Control (cutting with no hormone treatment) produced the highest shoot number (3.79), followed by Dynaroot 1 (3.54). The lowest number of shoots was observed in Dynaroot 2 (3.21) and Dynaroot 3 (3.00). Whereas,

cutting treated with Dynaroot 3 performed exceptionally lower than Dynaroot 2. The results therefore imply that the species might not really require application of more exogenous hormone to produce shoots. On the contrary, when Akwatulira et al. (2011) used *Warburgia ugandensis* stem cuttings, shoot success increased with increase in concentration of IBA.

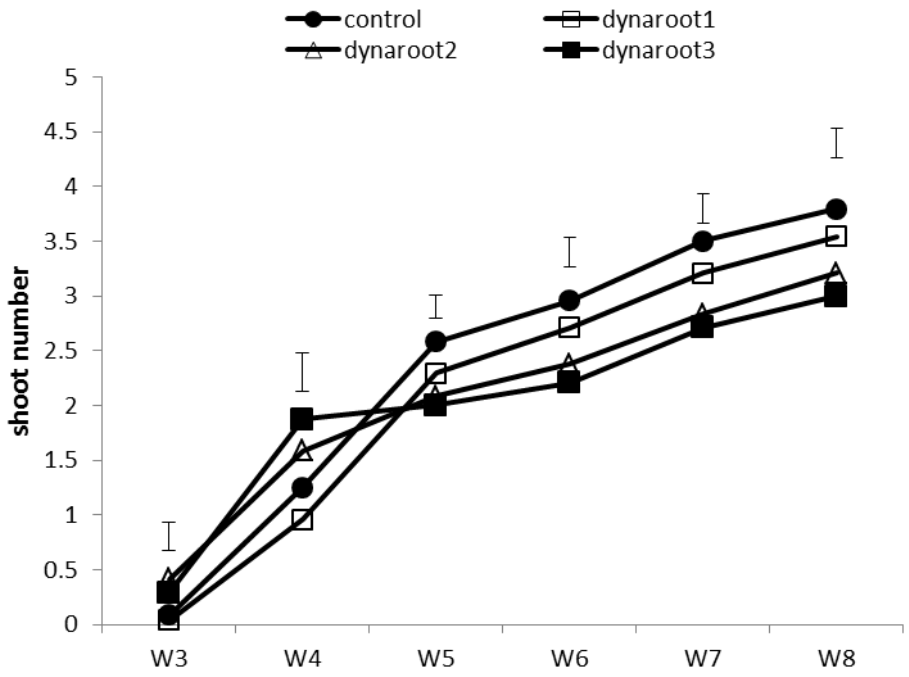

Figure 4.12: Effect of rooting hormone on shoot number of rose-scented geranium cuttings. The vertical bars are LSD at α = 0.05.

4.4.3 Interaction effect of stem length and rooting hormone on root holding ability (RHA) on growing media

Data recorded from this research showed that stem cutting length and rooting hormone had an effect on root holding ability on medium (Table 4.2). This suggests that both of these factors improve root holding ability with time in stem cutting propagation of rose-scented geranium. At Week 7 when Control (no-hormone) and Dynaroot 1,were used as treatments, both 10 cm and 12 cm cutting length gave 3 (medium, marginally acceptable) root holding ability, while 14 cm and 16 cm cutting length recorded 4 (tight, acceptable) root holding ability.

Table 4.2: Influence of cutting stem length and rooting hormone on root ability to hold rooting

*** Root holding ability (RHA) on media**							
		Weeks after planting					
Cutting stem length	**Hormone**	3	4	5	6	7	8
10 cm	Control (no-hormone)	1	2	2	3	3	4
	Dynaroot 1	1	2	2	3	3	4
	Dynaroot 2	1	2	2	3	4	5
	Dynaroot 3	1	2	2	3	4	4
12 cm	Control (no-hormone)	1	2	2	3	3	4
	Dynaroot 1	1	2	2	3	3	4
	Dynaroot 2	1	2	2	3	4	4
	Dynaroot 3	1	2	2	3	4	4
14 cm	Control (no-hormone)	1	2	2	3	4	4
	Dynaroot 1	1	2	3	4	4	5
	Dynaroot 2	1	2	3	4	5	5
	Dynaroot 3	1	2	3	4	5	5
16 cm	Control (no-hormone)	1	2	2	3	4	4
	Dynaroot 1	1	2	2	3	4	4
	Dynaroot 2	1	2	2	3	4	5
	Dynaroot 3	1	2	3	4	4	5

*** Root ability to hold medium (RHA) [determined by visual observation and rated at 1-5 scale: 1 (very loose, not acceptable); 2 (loose, not acceptable); 3 (medium, marginally acceptable); 4 (tight, acceptable); 5 (very tight, acceptable).**

Application of Dynaroot 2 and Dynaroot 3 on 14 cm cutting length resulted on 5 (very tight, acceptable) root holding ability. While, when the same treatments were applied on 10 cm, 12 cm and 16 cm cutting length, root holding ability recorded 4 (tight, acceptable). At Week 8, cuttings of 10 cm length gave 4 (tight, acceptable) root holding ability when Control, Dynaroot 1 and Dynaroot 3 were used. While, Dynaroot 2 treated cuttings recorded 5 (very tight, acceptable) root holding ability. Irrespective of the rooting hormone concentration used, root holding ability recorded 4 (tight, acceptable) root hold ability when 12 cm cuttings length were used.

The lowest root holding ability that was recorded on 10 cm and 12 cm long cuttings, irrespective of the hormone concentration used, can be ascribed to less maturity status accompanied by insufficient stored food material for root initiation. Hegde (1988), Good and Tukey (1966) as well as Husen and Pal (2007), in their research studies reported that poor performance of shorter stem cuttings is due to inadequate supply of nutrients and leaching of nutrients in shorter cuttings.

Cuttings of 14 cm stem length responded positively on IBA hormone application and recorded 5 (very tight, acceptable) root holding ability and cuttings with no-hormone (control) gave 4 (tight, acceptable) root holding ability. Cuttings of 16 cm length recorded maximum 5 (very tight, acceptable) root holding ability when cuttings were treated with Dynaroot 2 and Dynaroot 3. While control and Dynaroot 1 hormone gave 4 (tight, acceptable) root holding ability. The findings of the current study also observed that, IBA rooting hormone concentration played an important role in increasing root holding ability. For instance, on 14 cm and 16 cm cuttings length, root holding ability increased with an increase in IBA rooting hormone concentration. The reason for the best performance of the 14 and 16 cm long cuttings could be attributed to the production of high number of roots recorded for this cutting length. This could enhance nutrient absorption rate, which in turn results in improved root holding ability. The plantlets of this length also had maximum root length that could help the plantlets to thoroughly explore the growing media in the seedling trays. This can enhance absorption of the available nutrients, thereby increases root holding ability. The current results are in line with the findings of Reinhard (2003) and Gopale et al. (2010) that the more mature the cuttings, the easier to root. These results are also in agreement with those of Moon et al. (1991), Puri and Vermat (1996), Dole and Wilkins (1999), Husen (2004) as well as Awan et al. (2012).

The results of the study suggest that, with the use of IBA rooting hormone on 10 cm and 12 cm length and with or without rooting hormone on 14 cm and 16 cm cutting length, rose-scented geranium can be ready for transplanting on Week 7 after planting because of their tight root holding ability on growing media. It could be even earlier for cuttings of 14 cm length treated with Dynaroot 2, which can be ready for transplanting on Week 6 after establishment.

4.5 CONCLUSION AND RECOMMENDATION

Based on the results of the current study, it is concluded that stem cuttings with 14 cm and 16 cm length perform better for quicker root initiation and development, thereby ensuring survival of plantlets. While 16 cm cutting length were consistence in giving maximum leaves and shoots. Furthermore, 14 cm long stem cutting with Dynaroot 2 were reported do better in root holding ability on growing medium compared to all other cutting stem length treatments.

Results also showed that rooting hormone (IBA) enhances success of stem cutting propagation of rose-scented geranium. Cuttings treated with Dynaroot 3 and Dynaroot 2 showed better rooting, root fresh weight and leaf number while those treated with control (no-hormone) favoured shoot number and stem circumference. In addition, the results of the current experiment indicated that combination of long stem cuttings of 14 and 16 cm, and Dynaroot 3 or Dynaroot 2 improved rooting and the plantlet quality. Therefore, it is recommended that rose-scented geranium should be propagated through the combination of 14 cm cuttings length and treated with Dynaroot 2 of IBA rooting hormone.

Regarding impact of rooting media, Hygrotex consistently performed better, although its performances did not significantly differ from hygrotex + pine bark (1:1 v/v) on propagation of rose-scented geranium stem cuttings. Thus, depending on cost and availability, Hygrotex alone or mixed pine bark can be used as rooting media for rose-scent geranium stem cutting propagation. Both hygrotex and hygrotex + pine bark (1:1 v/v) are the best growing media for root formation and growth of rose-scented geranium, though hygrotex alone is less economical.

CHAPTER 5
EFFECT OF HEALING PERIOD ON ROOTING OF ROSE-SCENTED GERANIUM STEM CUTTINGS

5.1 ABSTRACT

Rose-scented geranium is both an essential oil and an economical plant species. Despite its usefulness in the perfume industry, adequate attention has not been given in improving its propagation and early transplanting of quality seedlings. Therefore, this study investigated the effect of wound healing period as well as IBA rooting hormone in the development of adventurous roots from stem cuttings and producing quality seedlings of rose-scented geranium. The experiment was conducted to assess the effect of different times of wound healing period and varied concentrations of IBA hormone on a 4 x 4 factorial experiment laid out in a randomised complete block design (RCBD) with three replicates. The treatments consisted of four groups of healing duration intervals of 24 hours (Days 0, Day 1, Day 2 and Day 3) and four rooting hormones (auxins, types of IBA) [(**1**) Dynaroot (1 – 1g/kg), (**2**) Dynaroot (2- 3g/kg), (**3**) Dynaroot (3-8g/kg) and (**4**) control (untreated with hormone)]. Cuttings were assessed on the following, root number; root length; root fresh mass; plant height (measured with a ruler); leaf number (counting); shoot number and stem circumference. To measurements the roots, the soil was removed from the rooted plantlets, their roots washed and separated from the cuttings before they were subjected to the root number, length and root fresh mass measurements. The root ability to hold medium was determined by visual observation and rated at 1-5 scale: **1** (very loose, not acceptable); **2** (loose, not acceptable); **3** (medium, marginally acceptable); **4** (tight, acceptable); **5** (very tight, acceptable). Where necessary, the data was subjected to Analysis of variance (ANOVA) and LSD at 5% probability level was used to compare the different means. The results obtained from the study revealed that rose-scented geranium had maximum number of roots when planted in Day 2 of the healing period and also resulted in maximum root holding ability. Longest roots were found when cuttings were planted on Day 3 followed by Day 2 of the healing period. While, Day 0 cuttings showed good response for stem circumference and shoot number. Due to its effective rooting and maximum root ability on Day 2 of the healing period, it is therefore, recommended that rose-scented can be propagated using cuttings that

have enough time to heal the wound that is Day 2 of the healing period. Increasing IBA hormone concentration up to Dynaroot 3 showed good response to rooting and other arial parameters except for stem circumference which was favoured by application of control. Therefore, it may be concluded that the propagation of rose-scented geranium requires wound healing period of about two days in room temperature and application of Dynaroot 3 IBA hormone before sucking cuttings in growing medium. Instead of Dynaroot 3, Dynaroot 2 can also be used because it is less economical and they all have similar effects on cuttings that have been healing for two days.

