RESPONSE OF FEVER TEA (*LIPPIA JAVANICA*) TO FERTIGATION FREQUENCY, GROWTH MEDIUM AND PROPAGATION METHOD

By

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Submitted in partial fulfilment of the requirements for the degree
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DECLARATION

I hereby declare that the thesis submitted for the degree M.Sc (Agriculture): Horticulture, University of Pretoria, is my own 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 by means of a comprehensive list of references.

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Kwena Winnie Mpati Date

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Abstract

Fever tea is one of the important medicinal plants belonging to the family Verbenaceae. The leaves of the plant are used as a remedy to treat malaria, stomach pains, colds and fever. There are different clones of *Lippia* species available with different medicinal value. Therefore, the purpose of this study was to determine protocols for propagation of fever tea so as to multiply an ideal clone. Vegetative propagation of this plant species by stem cuttings, requirements for seed germination and response of fertigation frequencies and growing medium to growth, yield and quality has not been studied. In this study, factors influencing effective propagation of fever tea were studied. Those factors were: cutting position (apical vs. basal), media (pine bark vs. sand), hormone (seradix No. 2 vs. no hormone) light and temperature requirement for fever tea seed germination and effect of fertigation frequencies (0.4 L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and growing media (pine bark vs. sand) on growth, oil yield and oil quality.

In vegetative propagation by stem cuttings, measurements made were number of roots per rooted cutting, fresh mass, stem circumference and number of leaves and the rate of rooting during four consecutive sampling dates (5, 10, 15 and 20 days after planting). Apical cuttings rooted earlier by 5 days than basal cuttings at 10 days but at 15-20 days after establishment, both cuttings had good rooting. Fresh mass was not affected by cutting position and rooting media, but cuttings performed slightly better when propagated in pine bark medium than sand medium. Basal cuttings resulted in thicker stems and more leaves as compared to apical cuttings. Seradix No.2 (0.3% IBA) hormone increased the fresh mass, stem circumferences, root number and leaf numbers on both apical and basal cuttings. For the establishment of fever tea stem cuttings, both apical and basal cuttings can be used but pine bark is the ideal medium. The cuttings can be ready for transplanting in 15-20 days after establishment and Seradix No. 2 (0.3% IBA) promotes rooting of fever tea cuttings.

The ideal combination of light and temperature for seed germination of fever tea was investigated. Germination was tested at constant temperature regimes (15, 20, 25 and 30ºC with continuous light or dark period and alternating temperatures of 20:30 and 16L: 8D (light: dark) combinations respectively. Seeds started to germinate after 8 days from incubation and the last germination was observed at 30 days from incubation. Germination percentages increased at 20-30 constant temperatures and 20/30 alternating temperatures but the difference amongst them was not significant. Higher germination of 86% were achieved when seeds were exposed to continuous light than alternating light and dark. Seeds failed to germinate in continuous darkness. Fever tea seeds were positively photoblastic.

The effect of fertigation frequency and growing medium on the growth, yield and quality of fever tea were investigated in a tunnel. Treatments used were five fertigation frequencies (0.5L/day, 1L/day, 2L/day, 2L/2nd day, and 2L/week) and two growing media (pine bark and sand). Measurements made were plant height, stem circumference and number of branches at 8, 16 and 32 weeks after planting. At 8 weeks after planting all fertigation frequencies improved fever tea growth except fertigation frequency of 2L/week. All the fertigation frequencies were ideal to sustain the growth and development of fever tea plants except 2L/week. At 16 weeks after planting there were interactive effects between fertigation frequencies and the growing medium for the plant height of fever tea. Plants fertigated with 2L/day grown in sand media grew taller than all the other fertigation frequencies. At 32 weeks after planting there was a significant effect on the plant height from the main effects of fertigation frequency and growing medium. Plants fertigated with 2L/day were significantly the tallest followed by plants fertigated with 0.5L/day, 1L/day, 2L/2nd day and 2L/week. When plants were younger better plant growth was obtained in pine bark media. Stem circumference and number of branches of fever tea were significantly affected by fertigation frequency and growth medium. At 16 and 32 weeks after planting, plants grown in sand media had thicker stems and more branches as compared to plants grown in pine bark media.
The essential oil of fever tea was extracted using hydro-distillation. Fertigation frequency did not affect oil yield. Plants grown in pine bark media yielded more oil than plants grown in sand medium. Microscopical studies using scanning electron microscope were investigated to determine the development of oil glands and trichomes on the abaxial (upper) and adaxial (lower) surfaces of the leaves as affected by fertigation frequency and the growing medium. There were no significant effects on the number of oil glands and trichomes developed on both surfaces of the leaves. Pine bark medium resulted in larger oil glands than sand medium