Keywords: Dynaroot; indole butyric acid (IBA); rooting hormone; rose-scented geranium; stem cuttings; wound healing period

5.2 INTRODUCTION

Research shows that wound of the cuttings tend to be inflicted when the end of the stem is cut off. According to Cline (1983), wound healing period is the survival time that the cutting can take before it is stuck in the substrate. Cuttings are usually stuck in the substrate immediately after being cut back. This is done to prevent bruising on the cutting that is said to be caused by infection in the wound (Hartmann et al. 2002).

Wound-induced roots are the major type of adventitious roots formed in most stem cuttings. Once the stem (or shoot) is removed from the mother plant, a series of wound responses occur and de novo adventitious root regeneration proceeds (Hartmann et al. 2002). At the wounded sites, sealing off of the wound (protection from desiccation and pathogen entries) occurs by the production of suberized, protective cells. Cells begin to divide and a layer of parenchyma cells (callus) then forms at the wound site (Kelly, 2009). The use of auxin during adventitious rooting enhances the formation of callus as well as promoting the formation of roots. Cells in the vicinity of the vascular cambium and phloem (near the source of hormones and carbohydrates) begin to divide and initiate adventitious roots (Davis, 1988).

Massive losses in geranium production during vegetative propagation have been reported due to poor quality seedlings because of poor rooting of cuttings (Leakey, 1990; Hartmann et al., 1997; Mamba and Wahome, 2010). In literature, there are claims that healing the basal end of cuttings for several hours before sticking them to rooting medium helps to improve rooting in plant species like pine apple (De Klerk et al., 1999); Fig tree (Takagaki et al., 2000); cacti species Cylindropuntia (Cholla) and Opuntia (Prickly pear) (Kelly, 2009).

However, the wound healing period of rose-scented geranium cuttings is not known. Therefore, the aim of this study was to investigate the wound healing period of rose-scented geranium stem cuttings and the effect of IBA rooting hormone application.

5.3 MATERIALS AND METHODS

5.3.1 Experimental site and facilities

The study was carried out at the Essential Amatole Nursery, at the University of Fort Hare Research Farm, Alice Campus (located at 32^0 47′3″S, 26^0 50′43″ E, and an altitude of 519 m.a.s.l), from February 2015 to April 2015. For the first three weeks (to facilitate root induction), the experiment was carried out in mist conditions on bottom-heated beds in a greenhouse (with polycarbonate roofing of about 40% shading effect). For the rest of the experimental period, the plants were grown in shade-cloth houses, with light 70% light penetration.

5.3.2 Experimental design and treatments details

The experiment was a 4 x 4 factorial experiment laid out in a randomised complete block design (RCBD) to block direct effect of the sun through the widow and wasreplicated three times. The treatments consisted of four groups of healing duration intervals of 24 hours [(**1**) Days 0, (**2**) Day 1, (**3**) Day 2 and (**4**) Day 3)] and four rooting hormones (auxins, types of IBA) [(**1**) Dynaroot (1 – 1g/kg), (**2**) Dynaroot (2- 3g/kg), (**3**) Dynaroot (3-8g/kg) and (**4**)

Control (untreated with hormone)]. The compositions of hormones used as treatment are presented in **Table 5.1.**

Table 5.1: Active ingredients in rooting hormone used

Commercial name	Composition
Control	4-indole-3-butyric acid: 0g/kg
Dynaroot 1	4-indole-3-butyric acid: 1g/kg
Dynaroot 2	4-indole-3-butyric acid: 3g/kg
Dynaroot 3	4-indole-3-butyric acid: 8g/kg

Source: Efekto Trading (2006)

5.3.3 Source of plant materials and cutting preparation

Softwood cutting were collected from mature healthy rose-scented geranium plants. After detaching the cutting from the mother plants, stem length of 14 cm (preliminary study comparing four cuttings stem length showed that 14 cm was an effective length for vegetative propagation of rose-geranium) were arranged with an equal number of nodes and uniform diameter. A mixture of pine bark and hygrotex (ratio1:1 v/v) was used as a rooting media (because a preliminary study comparing seven media types showed no significant effect on hygrotex and hygrotex + pine bark 1:1 v/v). These cuttings were separated into four groups of healing duration as follows:

1) Day 0: Cuttings put into growing media without healing period (Control),
2) Day 1: Cuttings put into growing media after 24-hrs healing period,
3) Day 2: Cuttings put into growing media after 48-hrs healing period and
4) Day 3: Cuttings put into growing media after 72-hrs healing period.

These four groups were further subdivided into four subgroups of rooting hormone. In the hormone application process, the basal ends of the cuttings were dipped in water then in the powder hormone, Dynaroot 1, 2, 3 of indole-3-butyric acid and untreated (control) plants were dipped in water only using a quick dip method for 1 minute before weighing them.

As a way to monitor growth progress, initial fresh mass was taken from all the cuttings before applying treatments. After taking fresh mass, the cuttings were exposed to the room temperature and dried up to heal the wound until it was stuck in the rooting media (Takagaki et al., 2000). The cuttings assigned to the healing period treatments were weighed again immediately before stacking the cuttings in the rooting media. The reason for weighing these stem cuttings was to obtain the amount of water loss during the healing period.

5.3.4 Plant culturing process

During the period of the experiment, the cuttings were grown under greenhouse conditions for three weeks, placed on a bottom-heating bed to hasten root initiation. After three weeks, the plantlets were moved into a shade-cloth house for another five weeks to promote further rooting and hardening. In the shade-cloth house, the seedling trays were placed on 1-meter high metallic parallel bars to prevent fungal infection and facilitate drainage. Throughout the experimental period, the temperature of the experimental site was measured using a thermograph. The measured weekly mean minimum and maximum temperatures were 23 ^{0}C to 30 ^{0}C and the average relative humidity was 81% during the experiment (February to April 2015). Air temperature and relative humidity were measured with a portable thermo-hygrometer throughout the durations of the experiments. Watering was done twice a day with a knapsack sprayer.

5.3.5 Data collection and statistical analysis

Starting from Day seven after planting, distractive sampling was done on weekly basis. At each sampling event, five cuttings were harvested from observation unit (50-socket trays). The following parameters were measured: root number, shoots and leaf number (counting); root length (measured with a ruler); stem circumference (measured with calliper). When measuring root growth parameters, the soil was removed from the rooted plantlets, and then the roots separated from the shoots before they were subjected to the measurements. The root

ability to hold medium was determined by visual observation and rated at 1-5 scale: **1** (very loose, not acceptable); **2** (loose, not acceptable); **3** (medium, marginally acceptable); **4** (tight, acceptable); **5** (very tight, acceptable). The same procedure used by Rajapakse et al., (1996) on *Chrysanthemum* cuttings was adopted. The data was subjected to Analysis of Variance (ANOVA) using the General Linear Model of SAS Programme (Statistical package version) (SAS Institute Inc. 1990). Where ANOVA detected significant difference, means were compared using Tukey's test, at 5% level of significance.

5.4 RESULTS AND DISCUSSION

The result from the study indicated that the interaction effect between factor A (healing period) and factor B (rooting hormone) was reported to be significant on leaf number and root fresh mass only (Table 5.2). Other parameters showed no significance effect on interaction of factor A by factor B. As a result, the main factors were discussed separately and the results are presented from figure 5.1 to figure 5.9. The effect between factor A and factor B on root holding ability (RHA) was determined by visual observation and rated at 1-5 scale and presented in table 5.3.

5.4.1 Root number of cuttings as affected by healing period and rooting hormones

There was no interaction between healing period and rooting hormones on the number of roots of rose-scented geranium cuttings. The results of the experiment indicated that the root number was significantly affected by healing period throughout the experimental period (Figure 5.1). At Week 3 and Week 4 after establishment, the number of roots was the highest on cuttings planted on Day 0 (control) followed by Day 1 (24 hours healing). Meanwhile, the lowest root number was recorded on cuttings planted on Day 2 and Day 3 of the healing period with no significant difference between each other.

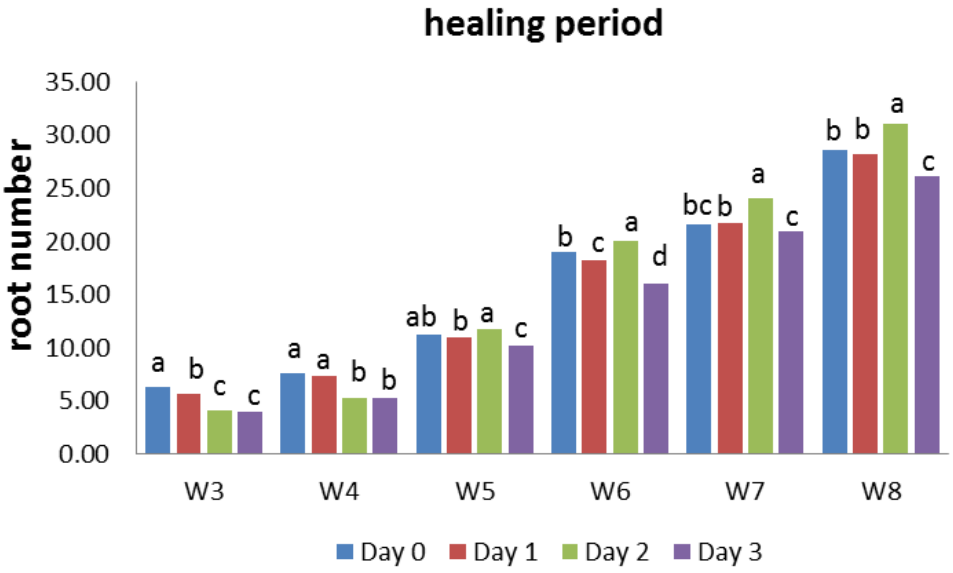

Figure 5.1: Effect of healing period on root number of rose-scented geranium cuttings. Bar graphs with the same letters in the same week are not significantly different from each other at α = 0.05 according to Tukey.

The research findings indicated that, as the rooting duration advance (from Week 5 to Week 8), the trend of root number also changed. For instance, in Week 8 after establishment, the cuttings planted on Day 2 of the healing period had significantly the highest number of roots (31.08) while, the second best was recorded for cuttings planted on Day 0 (28.58) and Day 1 (28.25) of the healing period and these had no significant difference amongst each other. Cuttings planted on Day 3 (26.17) of the wound healing period recoded the lowest root number of cutting which was different from the rest of the treatments. The main reason for the maximum number of roots on cuttings planted on Day 2 of the healing period was that the wound had enough time to heal the cut end such that the callus was already forming (Nolte, 2003). These finding suggests that, delayed planting (wound healing period, up to 48 hours) of some cuttings prior to sticking in rooting medium may be beneficial for increased rooting performance as compared to those cuttings that heal in the growing medium (Muralidhar et al., 2013).

Rooting hormone also had a significant effect on root number, especially on Week 4 to Week 8 after establishment (Figure 5.2). All cuttings treated with hormone showed greater root number than those cuttings with no hormone (control). Even though at week 7 and 8 there

were no statistical differences between cuttings treated with hormone, Dynaroot 3 treated cuttings (4.125) tended to have more roots than Dynaroot 1 (4.123) and Dynaroot 2 (4.092), respectively.

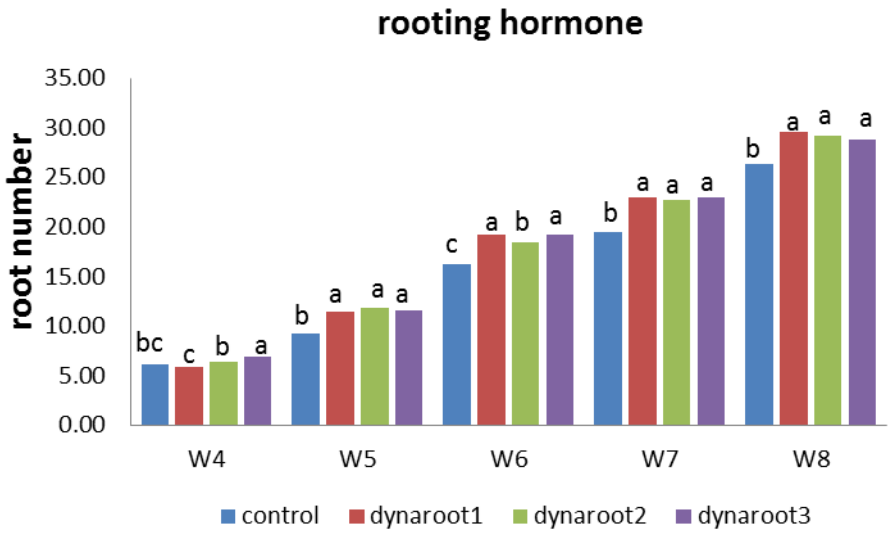

Figure 5.2: Effect of rooting hormone on root number of rose-scented geranium cutting. Bar graphs with the same letters in the same week are not significantly different from each other at α = 0.05 according to Tukey.

All cuttings treated with zero-rooting hormone (control) (3.667), root number was found to be significantly lower as compared to cuttings treated with hormone. These results support a research results reported by Mensèn et al. (1997). Similarly, Douglas (2008) concluded that the use of hormones promotes more root growth within a short period of time. Al- Barazi and Schwabe (1982) also reported that treating cuttings with auxins increased the percentage of rooting, root initiation, root number, as well as uniformity of roots in adult *Pistacia vera*.

5.4.2 Root length as affected by healing period and rooting hormones

The root length responded positively to different wound healing period tested (Fig.5.3). The results showed similar trends as the root number. At Week 3 to Week 5 after planting, mean root length was at its highest on cuttings planted on both Day 0 and Day 1 of the healing

period. On the other hand, cuttings planted on Day 2 and Day 3 had poor root length with no statistical difference between them.

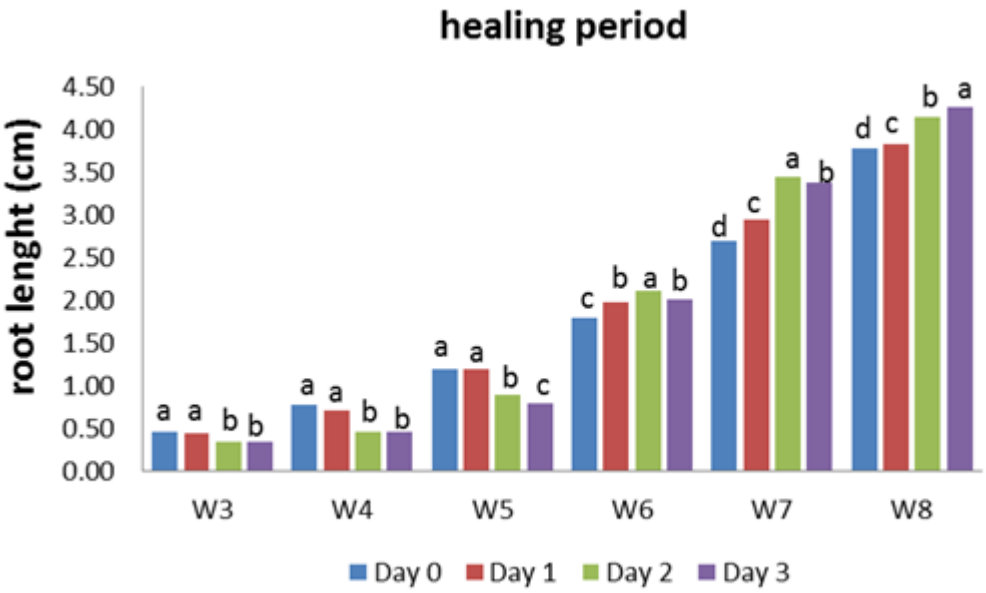

Figure 5.3: Effect of healing period on root length of rose-scented geranium cuttings. Bar graphs with the same letters in the same week are not significantly different from each other at α = 0.05 according to Tukey.

On the other hand, the results reversed after Week 6 showed that the greater root length increased with healing duration. As a result at Week 6 to Week 7 of the experiment, the greatest root length was found in the cuttings planted on Day 2 of the healing period, followed by cuttings planted in Day 3 of the healing period. Whereas the lowest root length was recorded on Day 0 of the healing period. Complimentary results were recorded at weeks 8 of the experiment; where cuttings planted on Day 3 of the healing period were found to give the longest root length and these were followed by cuttings planted on Day 2 of the healing period, and the lowest in Day 0 of the healing period. According to Cline (1983) when time for wound healing increased the end cut surface heal until the soft inner tissue calluses over, as evidenced by an increase in rooting and root elongation. Similar results were reported by

De Klerk et al., (1999) and Trail, 2001 on pineapple, where rooting was optimized by leaving the detached crown (tops) on the field for few days to allow the wound to heal and dry before planting.

In addition, the results of the study showed that the rooting hormone affected root length significantly as indicated in figure 5.4. At Week 3 to Week 4, cuttings treated with Dynaroot 3 and Dynaroot 2 had the greatest root length and had no significance amongst each other. The lowest root length was found in control and Dynaroot 1. This indicates that a decrease in hormone concentration may reduce length of the root. This is in agreement with the work of Memon et al (2013) who indicated that, the length of roots increased with the increasing concentration of NAA.

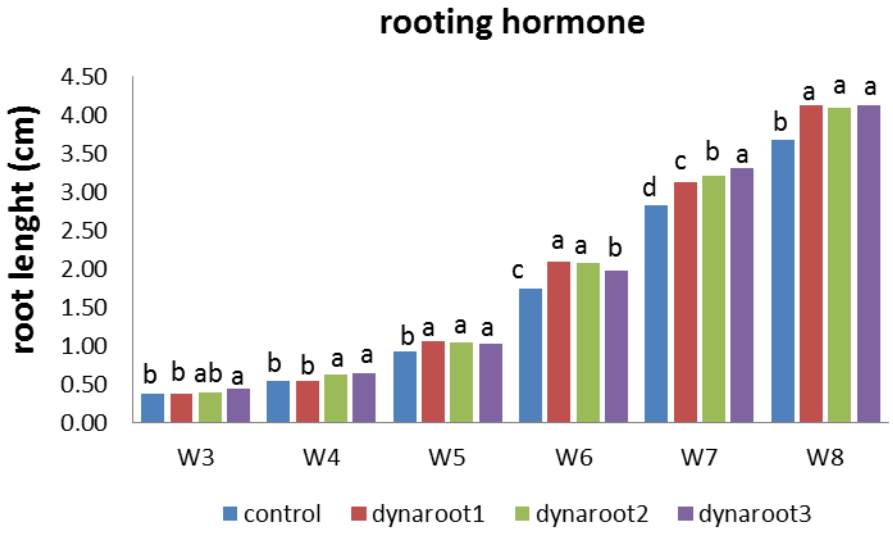

Figure 5.4: Effect of rooting hormone on root length of rose-scented geranium cuttings. Bar graphs with the same letters in the same week are not significantly different from each other at $\alpha = 0.05$ according to Tukey.

The emerging data also highlighted that, at Week 8 when cuttings were ready for transplanting, all cuttings treated with hormone performed significantly better than the control. Though statistically they showed no difference, cuttings treated with Dynaroot 3 (4.125) were observed to have a slightly greater root length followed by Dynaroot 2 (4.123) and Dynaroot 1 (4.092). This implies that treating stem cuttings with auxins can increase the root initiation, root length and number of roots. Even then, application of optimal hormone

concentration is very important for successful rooting of cuttings (Leakey et al., 1982). These results commensurate with those of Blythe et al. (2004) who obtained similar results where stem cuttings of *Ficus benjamina* and *Gardenia augusta* were used in percent rooting, root number and root length.

5.4.3 Shoot (branch) number of cuttings as affected by healing period and rooting hormone

The results of the study indicated that, the wound healing period affected shoot number of cuttings significantly. However, its significance was only noticeable from Week 5 to Week 8 after establishment (Figure 5.5). When cuttings were planted at the early days of the healing period (Day 0 and Day 1), the shoot number was found to be at its greatest. Though there were no statistical differences on both Day 0 and Day 1 cuttings, Day 2 cuttings were observed to have a greater number of shoots than Day 1 cuttings. The lowest shoots number was produced by cuttings planted on Day 2 and Day 3 healing period which had no significant difference amongst each other.

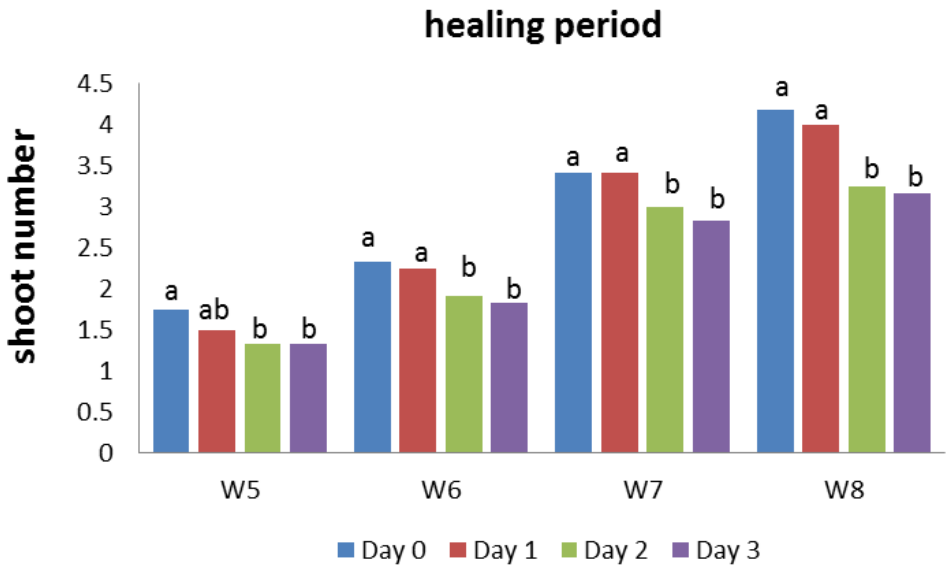

Figure 5.5: Effect of healing period on shoot number of rose-scented geranium cuttings. Bar graphs with the same letters in the same week are not significantly different from each other at α = 0.05 according to Tukey.