regardless of the treatments, and pine bark also yielded more oil percentages than sand medium. Based on this investigations for commercial production of fever tea essential oil pine bark media is recommended.

Chemical compounds of fever tea oil were also not affected by fertigation frequency or growth medium. In this study the chemical compounds detected from essential oils of fever tea were monoterpenes (i.e. α-pinene, sebinen, 1.8 cinede, myrcene, ipsenone and ipsedienone) and the sesquiterpenes (i.e. β-caryophyllene and germacrene-D). Compounds that gave the smallest chemical percentages and the shortest time to be detected were α-pinene, sebinen, 1.8 cinede, myrcene, ipsenone and ipsedienone. Compounds found with the highest chemical percentages with highest peaks were β-caryophyllene and germacrene-D.

Keywords: Cuttings, seed germination, fertigation frequency, growing medium, essential oil, chemical compounds, *Lippia javanica*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The genus *Lippia* belongs to the family Verbenaceae which includes approximately 200 species of herbs, shrubs and small trees (Tereblanche & Kornelius, 1996). Several authors include some species belonging to the genus *Aloysia, Lantana, Acantholippia, Phylla* or *Junelia* in the genus *Lippia* because all these genera are closely related (Munir, 1993). The plant is mainly distributed to large parts of southern Africa and extends into tropical African territories (Tereblanche & Kornelius, 1996). Fever tea is one of the most important medicinal plant scientifically known as *Lippia javanica*.

Leistner (2000) reported fever tea to be an erect woody shrub that grows up to 2 metres in height. The leaves are opposite or in whorls of 3(4), rarely alternate but mostly aromatic with a strong lemon smell. Its flowers are sometimes irregular, arranged in lax to dense form and have glubose spikes which become cylindrical as the fruit ripens. Each flower is supported by a bract. The fruit is small with a dry epicarp separating into 2 or have 1-seeded pyrenes. The seeds are without an endosperm.

The plant is traditionally utilized as a respiratory remedy (Morton, 1981). The leaf infusions are used for various chest ailments, influenza, measles, rashes, malaria, stomach problems and headaches (Tereblanche & Kornelius, 1996; Van Wyk, 1997). The Zulu communities use the leaves to treat febrile rashes and sometimes it is smeared on the body as a protection against dogs and crocodiles (Doke & Vilikazi, 1972). Xhosas use the leaves for fevers and measles (Watt & Breyer-Brandwijk, 1962). The VhaVenda use leaf infusions as anthelmintics for respiratory and febrile ailments and also as prophylactics against dysentery, diarrhoea and malaria (Mabogo, 1990). According to Gelfand, Mavi, Drummond & Ndemera (1985) in Zimbabwe leaves are used for a variety of ailments including asthma, headaches, febrile and respiratory complaints, convulsion, weak joints and sore eyes.

In Botswana roots are used as antidotes for suspected food poisoning and for bronchitis and sore eyes (Hedberg & Staugard, 1989). In West Africa, leaves and roots are used for fevers, headaches and skin diseases. It is also recommended as an effective mosquito repellent due to its insecticidal properties. And these properties could be potentially exploited to provide

pesticides that are safer, and considerably less environmentally damaging than synthetic chemicals (Mwangi, Addae-mensah, Muriuki, Munavu, Lwande & Hassanali, 1992).