The results of the study further show that in order to promote shoot number of cuttings, the cuttings must be immediately stuck in propagation medium to allow healing on the medium rather than in room temperature. Since the wound healing period is governed by an array of endogenous physiological factors (Hartmann et al. 2002), cuttings must survive physiological stress during healing period. But, if they are receiving little water or nutrient uptake they can be affected by the usually reduced stomata conductance (Druege and Kadner 2008; Pop et al. 2011). According to Hartmann et al. (2002) at Leakey (2002), cuttings are commonly put (stuck) into the rooting medium as soon as they are detached (harvested) from the mother plant to avoid water loss that is believed to lead to the death of cutting.

The observation of the study further illuminate that, there were differences between shoots number of stem cuttings on rooting hormone used, but statistically it had no differences (results not published). The observations also indicated that, shoot number increased with a decrease in IBA hormone concentration. Thus, the highest shoot number was found in Control, followed by Dynaroot 1, Dynaroot 2 and Dynaroot 3 respectively. Generally, cuttings without hormone application (control) had a higher number of shoots than cuttings with hormone.

5.4.4 Stem circumference as affected by healing period and rooting hormone

It emerged from the results that during the wound healing period, there were significant differences on stem circumference of cutting at Week 3 to Week 8 after planting (Figure 5.6). Cuttings planted on Day 0 of the healing period had significantly thicker stems followed by cuttings planted on Day 1 of the healing period. However, when cuttings were planted on Day 2 and Day 3 of the healing period they had the thinnest stem circumference and they had no statistical difference between each other. Cuttings are commonly put (stuck) into the rooting medium as soon as they are detached (harvested) from the mother plant to avoid water loss that is believed to lead to the death of cutting (Hartmann et al., 2002; Leakey,

2002). This effect may promote plant development (stem circumference) and growth (Hartmann et al., 2002).

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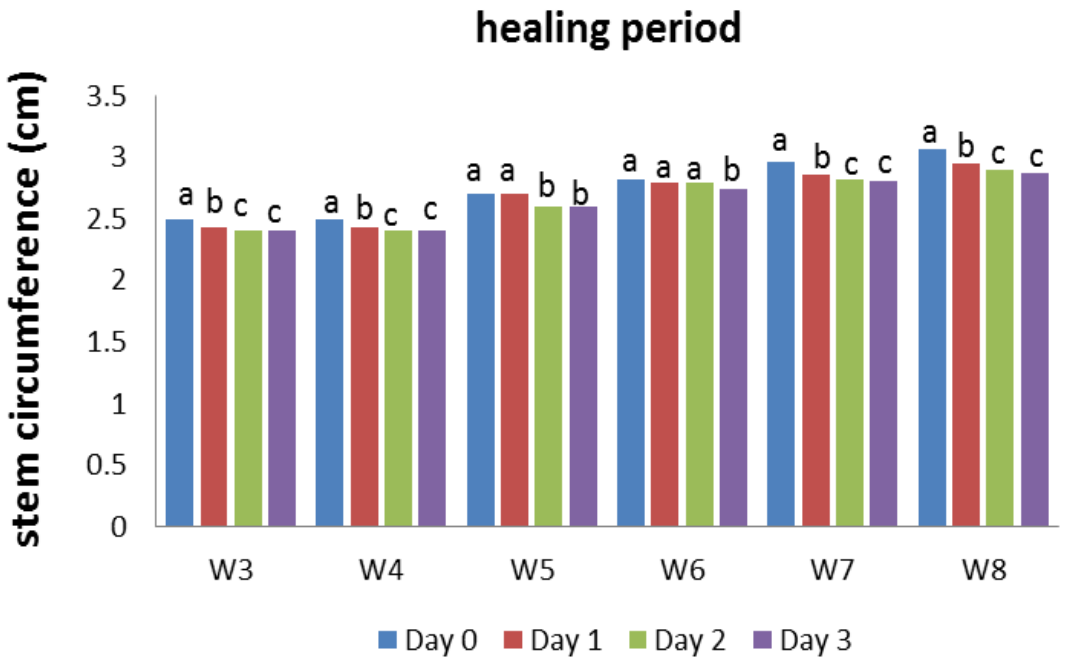

Figure 5.6: Effect of healing period on stem circumference of rose-scented geranium cuttings. Bar graphs with the same letters in the same week are not significantly different from each other at α = 0.05 according to Tukey.

The results of this experiment also indicated that the rooting hormone significantly affected stem circumference of rose-scented geranium cuttings (Fig. 5.7). Cuttings without hormone (control) had thicker stems than those cuttings treated with hormone. On the other hand, observations were made that, the thickness of stem decreased with an increase in rooting hormone concentration such that cuttings treated with Dynaroot 3 had the thinnest stem circumference.

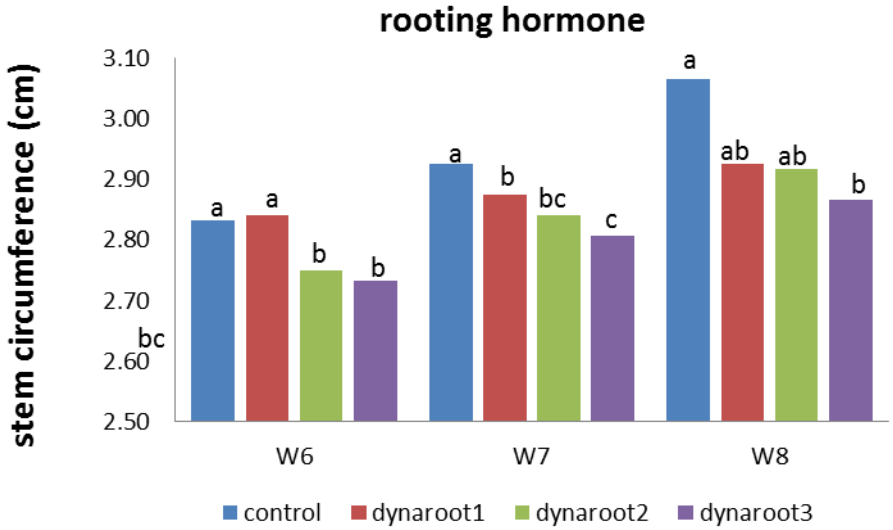

Figure 5.7: Effect of rooting hormone on stem circumference of rose-scented geranium cuttings. Bar graphs with the same letters in the same week are not significantly different from each other at α = 0.05 according to Tukey.

The decline in stem circumference with IBA concentrations is also a clear indication that some levels of hormone concentrations are inhibitory to stem development, as has been recorded in a number of other tree species (Leakey , 1990). Also, Tchiogio and Duguma (1998) discovered that, the addition of growth hormones had little overall effect on the stem cuttings of *Calliandra calothyrsus*. The effect of different auxin concentrations, on rooting ability and stem of leafy stem cuttings of *Milicea excelsa* were also investigated by Ofori et al. (1996). The overall results indicated that, in order to promote stem circumference of rose-scented geranium cuttings during propagation, cuttings should therefore be treated with no-hormone (control).

5.4.5 Leave number of cuttings as affected by healing period and rooting hormone

The effect of wound healing period on number of leaves on rose-scented geranium cuttings is shown in Figure 5.8. Wound healing period tested was identified in this experiment as an important factor that influences number of leaves of cuttings. There were significant

differences ($P < 0.05$) between the performances of the different healing durations (Day 0, Day 1, Day 2 and Day 3). However, its statistical significance was only found on Week 4 up to the termination of the experiment.

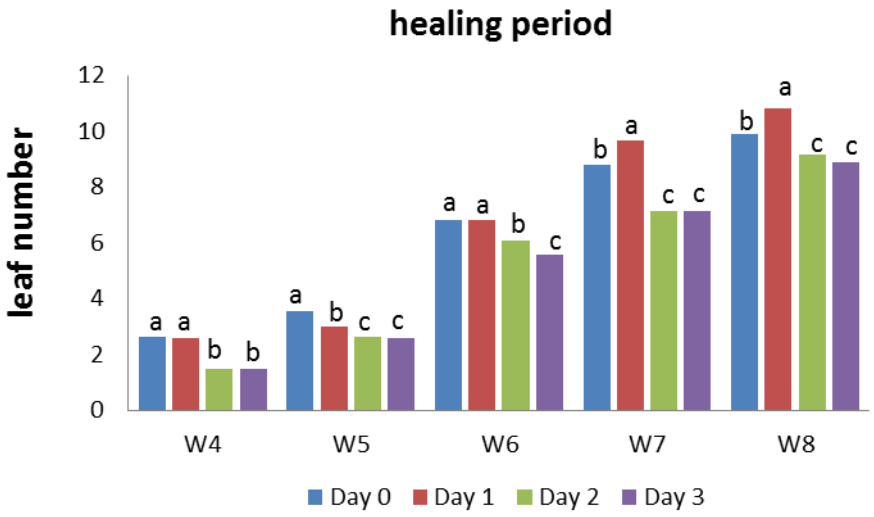

Figure 5.8: Effect of healing period on leaf number of rose-scented geranium cuttings. Bar graphs with the same letters in the same week are not significantly different from each other at $\alpha = 0.05$ according to Tukey.

From the data collected, it could be deduced that, for the first three significant sampling (at Week 4-6) results showed that cuttings planted within the 24 hours after they were detached from the mother (Day 0 and Day 1) had a greater number leaf of leaves and showed no significantly difference amongst each other. While cuttings planted on Day 2 and Day 3 of the healing period produced the lowest leaf number and they did not show any significant differences in their ability to influence leaf number.

However, at Week 8, plants showed significantly greater leaf number from cuttings planted on Day 1 of the healing period than all other treatments. For instance, Day 0 cuttings were found to be the second best performing treatment and they were significantly greater than cuttings planted in Day 2 and Day 3, both these cutting gave the lowest leaf number. Results of this study show that number of leaves was promoted when wounded cutting was immediately planted in the soil or allowed to heal for less than two days in the room

temperature. Thompson (2003) believed that soil moisture had a major effect on vegetative growth of the plants, which means that wound healing would also occur in the soil with warm temperature and by being well-watered.

On the other hand, even though the leaf number did not respond well to the use of different IBA root hormone, visual observation showed that cuttings treated with no-hormone (control) had greater number of leaves as compared to cuttings treated with hormone. The use of external (exogenous) hormone in stimulating leaf number is not necessary in this species. The findings from this study agree with Oni and Ojo (2002) who reported that *Massularia acuminata* is amenable to cloning with or without auxin treatment.

5.4.6 Roots fresh mass as affected by healing period and rooting hormone

The root fresh mass of cuttings differed amongst wound healing period tested. From Week 3 to Week 5 cuttings planted on Day 0 and Day 1 of the healing period did not show any significant differences in their ability to influence root weight. Cuttings planted on both Day 0 and Day 1 of the healing period produced highest fresh weight, the second best root fresh weight was found in cuttings planted on Days 2 and Day 3 of the healing period. Whereas, in Week 6 to Week 8, healing treatment Day 2 had the highest root fresh mass which was significantly higher than all other treatments. The second highest root fresh mass was recorded on Day 0 cuttings and the least root fresh mass was obtained from the cuttings planted on Day 3 of the healing period. The reason for the best performance of Day 2 cuttings could be attributed to maximum number of roots as well as root length observed in this treatment (Day 2), that may have played a critical role on greatest root fresh mass.