Fever tea is rich in volatile oil and numerous monoterpenoids have been identified, including myrcene, caryophyllene, linalool, p-myrcene and ipsidienone. There are various organic acids and alcohols occurring in the plant. Iridiod glycosides and toxic triterpenoids have been detected in some *Lippia* species. Volatile oils are found to have decongestant and antiseptic effects but the fever reducing and possible pain relieving activities need further study (Watt & Breyer-Brandwijk, 1962; Van Wyk, Van Oudshoorn & Gericke 1997).

There are different clones of *Lippia* species available with different medicinal value. However, vegetative propagation of this plant using stem cuttings so as to multiply an ideal clone and the effect of rooting media and hormone on cuttings has not been studied. Furthermore, the germination requirement of fever tea and the effect of fertigation frequencies on growth, yield and quality of fever tea is not known.

The objectives of this study were to

- Determine the effect of cutting position (apical vs. basal), rooting media (sand vs. pine bark) and rooting hormone (seradix no.2 vs. non seradix no.2) of the stem cuttings of fever tea
- Determine the ideal seed germination temperature and light combinations for fever tea
- Determine the effect of fertigation frequency and growing medium on growth, yield and quality of fever tea

CHAPTER 1

LITERATURE REVIEW

1.1 **Introduction**

A medicinal plant is a plant that contains valuable chemical compounds known to confer well being to people and cure illness. Seventy percent of South African population make use of traditional remedies. Medicinal plants have been used over many centuries, however, many people still rely on them even in this sophisticated world of our present time. They have always been considered important and effective for the treatment of many different diseases in Africa and other parts of the world (Adimoelja, 2000). Because of the fear of diseases and death, every cultural group has responded by developing a medicinal system that makes use of natural products to cure various ailments, and undoubtedly plants play a major role (Ellis, 1996). In South Africa a large part of the day-day medicine is still derived from plants and large volumes of plants and their extracts are sold in the informal and commercial sector of the economy (Van Wyk *et al.*, 1997).

Plant propagation is the multiplication of plants by both sexual and asexual reproduction. Successful propagation of plants requires knowledge of plant growth, its development, morphology, different kinds of plants and various possible methods by which certain plants can be propagated. Through ancient civilization man discovered plant regeneration through propagation techniques and one of it was vegetative propagation (Hartmann & Kester, 1983). Reasons for using vegetative propagation are ease and convenience during propagation, shorter time to reproductive maturity, combination of more than one genotype into a single plant, and for control of growth phases and morphology (Hartmann, Kester, Davies & Genever, 1997). Among these propagation techniques propagation by cuttings was the easiest method to be chosen (Hartmann & Kester, 1990). This method of propagation was discovered to be advantageous due to the fact that many new plants can be started in a limited space from a few stock plants, and it is inexpensive, rapid and simple. In other words, it does not require special techniques necessary in grafting, budding or micro propagation. Another method of plant propagation was through seeds. Seed germination is affected by environmental factors such as availability of moisture, light, temperature and these factors vary with plant species (Hartmann & Kester, 1983; Copeland &McDonald, 1985; Hartmann *et al.*, 1997).

1.2 **Vegetative propagation by stem cuttings**

Many plants can be propagated by different types of cuttings with satisfactory results. In propagation by stem cuttings, segments of shoots containing lateral or terminal buds are obtained with the expectation that under the proper conditions, adventitious roots will develop and thus produce independent plants. Cuttings prepared from the soft, succulent, new spring growth of deciduous or evergreen species may properly be classed as softwood cuttings (Hartmann *et al.,* 1990).