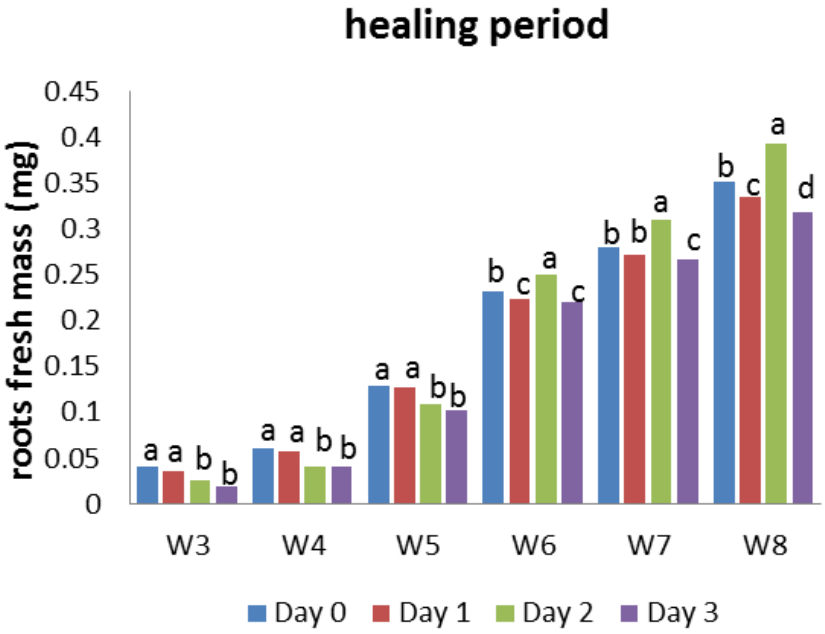

Figure 5.9: Effect of healing period on root fresh mass of rose-scented geranium stem cuttings. Bar graphs with the same letters in the same week are not significantly different from each other at $\alpha = 0.05$ according to Tukey.

In the current research, rooting hormone IBA did not affect root fresh mass of rose-scented geranium cuttings. Even though there were no significant differences, cuttings performed slightly better when hormone was applied than those cuttings with no-hormone (control). Such that, Dynaroot 3 was observed to have the highest root weight followed by Dynaroot 2 and Dynaroot 1 respectively while, control had least root weight.

5.4.7 Interaction effect of rooting hormone and healing period on root fresh mass and leaf number

There were interactions between rooting hormone and healing period on leaf number of rose-scented geranium cuttings at Week 8 after establishment (Table 5.2). The highest leaf number was recorded when cuttings were planted on Day 3 of the healing period regardless of the root hormone concentration used, however, the treatment combination of Day 3 by control gave significantly the greatest leaf number as compared to all other treatments used. These results show that, leaf number can be promoted with the extension of wound healing period

up to Day 3 using zero- hormone application as a treatment. This may be attributed to the fact that, prolonged drying period possibly allowed the surface cut of the cutting to heal or be air dried until the soft inner tissue calluses over thereby inducing rooting, areal parts of the plant and promoting plant growth and development (Nolte, 2003).

Meanwhile, cuttings planted on Day 1 and Day 2 of the healing period and treated with control gave the lowest number of leaves as compared to IBA treated cuttings. Furthermore, application of IBA rooting hormone on Day 0 and Day 1 planted cuttings had no significant difference amongst each other. Similar results were reported by Kelly (2009) on agaves, affirming that, rooting was promoted when wounded endings of the cutting were dusted with sulphur.

There were also interactions between rooting hormone and healing period on root fresh mass of rose-scented geranium cuttings at Week 8 of the experiment (Table 5.2). Root fresh mass was significantly higher when Day 2 by Dynaroot 1 treatment combination was used as compared to all other treatments, moreover, Day 2 cuttings were consistence in giving the highest root mass irrespective of the rooting hormone used. Furthermore, Day 3 cuttings were also promoted by application of Dynaroot 1 as compared to other treatments combination. On the other hand, when Dynaroot 1 was applied on Day 0 and Day 1 cuttings, minimum root fresh mass was observed, while Dynaroot 3 gave the maximum root fresh mass on Day 0 and Day 1 planted cuttings. It was further observed that, early wound healing cuttings of Day 0 and Day 1 promoted fresh mass with an increase in rooting hormone concentration. The results of the study conclude that, root fresh mass was improved by combination of prolonged wound healing period (Day 2) and minimum application of IBA rooting hormone (Dynaroot 1). According to Hartmann et al, (2002) when a plant is wounded, auxins collect briefly around the wound and alter the nature of cell division in the cambium so that it begins to form embryonic root tissue.

Table 5.2: Interaction effect of rooting hormone and healing period on root fresh weight and leaf number of rose-scented geranium cuttings at week 8 after establishment

Wound healing period	Rooting treatment	Leaf number	Root fresh mass (mg)
Day 0	Control	9.917 CDE	0.333 EFG
	Dynaroot 1	10.83 ABC	0.343 DER
	Dynaroot 2	9.167 DE	0.367 BCD
	Dynaroot 3	8.917 E	0.363 BCDE
Day 1	Control	6.588 F	0.320 FG
	Dynaroot 1	9.833 CDE	0.327 FG
	Dynaroot 2	9.583 CDE	0.347 DEF
	Dynaroot 3	9.500 DE	0.350 CDEF
Day 2	Control	6.588 F	0.390 AB
	Dynaroot 1	10. 00 CDE	0.413 A
	Dynaroot 2	9.333 DE	0.390 AB
	Dynaroot 3	9.000 E	0.380 BC
Day 3	Control	12.00 A	0.307 G
	Dynaroot 1	11.33 AB	0.340 DEF
	Dynaroot 2	10.33 BCD	0.320 FG
	Dynaroot 3	9.667 CDE	0.307 G
Grand mean		9.708	0.350
CV (%)		4.29	3.64
LSD (P < 0.05)		0.2408	0.00577
Wound healing period (WHP)		*	*
Rooting hormone (RH)		*	*
WHP x RH		*	*

Mean within a column followed by the same letter are not significantly different at 5% level.
Ns= Not significant, * = P ≤ 0.05.

5.4.8 Root ability to hold medium as affected by healing period and rooting hormone

Visual observation showed that, root holding ability was affected by interaction effect between healing period and rooting hormone from Week 5 until the termination of the experiment (Table 5.3). Cuttings planted on Day 2 and treated with Dynaroot 3 had the highest root holding ability that observed at Week 5 (3- medium, marginally acceptable) and Week 6 (4- tight, acceptable) after planting. Meanwhile, at Week 5, regardless of the rooting hormone used, cuttings planted on Day 0, Day 1 and Day 3 healing period had the lowest root holding ability (2-loose, not acceptable) as compared to Day 2 planted cuttings. Furthermore, at Week 6 after planting, data shows that, cuttings planted on Day 0 and Day 1 of the healing period had the lowest root holding ability (3- medium, marginally acceptable) which was

observed to be better than that of cutting planted on Day 3 (2- loose, not acceptable) of the healing period.

Table 5.3: Influence of healing period and rooting hormone on root ability to hold medium on cuttings

*** Root holding ability on medium**

		Weeks after planting					
Healing period	**Hormone**	3	4	5	6	7	8
Day 0	Control (no-hormone)	1	2	2	3	3	4
	Dynaroot 1	1	2	2	3	3	4
	Dynaroot 2	1	2	2	3	4	5
	Dynaroot 3	1	2	2	3	4	5
Day 1	Control (no-hormone)	1	2	2	3	3	4
	Dynaroot 1	1	2	2	3	3	4
	Dynaroot 2	1	2	2	3	4	4
	Dynaroot 3	1	2	2	3	4	5
Day 2	Control (no-hormone)	1	2	2	3	4	4
	Dynaroot 1	1	2	2	3	4	5
	Dynaroot 2	1	2	2	3	4	5
	Dynaroot 3	1	2	3	4	5	5
Day 3	Control (no-hormone)	1	2	2	3	5	5
	Dynaroot 1	1	2	2	3	4	4
	Dynaroot 2	1	2	2	2	3	3
	Dynaroot 3	1	2	2	2	3	3

*** root ability to hold medium [determined by visual observation and rated at 1-5 scale: 1 (very loose, not acceptable); 2 (loose, not acceptable); 3 (medium, marginally acceptable); 4 (tight, acceptable); 5 (very tight, acceptable).**

At Week 7 of the experiment, cuttings planted on Day 0 and Day 1 of the healing period and treated with control and Dynaroot 1 showed marginally acceptable (3: medium) root holding ability. Whereas, Day 2 planted cuttings that were treated with control and Dynaroot 1 showed acceptable (4- tight, acceptable) root holding. On the other hand, when cuttings were planted on Day 3 of the healing period and treated with control, results showed very tight (5- acceptable) root holding ability on medium.

Furthermore, data shows that, at Week 8 after establishment, cuttings planted on Day 0, Day 1 and Day 2 of the healing period and treated with Dynaroot 1, were observed to have a tight root holding ability on medium. However, when Day 2 healing cuttings were treated with all IBA rooting hormone concentration except the control, the root holding ability was observed to be very tight on root holding ability of medium. This clarifies that, root holding ability was improved with an increase in wound healing time up to Day 2 and an application of IBA root hormone. But for further healing up to Day 3, root holding ability was improved with a decrease in IBA root hormone concentration such that control treated cuttings gave the highest root holding ability as compared to other treatments. Correlated results were found with potato tuber where by rooting increased with an increase in wound healing time. For instance, for the potato tuber, wound healing period may take three to five days at room temperature or under good conditions (Chase, 1983). This may be attributed to the fact that, prolonged drying period possibly allowed the surface cut of the cutting to heal or be air dried until the soft inner tissue calluses over and rooting is promoted. Further observations, showed that, auxin treated cuttings before healing encouraged a large number of roots which in turn promoted root holding ability of medium (Hartmann et al., 2002).

5.5 CONCLUSION AND RECOMMENDATION

Vegetative propagation of rose-scented geranium was possible and successful in this study. However, there are certain factors that should be considered which affect rooting of stem cuttings. These factors are wound healing period (Day 0; 1; 2 and 3) and application of different types of IBA rooting hormone concentrations. Roots development of rose-scented geranium was confirmed to depend on healing period of stem cuttings before planting. Room temperature in wound - healing helps the cuttings to produce more roots at transplanting stage. In this study, the maximum rooting was found in cuttings that passed through 48 hours of the healing period. While, Day 0 cuttings showed good response for stem circumference and shoot number.

The results of this study also indicated that, rose-scented geranium was also influenced by rooting hormone, when IBA rooting hormone was applied, rooting of cuttings was enhanced. Cuttings treated with Dynaroot 3 produced more roots as well as other arial parameters except for stem circumference which was on its highest when cuttings treated with without hormone (control). There was also an indication from the results that rose-scented geranium requires wound healing period of about three days in room temperature and application of IBA hormone before sucking cuttings in growing medium. For instance, cuttings that had healed for 48 hours and treated with Dynaroot 3 performed significantly better on rooting and development of the plant. When Day 2 cuttings were treated with Dynaroot 3 or Dynaroot 2 they showed highest root holding capacity which motivated for early transplanting of the seedling.

The emerging results show that cuttings can be transplanted from Week 7 after establishment. This is because at this time the cuttings achieve acceptable root holding ability irrespective of the healing period or hormone used. Transplanting can even be done earlier (Week 5 or Week 6) on cuttings that have healed for three days and treated with Dynaroot 3 due to acceptable root holding ability on medium. The results of this research may also be used as a

basis for propagation by stem cutting even in other herbaceous species to meet future seedling demands.