1.2.1 **Position of cuttings**

Cutting position is the most important variable, which was found to be closely related to rooting ability in a number of species (Leakey & Mohammed, 1985). In many vegetatively propagated species, older lignified woody cuttings are more difficult to root than newly formed stems (Hartmann *et al*., 1990). Cuttings can be divided in terms of their position into apical, medial and basal cuttings. The effect of position seems to be species dependent, since in some species basal or medial cuttings root best, whereas in others, apical cuttings root best (Hartmann *et al*., 1997).

Apical cuttings

Apical cuttings are mostly softwood or semi-hardwood cuttings with developing apex and associated young leaves, which may produce rooting promoters that could be inhibitors if present in excess (Hansen, 1986; Leakey & Coutts, 1989). Studies were undertaken on the influence of position on cuttings of *Syzygium paniculatum.* The results showed that development of roots was higher among those taken from the median and lower parts of the plant than from the apical part. The natural accumulation of endogenous auxin was favourable to the development of roots in the basal than in the apical part (Lebrun, Toussaint & Roggemans, 1998).

Medial cuttings

The proportion of *Sygium paniculatum* cuttings that developed roots was significantly higher among those taken from the medial parts of the plant than from the apical part. Furthermore,

roots developed earlier on cuttings originating from basal and medial parts of the stock plant than on those from the apex (Lebrun *et al*., 1998). A similar result was reported by Hansen (1988) when the upper and basal cuttings of *Strephanotis floribunda* were produced with fewer roots than medial cuttings.

Basal cuttings

Basal cuttings are usually made just below the node; they can either be from semi-hardwood or softwood cuttings. In many vegetatively propagated plants highest rooting were found when cuttings were taken from the basal portion of the shoot (Hansen, 1986; Hartmann *et al*., 1997). Good results were found when three cultivars of high bush blueberry (*Vaccinium corymbosum*) were taken from three nodal positions (sub-apical, medial and basal) and basal cuttings produced significantly higher rooting percentage than the other types of cuttings (Hartmann *et al*., 1990).

The good rooting ability of basal cuttings could be due to the somewhat higher food reserves as total sugars or it could have been due to the accumulation of natural auxins in the shoot bases or other root promoting factors with the relatively low levels of rooting inhibitors (Al-Saqri & Alderson, 1996). Rooting was initiated as early as the third week on basal cuttings, in the fourth week on median ones and in the sixth on the apical ones when comparing the influence of cutting position on *Syzygium paniculatum* (Lebrun *et al*., 1998).

1.2.2 **Influence of growth hormones on stem cuttings**

It has been widely documented that auxins promote adventitious root development of stem cuttings through their ability to promote the initiation of lateral primordia and to enhance transport of carbohydrates to the cutting base (Leakey, 1983; Hartmann *et al*., 1990). However, experiments with a range of tree species have indicated highly contrasting responses to IBA addition. For example, the optimum IBA concentration for rooting *Triplochiton scleroxylon* was found to be 0.4% (Leakey, 1983) and for rooting *Milisia excelsa* it was found to be 0.2%.

The purpose of treating cuttings with auxin is to increase the percentage of rooting, root initiation, number of roots and uniformity of rooting (Al-Barazi & Schwabe, 1982). It also

accelerates the translocation of nutrients from upper part of the cuttings to their basal ends by increasing the activity of enzymes. This increases hydrolysis of carbohydrates by providing enough energy in rooting respond of the cells (Arya, Tomar & Tokyt, 1994). As reported by Al-Barazi & Schwabe (1982), occasionally IBA treatment seems to stimulate cell division in the ray cells between the primary bundles to improve root initiation and to increase uniformity of rooting.

According to Jwanda, Singh, Singh, & Bal (1991) the highest rooting percentage of plum cuttings was recorded in the treatment of 0.01% IBA followed by 0.005% IBA, whereas it was the lowest in the control. Ofori, Newton, Leakey & Grace (1996) also reported that 0.002% IBA treatment increased the final rooting percentage of *Milicia excelsa* by 9% above that of the control. Puri & Vermat (1996) also supported this when they reported that the application of auxin to cuttings of *Dolbergia sisso* triggered and enhanced rooting.