CHAPTER 6
GENERAL DISCUSION AND CONCLUSION

The present study was set out to investigate the stem cutting propagation protocol for rose-scented geranium (*Pelargonium graveolens*). And efforts were made to develop an efficient, rapid and inexpensive method for large scale propagation of rose-scented geranium. The study was carried out at the Essential Amatole Nursery, at the University of Fort Hare Research Farm, Alice Campus (located at 32^0 47′3″S, 26^0 50′43″ E, and an altitude of 519 m.a.s.l). The investigated variables were: growing medium ((**1**) mixture growing medium; (**2**) sand; (**3**) pine bark; (**4**) hygrotex; (**5**) hygrotex + sand; (**6**) hygrotex + pine bark and (**7**) hygrotex + sand + pine bark), rooting hormones (auxins, types of IBA) applied as treatments were (**1**) Dynaroot (1 – 1g/kg); (**2**) Dynaroot (2- 3g/kg); (**3**) Dynaroot (3-8g/kg) and (**4**) Control (untreated with hormone), cutting length [(**1**)10 cm; (**2**)12cm; (**3**)14cm and (**4**)16 cm) and wound healing duration intervals of 24 hours: (Days 1; Day 2; Day 3 and Day 4).The results of this study indicated that it is possible to successfully achieve propagate protocol of rose-scented geranium. The optimum rooting of this plant can be achieved if factors that affect rooting are considered. These factors are propagation medium, rooting hormone, stem cutting length and wound healing period.

Growing media and rooting hormone were found to play an important role in rooting and development of rose-scented geranium cuttings (Chapter 3). Hygrotex was found to be the best rooting media for rose-scented geranium stem cutting propagation. The choice of medium component used plays a significant role on rooting performance and growth of the plant (Hartmann and Kester, 1997; Hartmann et al., 2002). Cuttings, however, showed a good response to hygrotex in root development while hygrotex and pine bark (v/v 1:1) was efficient in producing more leaves, leaf area, plant height and other aerial parameters. Although there are various materials that can be used for propagation of rose-scented geranium stem cuttings, it is recommended that this plant should be propagated through commercial rooting media – hygrotex. Alternatively, hygrotex + pine bark (1:1) may also be tried out in situations where hygrotex is in short supply. The good choice of rooting enhancer is very essential, especially as there is a wide array of rooting hormones in the market. Even though the choice of the propagation medium is important for propagators, rooting of cuttings

does not only depend on the type of rooting media alone but, on other factors such as availability and cost of material, type of species used, part of plant used, time of planting and season. (Leakey, 1990; Mamba and Wahome, 2010). Rooting hormone also had a major effect on rooting of rose-scented geranium cuttings. Consequently, the effectiveness of auxins in promoting root initiation has been shown in a variety of plant species to break root apical dominance induced by cytokinin (Cline, 2000). Dynaroot 3 increased rooting and other aerial parts of this plant, while, no-hormone (control) treatment promoted stem circumference. Similarly, Stafanini (2004) in his results witnessed the effect of different rooting hormone dose on Aloysia triphylla (L' Hérit). However, due to commercial constraints, Dynaroot 2 may be used in place of Dynaroot 3. The results of the study revealed that, rooting performance is not only influenced by the type of medium used for propagation, but also the interaction of rooting hormone as well (Hartmann et al., 1990). As a result, a combination of IBA hormone concentration (Dynaroot 3) and hygrotex should be promoted for mass propagation of this herbaceous plant.

An ideal stem cutting length and rooting hormone are other major factors of importance in rooting of stem cuttings (Chapter 4). Stem cuttings with 14 cm and 16 cm length performed better for quicker root initiation and development, thereby ensuring survival of plantlets. Similarly, Kowalczyk and Kobryn (1999) observed that long intermodal length (10 cm) gave the highest root length and root number than short (5 cm) intermodal stem cuttings of *Solanum muricatum*. Furthermore, 14 cm cutting stem length were reported to be greater on root ability to hold medium as compared to all other treatments. This has led to the peculations that the long stem cuttings are most likely to develop roots faster and give a more vigorous plant than the shorter ones (Welch- Keesey and Lerner, 2002). The results also showed that rooting hormone (IBA) enhances success of stem cutting propagation of rose-scented geranium. Cuttings treated with Dynaroot 3 and Dynaroot 2 showed better rooting, root fresh weight and leaf number. While, those treated with control (no-hormone) favoured shoot number and stem circumference. On the other hand, the impact of rooting media indicated that, hygrotex consistently performed better, although its performances did not significantly differ from hygrotex + pine bark (1:1 v/v) on propagation of rose-scented geranium stem cuttings. Both hygrotex and hygrotex + pine bark (1:1 v/v) are the best growing media for root formation and growth of rose-scented geranium, though hygrotex alone is more economical. Therefore, it is recommended that rose-scented geranium should

be propagated through the combination of 14 cm cuttings length and treated with Dynaroot 2 IBA rooting hormone.

Even though there was no interaction between wound healing period and rooting hormone. The wound healing period alone and rooting hormone significantly affected rooting and growth of rose-scented geranium stem cuttings (Chapter 5). This essential oil plant requires wound healing period of about three days in room temperature. The study showed that maximum rooting and plant development was found in cuttings of Day 2 wound healing period. According to Ahkami et al. (2009), when the wound is permitted to heal for hours or even weeks, the callus dries and becomes hard then when it comes in contact with moist soil, the callus remains somewhat soft, and the burgeoning roots beneath it can emerge. To optimize propagation of pineapple, the crowns are set aside for few days to allow the wound to heal and dry before planting (De Klerk et al., 1999). While, Day 0 cuttings showed good response for stem circumference and shoot number. Application of IBA hormone before sucking cuttings in growing medium promoted rooting. Dynaroot 3 treated cuttings favoured highest rooting and plant development. Moreover, cuttings planted on Day 2 of the healing period and treated with IBA rooting hormone showed the highest root holding capacity which motivated for early transplanting of the seedling. On the basis of these results, this plant can be easily regenerated from the combination of Day 2 treated with IBA rooting hormone. This protocol could also be easily utilised for quality seedling development of this essential oil plant and ornamental and herbaceous species to meet future seedling demands in vegetative propagation.

GENERAL SUMMARY

Rose-scented geranium (*Pelargonium graveolens*), is a high value essential oil plant used in the perfumery, cosmetic, aromatherapy and food flavouring industries. It is well known for its medicinal and fragrance properties. Three separate experiments were undertaken to determine factors influencing effective propagation of rose-scented geranium. These factors were: rooting media, root hormone, cutting length and wound healing period on rooting and development of rose-scented geranium stem cuttings. This study was carried out in a glasshouse with heated beds and further taken to shade house at the Essential Amatole Nursery, located at the University of Fort Hare Research Farm. In a glasshouse with heated beds and further taken to shade house. A protocol for vegetative propagation of rose-scented geranium was optimized with the use of subsequent propagation medium, ideal rooting hormone concentration, stem cutting length and ideal wound healing period.

The stem cuttings grown in Hygrotex recorded the maximum mean number of roots, highest root length, maximum fresh weight, and highest shoot number. While, hygrotex + pine bark (v/v 1:1) was efficient in producing more leaves, plant height and other aerial parameters. The river sand performed significantly poor compared to all media used. On the other hand, Dynaroot 3 was recorded as the best rooting enhancer for quicker regeneration. However, a combination of control and hygrotex interacted significantly better and recorded the greatest stem circumference than any treatment. Nevertheless, hygrotex and Dynaroot 3 were identified as the best combination for successful rooting of rose-scented geranium stem cuttings.

Stem cuttings of 14 cm and 16 cm length were suitable for quicker regeneration of this plant. In addition, cuttings treated with Dynaroot 3 and Dynaroot 2 showed better rooting and leaf number while those treated with control (no-hormone) favoured root mass, shoot number and stem circumference. It is recommended that Rose-scented geranium should be propagated through the combination of 14 cm cuttings length and treated with Dynaroot 2 IBA rooting hormone. Both hygrotex and hygrotex + pine bark (1:1 v/v) are the best growing media for root formation and growth of rose-scented geranium, though hygrotex alone is more economical.

Responses of wound healing period and rooting hormone on rooting of rose-scented geranium were investigated (Chapter 5). The result obtained from the study revealed that rose-scented geranium rooted easily when planted in Day 2 of the healing period. While, Day 0 cuttings showed good response for stem circumference and shoot number. In addition, an increase in IBA rooting hormone favoured rooting of this plant, such that root holding ability was promoted by IBA rooting hormone on Day 2 planted cutting. The study therefore, recommends that, rose-scented geranium can be propagated using cuttings that have enough time to heal the wound that is Day 2 cuttings and application of rooting hormone.

Recommendations for propagators at Essential Amathole, is that they should consider using hygrotex or hygrotex + pine bark (1:1 v/v) instead of Mixture rooting medium [pine bark (8 bags) + sand (2 bags) + lime (4 kg) + coconut (10 blocks) + talborne (6.25 kg) + bone meal (2 kg)] as a growing medium. The reason is that, it is expensive and has less effect on roots formation when compared to hygrotex and hygrotex + pine bark (1:1 v/v). Moreover, they should consider application of IBA hormone (Dynaroot 3 or Dynaroot 2) to cuttings prior planting. Hygrotex + pine bark (1:1 v/v) with Dynaroot 2 IBA hormone concentration should be used by poor farmers who propagate their own seedlings because of its effectiveness on rooting and development and economical affordability.

Furthermore, it is recommended that, instead of using very short stem cutting length of 9-11cm which resulted in poor rooting of cuttings and less effect on root holing ability. Propagators must use 14 to 16 cm stem cutting length for effective and good quality seedlings. These are expected to be ready for transplanting at week 6 after establishment. This is because its higher root holding ability qualifies it to be transplanted as well as other improved parameters

In addition, as a measure to improve seedling quality, cuttings may be allowed to heal at room temperature for at least two to three days. Moreover, this process will also work as an advantage for farmers who would like to harvest and prepare cuttings before heavy rains come. Lastly, the study recommends that cuttings should be allowed to heal for two days with IBA rooting hormone (Dynaroot 2) application for efficient rooting. Hence, studying the effect of different temperature during wound healing period under controlled conditions could be helpful for further seedling quality-improving efforts.

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APPENDIX A

WEATHER DATA FOR THE EXPERIMENTAL SITE– MINIMUM AND MAXIMUM TEMPERATURES AND AVERAGE RELATIVE HUMIDITY (RH)

Table A 1: Average minimum and maximum temperatures and average relative humidity (RH) inside and outside of the glass house and shade house during the experimental period (23 September to 11 November 2014)

Growth System		Minimum ^{0}C		Maximum ^{0}C		Average RH (%)	
	Weeks	Inside	Outside	Inside	Outside	Inside	Outside
Glass house	1	21.51	8.74	28.00	24.93	85.00	59.93
	2	21.55	9.66	30.00	25.33	85.55	59.88
	3	21.55	9.42	30.00	23.54	85.55	65.27
Shade house	4	15.44	9.66	27.93	23.31	75.88	67.91
	5	15.54	9.45	28.66	24.62	75.77	65.39
	6	15.89	9.33	28.55	24.82	80.22	65.03
	7	20.76	18.08	28.22	24.31	80.22	66.19
	8	20.65	18.45	29.61	25.42	77.88	66.66

Weeks 1 to 3 of the experiment was conducted in the glass house from 23 September to 7 October and then continued in the shade house on week 4 to 8 from 14 October to 11 November 2014.