Even though auxin promote adventitious root development of stem cuttings, the use of auxins at the appropriate concentration is essential because the wrong concentration can inhibit rooting or it can act as a growth retardant when applied in higher concentrations (Hartmann *et al*., 1990; Wiessman-Ben & Tchoundjeu, 2000). Hartmann *et al*. (1990) supported this when they reported that rooting response of cuttings was found to be dependent on the concentration of auxin. High concentration added to the endogenous auxin of the plant, which itself increases as a consequence of taking cuttings, might have led to a level which disturbed the hormonal metabolism and inhibited rizogenesis (Lebrun *et al*., 1998).

It is evident that the cuttings of different plants require different optimum concentration of auxins for the best rooting. *Cordia alliodora* was found to require a concentration of 1.6% IBA and 0.8% for the optimum rooting percentage but it failed to root when no auxin was applied (Mesen, Newton, & Leakey, 1997). Similar results were reported by Leakey & Mohammed (1985) in rooting of *Triplochiton scleroxylon*. On the other hand, successful rooting without applied auxin has been reported in a number of other tropical tree species, such as *Nauclea dierrichii* and *Vochsia hondurensis* (Leakey, 1990), *Shroea macrophlla* (Lo, 1985), *Milicia excelsa* (Ofori *et al*., 1996). This implies that these species are well supplied with the endogenous auxin (Ofori *et al*., 1996). Furthermore, rooting percentage of many plants was found to decline with the successive increase in IBA concentration, such that the percentage of roots in cuttings with the lower concentration was higher than those with the

higher concentration (Leakey, 1990). For example, application of 0.8% and 1.6% of IBA to the cuttings of *Milisia excelsa* generally inhibited rooting (Ofori *et al*., 1996). Deen & Mahmoud (1996) also stated that auxin stimulate root formation of Rosemary (*Rosmarinus officinalis* L.) if applied in dilute concentration and tended to produce the longest and heaviest mass of roots. Application of higher dosage (0.2%) appeared vigorously to cause visible damage, root weight reduction and inhibitory effect in rooting of Rosemary cuttings. Rooting of cuttings is also affected when rooting hormones are used in combination with each other (Smalley, Dirr & Armitage, 1991).

Deen & Mahmoud (1996) showed that terminal and basal cuttings of Rosemary (*Rosmarinus officinalis* L.) produced more number of roots/ cuttings with auxin application compared to the saponin concentration or the control. Puri & Vermat (1996) supported this report when NAA treated cuttings of *Dalbergia sisso* showed higher response than IBA treated cuttings, but the difference was not significant.

Struve & Arnold (1986) studied the effect of esters of IBA to increase rooted cutting quality of red maple 'Red Sunset' softwood cuttings. Softwood cuttings of the red maple (*Acer rubrum*) were given 3-sec basal dips in five auxins at four concentrations. Auxins used were IBA, K-IBA, N-IBA, P-IBA and P-ITB. All auxins were prepared in 10, 20, 30 and 40 mM concentrations. Cuttings treated with 30 mM IBA had the highest percentage of rooting (70%) and other treatments exceeding 50% rooting were 20 mM K-IBA and 30 mM P-IBA and P-ITB. Cuttings treated with 30 and 40 mM P-IBA had more roots per rooted cutting and more total root length compared with 30 mM IBA treated cuttings. Hassig (1983) experimented with 3 aryl esters of IBA and IAA. The aryl esters of IBA, NP-IBA, P-ITB and P-IBA increased rooting response in mung bean cuttings and stem cuttings of jack pine seedlings and trees. NP-IBA was the most effective auxin tested; it decreased time to first root generation and increased percentage of rooting, number of roots per cutting and total root length per cutting compared with IBA.

Schrader & Graves (2000) experimented on the propagation of *Alnus maritima* from softwood cuttings. The cuttings were treated with IBA at 0, 1 and 8 g/kg and placed under intermittent mist in the greenhouse for 9 weeks. The results showed that the use of IBA at 8 g/kg caused a greater rooting percentage (68%), root count (7.2) and root length (39.2 mm) than did the other IBA rates when applied to cuttings. It was concluded that softwood cuttings from

mature plants are an effective way to multiply clones, particularly when cuttings are collected early in the season and treated with IBA at 8 g/kg.

Read & Yang (1991) studied the effect of plant growth regulators on rooting softwood cuttings of *Ligustrum vulgaris* and *Viburnum dentatum*. Giberrellic acid (GA_3) and indolebutyric acid (IBA) were applied on the cuttings. The results showed that application of GA_3 and IBA in the forcing solution strongly influenced rooting responses of the softwood cuttings. IBA increased root number per cutting and promoted root elongation, while GA_3 inhibited rooting.

McGuigan, Blazich & Ranney (1996) studied propagation of stem cuttings of two clones (Clone 1 and 2) of *Quercus phillyreoides*. All the cuttings were treated with selected concentrations and formulations of IBA and placed under intermittent mist for rooting. The greatest rooting for both clones was achieved with softwood cuttings with 97% and 56% rooting for Clones 1 and 2 respectively treated with 8000 ppm (0.8%) IBA in talc. For both clones, rooting of semi-hardwood cuttings was poor, which was the same for hardwood cuttings of Clone 2. Auxin treatment generally increased root number. Greater over winter survival was observed for rooted softwood cuttings, which produced a flush of new growth following rooting in comparison to semi-hardwood cuttings that did not flush following rooting.

Wiesman & Lavee (1995) experimented on the enhancement of IBA stimulatory effect on rooting of an olive cultivar stem cuttings. Three groups of olive cultivars were characterised as showing low, moderate or high rooting percentage after application of IBA. To improve the rooting percentage of olive cuttings, urea phosphate (UP) and paclobutrazol (PB) were tested in combination with IBA. It was found that UP alone did not stimulate rooting of olive cuttings. However, when applied together with IBA it significantly enhanced the rooting of cuttings of the cultivar Manzanillo. PB alone had a weak effect on rooting of cuttings but in combination with IBA it improved the rooting of cuttings of cultivars Manzanillo and Souri. A triple combination of IBA, UP and PB provided the most effective treatment for the improvement of rooting percentage. IBA treatments increased the number of roots per cutting in comparison with the control but decreased the length of roots of cultivar Barnea. Thus, UP and PB were shown to enhance the effect of IBA in stimulation of rooting of olive cuttings.

The effect of five different concentrations of IBA (0, 0.2, 0.4, 0.8 and 1.6%) on the rooting percentage and the number of roots per rooted cuttings of single-node, leafy stem cuttings of *Cordia alliodora* were investigated. Nine weeks after insertion, highly significant differences in rooting percentage were found between IBA concentrations with a value of 70% recorded in cuttings treated with 1.6% IBA, compared with only 10% for the control cuttings. The effect of IBA concentration on number of roots per rooted cutting was also significant. It was confirmed that rooting was highly dependent on IBA concentration (Mesen *et al*., 1997).

1.2.3 **Effect of growing media on stem cuttings**

Cuttings of many species root successfully in a variety of rooting media but those that are more difficult to root may be greatly influenced by the kind of rooting medium used, not only in percentage of cuttings rooted but in the quality of root system formed (Leakey, 1990). The rooting media should hold the cutting in place during the rooting period, provide moisture for the cutting, permit penetration and exchange of air at the base of the cutting and reduce light penetration to the cutting base (Hartmann *et al*., 1990). Most frequently used mediums contain combinations of sand, peat, sphagnum moss, vermiculite, perlite, compost and shredded bark (Hartmann *et al*., 1997).