Table A 2: Average minimum and maximum temperatures and average relative humidity (RH) inside and outside of the glass house and shade house during the experimental period (25 November to 13 January 2015)

Growth system	Weeks	Minimum ^{0}C		Maximum ^{0}C		Average RH (%)	
		Inside	Outside	Inside	Outside	Inside	Outside
Glass house	1	30.55	18.08	38.00	26.13	85.88	65.77
	2	32.22	20.68	39.55	26.80	90.55	67.91
	3	32.22	21.68	39.55	27.44	90.55	67.66
Shade house	4	30.45	21.22	35.45	26.32	85.33	66.88
	5	30.45	20.55	34.55	28.77	85.44	66.99
	6	31.43	20.45	36.00	27.33	81.00	67.55
	7	30.55	16.55	37.55	30.09	81.00	62.39
	8	30.55	15.88	37.34	30.55	85.44	62.40

Weeks 1 to 3 of the experiment was conducted in the glass house from 25 November to 9 December 2014 and then continued in the shade house on week 4 to 8 from 16 December to 13 January 2015.

Table A 3: Average minimum and maximum temperatures and average relative (RH) humidity inside and outside of the glass house and shade house during the experimental period (18 February to 8 April 2015)

Growth system	Weeks	Minimum ^{0}C		Maximum ^{0}C		Average RH (%)	
		Inside	Outside	Inside	Outside	Inside	Outside
Glass house	1	26	14.24	33.55	26.21	88.77	69.03
	2	28	14.55	33.55	26.44	88.77	69.44
	3	28	13.96	33.55	27.11	88.75	68.19
Shade house	4	24.93	12.55	30.33	26.55	80.00	68.55
	5	25.66	12.33	30.11	26.88	80.44	67.44
	6	26.55	13.65	28.44	26.56	75.67	68.44
	7	26.22	9.86	28.00	22.55	72.88	70.37
	8	27.61	10.44	26.00	23.00	72.90	70.66

Weeks 1 to 3 of the experiment was conducted in the glass house from 18 February to 4 March and then continued in the shade house from 11 March to 8 April 2015.

APPENDIX B

Table B1: Summary of ANOVA table for the effects of growing media and rooting hormone in the glass house and shade house from week 3 to week 8 of the experiment (7 October to 11 November 2014)

Weeks	Source of variance)	Degree of freedom	Root number	Root length (cm)	Root fresh weight (mg)	Stem circumference (cm)	Leaf number	Shoot number	Plant height (cm)
3	Factor A	6	0.0000*	0.0000*	0.0000*	0.0000*	0.0127*	0.0886^{NS}	0.0000*
	Factor B	3	0.0000*	0.0000*	0.0000*	0.0000*	**NS**	0.1001^{NS}	0.0000*
	AB	18	0.0000*	0.0000*	0.0088*	0.0110*	0.0324*	0.3146^{NS}	0.2807^{NS}
	Error	56	–	–	–	–	–	–	–
4	Factor A	6	0.0000*	0.0000*	0.0000*	0.0002*	0.0000*	0.0000*	0.0000*
	Factor B	3	0.0000*	0.0000*	0.0000*	0.0000*	0.1866^{NS}	0.0522^{NS}	0.0000*
	AB	18	0.0000*	0.0002*	**NS**	0.0063*	**NS**	0.4511^{NS}	**NS**
	Error	56	–	–	–	–	–	–	–
5	Factor A	6	0.0000*	0.0000*	0.0000*	0.0369*	0.0000*	0.0000*	0.0000*
	Factor B	3	0.0000*	0.0000*	0.0000*	0.0000*	0.3798^{NS}	**NS**	0.0000*
	AB	18	0.0000*	0.0000*	**NS**	0.0000*	**NS**	0.1181^{NS}	**NS**
	Error	56	–	–	–	–	–	–	–
Weeks	**Source of variance)**	**Degree of freedom**	**Root number**	**Root length**	**Root fresh weight**	**Stem circumference**	**Leaf number**	**Shoot number**	**Plant height**
	Factor A	6	0.0000*	0.0000*	0.0000*	0.0460*	0.0000*	0.0000*	0.0000*

				(cm)	(mg)	(cm)			(cm)
6	Factor B	3	0.0000*	0.0000*	0.0000*	0.0000*	0.0767^{NS}	0.1653^{NS}	0.0000*
	AB	18	0.0047*	0.0000*	**NS**	0.0000*	0.4706^{NS}	0.2088^{NS}	**NS**
	Error	56	–	–	–	–	–	–	–
7									
	Factor A	6	0.0000*	0.0000*	0.0000*	0.0567^{NS}	0.0000*	0.0000*	0.0000*
	Factor B	3	0.0000*	0.0000*	0.0000*	0.0000*	0.0019*	0.0860^{NS}	0.0000*
	AB	18	0.0007*	0.0000*	**NS**	0.0000*	**NS**	0.0962^{NS}	**NS**
	Error	56	–	–	–	–	–	–	–
8									
	Factor A	6	0.0000*	0.0000*	0.0000*	0.0017*	0.0000*	0.0000*	0.0000*
	Factor B	3	0.0000*	0.0000*	0.0000*	0.0000*	0.0019*	0.0860^{NS}	0.0000*
	AB	18	0.0061*	0.0000*	**NS**	0.0029*	**NS**	0.0962^{NS}	**NS**
	Error	56	–	–	–	–	–	–	–

Not significant (NS), significant at 0.05 (*) , Where *Factor A is medium and Factor B is rooting hormone*

APPENDIX C

SUMMARISED ANALYSIS OF VARIANCE (ANOVA) TABLES

Table C 1: Summary of ANOVA table for the effect of stem length, rooting hormone and propagation media on rooting and growth parameters of rose-scented geranium on Week 1 of the experiment

		F-probability levels for:					
Source of variance	Degree of freedom	Root number	Root length (cm)	Root fresh weight (mg)	Stem circumference (cm)	Leaf number	Shoot number
STL	3	0.0671 ^{NS}	0.0561 ^{NS}	0.2234 ^{NS}	0.2311 ^{NS}	0.08901 ^{NS}	0.1501 ^{NS}
HORM	3	0.1201 ^{NS}	0.2301 ^{NS}	0.1156 ^{NS}	0.3035 ^{NS}	0.2001 ^{NS}	0.1206 ^{NS}
STL* HORM	9	0.0944 ^{NS}	0.9755 ^{NS}	0.0988 ^{NS}	0.1481 ^{NS}	0.1206 ^{NS}	0.0606 ^{NS}
MED	1	0.1042 ^{NS}	1.0000 ^{NS}	0.6640 ^{NS}	0.4855 ^{NS}	1.0000 ^{NS}	0.5486 ^{NS}
STL*MED	3	0,0666 ^{NS}	0.7066 ^{NS}	0.1002 ^{NS}	0.9185 ^{NS}	1.0000 ^{NS}	0.3594 ^{NS}
HORM*MED	3	0.9602 ^{NS}	0.5756 ^{NS}	0.2714 ^{NS}	0.9185 ^{NS}	1.0000 ^{NS}	0.6135 ^{NS}
STL*HORM*MED	9	0.8805 ^{NS}	0.4156 ^{NS}	0.4535 ^{NS}	0.9785 ^{NS}	1.0000 ^{NS}	0.9481 ^{NS}
Error	64	-	-	-	-	-	-

Ns= Not significant, * = P ≤ 0.05.*Where STL- stem cutting length; HORM- rooting hormone; MED- propagation medium*

Table C 2: Summary of ANOVA table for the effect of stem length, rooting hormone and propagation media on rooting and growth parameters of rose-scented geranium on Week 2 of the experiment

		F-probability levels for:					
Source of variance	Degree of freedom	Root number	Root length (cm)	Root fresh weight (mg)	Stem circumference (cm)	Leaf number	Shoot number
STL	3	0.1301 ^{NS}	0.3201 ^{NS}	0.064 ^{NS}	0.2111 ^{NS}	0.0791 ^{NS}	0.121 ^{NS}
HORM	3	0.0941 ^{NS}	0.2301 ^{NS}	0.1156 ^{NS}	0.2335 ^{NS}	0.0671 ^{NS}	0.1206 ^{NS}
STL* HORM	9	0.0944 ^{NS}	0.9755 ^{NS}	0.0988 ^{NS}	0.1481 ^{NS}	0.1206 ^{NS}	0.0606 ^{NS}
MED	1	0.1042 ^{NS}	1.0000 ^{NS}	0.6640 ^{NS}	0.4855 ^{NS}	1.0000 ^{NS}	0.5486 ^{NS}
STL*MED	3	0,0666 ^{NS}	0.7066 ^{NS}	0.1002 ^{NS}	0.9185 ^{NS}	1.0000 ^{NS}	0.3594 ^{NS}
HORM*MED	3	0.9602 ^{NS}	0.5756 ^{NS}	0.2714 ^{NS}	0.9185 ^{NS}	1.0000 ^{NS}	0.6135 ^{NS}
STL*HORM*MED	9	0.8805 ^{NS}	0.4156 ^{NS}	0.4535 ^{NS}	0.9785 ^{NS}	1.0000 ^{NS}	0.9481 ^{NS}
Error	64	-	-	-	-	-	-

Ns= Not significant, * = P ≤ 0.05.*Where STL- stem cutting length; HORM- rooting hormone; MED- propagation medium*

Table C 3: Summary of ANOVA table for the effect of stem length, rooting hormone and propagation media on rooting and growth parameters of rose-scented geranium on Week 3 of the experiment

		F-probability levels for:					
Source of variance	Degree of freedom	Root number	Root length (cm)	Root fresh weight (mg)	Stem circumference (cm)	Leaf number	Shoot number
STL	3	0.0001*****	0.0001*****	0.0234*****	0.0011*****	0.0001*****	0.0001*****
HORM	3	0.0001*	0.0001*****	0.0156*****	0.0035*****	0.0001*****	0.0006*****
STL* HORM	9	0.0944^{NS}	0.9755^{NS}	0.0988^{NS}	0.1481^{NS}	0.1206^{NS}	0.0606^{NS}
MED	1	0.1042^{NS}	1.0000^{NS}	0.6640^{NS}	0.4855^{NS}	1.0000^{NS}	0.5486^{NS}
STL*MED	3	0,0666^{NS}	0.7066^{NS}	0.1002^{NS}	0.9185^{NS}	1.0000^{NS}	0.3594^{NS}
HORM*MED	3	0.9602^{NS}	0.5756^{NS}	0.2714^{NS}	0.9185^{NS}	1.0000^{NS}	0.6135^{NS}
STL*HORM*MED	9	0.8805^{NS}	0.4156^{NS}	0.4535^{NS}	0.9785^{NS}	1.0000^{NS}	0.9481^{NS}
Error	64	-	-	-	-	-	-

Ns= Not significant, * = P ≤ 0.05.*Where STL- stem cutting length; HORM- rooting hormone; MED- propagation medium*

Table C 4: Summary of ANOVA table for the effect of stem length, rooting hormone and propagation media on rooting and growth parameters of rose-scented geranium on Week 4 of the experiment

		F-probability levels for:					
Source of variance	Degree of freedom	Root number	Root length (cm)	Root fresh weight (mg)	Stem circumference (cm)	Leaf number	Shoot number
STL	3	0.0001*****	0.0001*****	0.04111*****	0.0001*****	0.0001*****	0.0001*****
HORM	3	0.0001*****	0.0001*****	0.03556*****	0.0035*****	0.0001*****	0.0001*****
STL* HORM	9	0.0172^{NS}	0.2689^{NS}	0.5465^{NS}	0.1481^{NS}	0.1100^{NS}	0.0583^{NS}
MED	1	0.1042^{NS}	0.0988^{NS}	0.9226^{NS}	0.4821^{NS}	0.6544^{NS}	1.0000^{NS}
STL*MED	3	0.5833^{NS}	0.7647^{NS}	0.0655^{NS}	0.9185^{NS}	0.9881^{NS}	0.4298^{NS}
HORM*MED	3	0.8239^{NS}	0.1212^{NS}	0.7422^{NS}	0.9185^{NS}	0.6836^{NS}	0.5756^{NS}
STL*HORM*MED	9	0.5819^{NS}	0.7059^{NS}	0.4363^{NS}	0.9785^{NS}	0.8691^{NS}	0.9517^{NS}
Error	64	-	-	-	-	-	-

Ns= Not significant, * = P ≤ 0.05.*Where STL- stem cutting length; HORM- rooting hormone; MED- propagation medium*

Table C 5: Summary of ANOVA table for the effect of stem length, rooting hormone and propagation media on rooting and growth parameters of rose-scented geranium on Week 5 of the experiment

		F-probability levels for:					
Source of variance	Degree of freedom	Root number	Root length (cm)	Root fresh weight (mg)	Stem circumference (cm)	Leaf number	Shoot number
STL	3	0.0002*****	0.0008*****	0.0192*****	0.0011*****	0.0001*****	0.0001*****
HORM	3	0.0001*****	0.0001*****	0.0134*****	0.3987 **NS**	0.0001*****	0.0001*****
STL* HORM	9	0.9628 **NS**	0.7819 **NS**	0.2475 **NS**	0.1445 **NS**	0.0842 **NS**	0.0489 **NS**
MED	1	1.0000 **NS**	1.0000**NS**	1.0000NS	0.4845 **NS**	0.5110 **NS**	0.7248 **NS**
STL*MED	3	1.0000 **NS**	1.0000**NS**	1.0000NS	0.9185 **NS**	0.4998 **NS**	0.7124 **NS**
HORM*MED	3	1.0000 **NS**	1.0000**NS**	1.0000NS	0.9185**NS**	0.3902 **NS**	0.9450 **NS**
STL*HORM*MED	9	1.0000 **NS**	1.0000**NS**	1.0000NS	0.9785**NS**	0.9987 **NS**	0.8969 **NS**
Error	64	_	_ _	_	-	-	-

Ns= Not significant, * = P ≤ 0.05.*Where STL- stem cutting length; HORM- rooting hormone; MED- propagation medium*

Table C 6: Summary of ANOVA table for the effect of stem length, rooting hormone and propagation media on rooting and growth parameters of rose-scented geranium on Week 6 of the experiment

		F-probability levels for:					
Source of variance	Degree of freedom	Root number	Root length (cm)	Root fresh weight (mg)	Stem circumference (cm)	Leaf number	Shoot number
STL	3	0.0001*****	0.0001*****	0.0011*****	0.0001*****	0.0001*****	0.0001*****
HORM	3	0.0001*****	0.0001*****	0.0101*****	0.3433 **NS**	0.0001*****	0.0001*****
STL* HORM	9	0.1929 **NS**	0.0902**NS**	0.0883**NS**	0.4494 **NS**	0.5262 **NS**	0.0484 **NS**
MED	1	0.9381 **NS**	0.9276**NS**	0.6702**NS**	0.3211 **NS**	0.0810 **NS**	0.5657**NS**
STL*MED	3	0.9993 **NS**	0.9989**NS**	0.9075**NS**	0.3987**NS**	0.0498 **NS**	0.9533**NS**
HORM*MED	3	0.9966 **NS**	0.9989**NS**	0.9389**NS**	0.3987**NS**	0.9305 **NS**	0.8013**NS**
STL*HORM*MED	9	1.0000 **NS**	1.0000**NS**	0.9985**NS**	0.4494**NS**	0.3737 **NS**	0.9267 **NS**
Error	64	_	_	_	-	-	-

Ns= Not significant, * = P ≤ 0.05.*Where STL- stem cutting length; HORM- rooting hormone; MED- propagation medium*

Table C 7: Summary of ANOVA table for the effect of stem length, rooting hormone and propagation media on rooting and growth parameters
of rose-scented geranium on Week 7 of the experiment

		F-probability levels for:					
Source of variance	Degree of freedom	Root number	Root length (cm)	Root fresh weight (mg)	Stem circumference (cm)	Leaf number	Shoot number
STL	3	0.0001*****	0.0001*****	0.0201*****	0.0001*****	0.0001*****	0.0001*****
HORM	3	0.0001*****	0.0001*****	0.0081*****	0.0001*****	0.0001*****	0.0001*****
STL* HORM	9	0.1711 ^{NS}	0.8939^{NS}	0.0650^{NS}	0.0494 ^{NS}	0.4247 ^{NS}	0.2975 ^{NS}
MED	1	0.0988 ^{NS}	0.8664^{NS}	0.3703^{NS}	0.6074 ^{NS}	0.0510 ^{NS}	0.0899^{NS}
STL*MED	3	0.1297 ^{NS}	0.0993^{NS}	0.6022^{NS}	0.3337^{NS}	0.0433 ^{NS}	0.6463^{NS}
HORM*MED	3	0.7380 ^{NS}	0.5333^{NS}	0.6029^{NS}	0.7221^{NS}	0.7781 ^{NS}	0.9533^{NS}
STL*HORM*MED	9	0.9977 ^{NS}	0.9797^{NS}	0.9953^{NS}	0.7738^{NS}	0.3213 ^{NS}	0.5090 ^{NS}
Error	64	_	_	_	-	-	-

Ns= Not significant, * = P ≤ 0.05.*Where STL- stem cutting length; HORM- rooting hormone; MED- propagation medium*

Table C 8: Summary of ANOVA table for the effect of stem length, rooting hormone and propagation media on rooting and growth parameters of rose-scented geranium on Week 8 of the experiment

		F-probability levels for:					
Source of variance	Degree of freedom	Root number	Root length (cm)	Root fresh weight (mg)	Stem circumference (cm)	Leaf number	Shoot number
STL	3	0.0001*****	0.0001*****	0.0412*****	0.0001*****	0.0001*****	0.0001*****
HORM	3	0.0001*****	0.0001*****	0.0381*****	0.0001*****	0.0001*****	0.0001*****
STL* HORM	9	0.1465 **NS**	0.9749 **NS**	0.0536**NS**	0.0494 **NS**	0.1294 **NS**	0.3475 **NS**
MED	1	0.0998 **NS**	0.5443 **NS**	0.9272**NS**	0.6074 **NS**	0.0710 **NS**	0.3897**NS**
STL*MED	3	0.1183 **NS**	0.0010 **NS**	0.9993**NS**	0.3337**NS**	0.1644 **NS**	0.3190**NS**
HORM*MED	3	0.8483 **NS**	0.3544 **NS**	1.0000**NS**	0.7221**NS**	0.6403 **NS**	0.9689**NS**
STL*HORM*MED	9	0.9902 **NS**	0.9986**NS**	1.0000**NS**	0.7738**NS**	0.5537 **NS**	0.1999**NS**
Error	64	_	_	_	-	-	-

Ns= Not significant, * = P ≤ 0.05.*Where STL- stem cutting length; HORM- rooting hormone; MED- propagation medium*

APPENDIX D

SUMMARISED ANALYSIS OF VARIANCE (ANOVA) TABLES

Weeks	Source of variance	Degree of freedom	Root number	Root length (cm)	Root fresh weight (mg)	Stem circumference (cm)	Leaf number	Shoot number	Plant height (cm)
1	WHP	3	0.0667 NS	0.0667 NS	0.8999 NS	0.3665 NS	0.2667 NS	0.3667 NS	0.4343 NS
	RH	3	0.3222 NS	0.3222 NS	0.9886 NS	0.0998 NS	0.3667 NS	0.5322 NS	1.0000 NS
	WHP*RH	9	0.4650 NS	0.4650 NS	0.6772 NS	0.6650 NS	0.344 NS	1.0000 NS	1.0000 NS
	Error	30	–	–	–	–	–	–	–
2	WHP	3	0.0667 NS	0.0667 NS	1.0000 NS	1.0000 NS	0.3442 NS	0.4637 NS	0.1667 NS
	RH	3	0.5222 NS	0.5222 NS	1.0000 NS	1.0000 NS	0.0988 NS	0.9322 NS	0.1122 NS
	WHP*RH	9	0.6650 NS	0.6650 NS	1.0000 NS	0.9666 NS	1.0000 NS	1.0000 NS	0.3450 NS
	Error	30	–	–	–	–	–	–	–
3	WHP	3	0.0000*	0.0000*	0.0000*	0.0000*	0.0049*	**NS**	0.2501 NS
	RH	3	**NS**	0.0473*	0.0000*	0.1515 NS	**NS**	0.0063*	0.1515 NS
	WHP*RH	9	0.0563 NS	0.2706 NS	0.2152 NS	0.0913 NS	0.0000*	**NS**	0.0913 NS
	Error	30	–	–	–	–	–	–	–
4	WHP	3	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0987 NS	**NS**
	RH	3	0.0005*	0.0000*	0.0000*	0.1515 NS	0.0000*	0.0000*	0.0767 NS
	WHP*RH	9	0.1355 NS	0.2655 NS	**NS**	0.0913 NS	0.0436*	**NS**	0.1913 NS
	Error	30	–	–	–	–	–	–	–

5	WHP	3	0.0002*	0.0000*	0.0000*	0.0223*	0.0000*	0.0093 *	0.2871 NS
	RH	3	0.0005*	0.0110*	0.0576 NS	0.1812 NS	0.0001*	0.2921 NS	0.3515 NS
	WHP*RH	9	0.0788 NS	0.14400 NS	0.0000*	0.1887 NS	0.0974 NS	**NS**	0.2913 NS
	Error	30	–	–	–	–	–	–	–
6	WHP	3	0.0000*	0.0000*	0.0002*	0.0157*	0.0000*	0.0040 *	0.2501 NS
	RH	3	0.0000*	0.0000*	0.0523 NS	0.0000*	**NS**	0.0489 NS	**NS**
	WHP*RH	9	0.04889 NS	0.1882 NS	0.0098*	**NS**	0.0001*	0.2306 NS	**NS**
	Error	30	–	–	–	–	–	–	–
7	WHP	3	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0001*	0.9232 NS
	RH	3	0.0000*	0.0000*	0.0523 NS	0.0000*	0.0463 NS	0.2016 NS	0.0614 NS
	WHP*RH	9	0.0877 NS	0.14500 NS	0.0000*	0.0000*	0.0000*	**NS**	**NS**
	Error	30	–	–	–	–	–	–	–
8	WHP	3	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000 *	0.0861 NS
	RH	3	0.0002*	0.0000*	**NS**	0.0000*	0.0460 NS	0.0611 NS	**NS**
	WHP*RH	9	0.1361 NS	0.3771 NS	0.0026*	0.0000*	0.0000*	0.0732 NS	**NS**
	Error	30	–	–	–	–	–	–	–

Ns= Not significant, * = P ≤ 0.05, *Where, WHP wound healing period; RH- rooting hormone-*