Studies were undertaken in rooting stem cuttings of *Clematis vitalba* in different substrates (perlite, peat-perlite, and sand-perlite). The rooting percentage and the rate of rooting were higher in pure perlite and lower in peat perlite. The number of primary roots and root dry mass of stem cuttings were higher in sand-perlite and pure perlite (Kreen, Svensson & Rumpunen, 2002).

Rosa centifolia cuttings showed that root length and rooting percentage have some dependence on the type of medium used. Peat perlite resulted in a significantly higher rooting percentage and length than in vermiculite, although the media did not affect mean root number per rooted cutting (Al-Saqri & Alderson, 1996). The same results were observed in *Milicia excelsa* when cuttings inserted in pure sawdust and in a mixture of coarse sand and sawdust displayed higher rooting percentage and higher mean number of roots per rooted cuttings than those in coarse sand and fine sand (Ofori *et al*., 1996). Some authors reported that the type of media could affect the position of cuttings. In apical and basal cuttings of *Rosa centifolia* there was a significant reduction in percentage of rooting in vermiculite, while

the medial and sub-apical cutting in peat-perlite had more roots than in other treatments (Al-saqri & Anderson, 1996).

The effect of rooting substrate on *Cordia alliodora* was also studied. The three rooting media used were sawdust, gravel and sand. The three rooting media showed clear differences in their relative proportions of solids, water and air. The water component was higher in sawdust (53.8%) than in sand (17.6%) and gravel (4.5%). The air content was similar in sawdust (30.3%) and gravel (30.5%) and relatively low (5.5%) in sand. Rooting percentages were relatively high with values over 75% in all medias.

However, highly significant differences were found in rooting percentages of all the medias. The rooting percentage in both gravel and sand were significantly higher than in sawdust. In general, media with relatively high water content such as sawdust are associated with higher rates of water uptake in the cutting and consequently higher rooting percentage. However, water can present a major diffusion barrier to oxygen and excess water may, thereby, result in anoxia within the cutting base (Loach, 1992). Rotting of the cutting base in sawdust was frequently observed indicating that *C. alliodora* requires a relatively well-aerated medium for cutting survival. The fewer roots per rooted cutting recorded in sawdust may also reflect anoxia as recorded with *Chrysanthemum* (Hartmann *et al.,* 1990) and Cupressocyparis *leylandii* cuttings (Loach, 1992). Both the rooting percentage and the number of roots per cutting were reduced when sawdust was used, in comparison with sand and gravel indicating that rooting substrates with relatively low water holding capacities may be appropriate for propagation of these species (Mesen *et al.,* 1997).

The effect of leaf areas on cuttings of *Milisia excelsa* has also been studied. In many species, the presence of leaves on cuttings exerts a strong stimulatory influence on root initiation (Hartmann *et al.,* 1990). This reflects the role of foliage as a source of both auxins and carbohydrates. In *Triplochiton scleroxylon* the inability of leafless cuttings to root was associated with rapid depletion of carbohydrates in the stem, emphasizing the importance of the leaf as a carbohydrate source. The fact that 30% of leafless cuttings of *Milisia excelsa* were found to root suggest that both current assimilates and carbohydrates produced pre-severance and stored in the stem may influence the rooting of this species (Ofori *et al*., 1996). In species such as *Nauclea didrichii* (Leakey, 1990) and *Terminali spinosa* (Newton, Muthoka & Dick 1992) variation in leaf area had little effect on the final rooting percentage.

In contrast, variation in cutting leaf area had a substantial effect on the percentage rooting of *Milisia excelsa*. This confirms with results obtained for relatively difficult-to-root species such as *Triplochiton scleroxylon, Cleistopholis glauca*, *Terminalia ivorensis* and *Khaya ivorensis* where optimum leaf areas for rooting were found to be 50 cm², 50 cm², 100 cm² and 10 to 30 cm² respectively (Leakey, 1983).

1.2.4 **Environmental factors affecting root development on stem cuttings**

The environmental factors that affect rooting of stem cuttings are light, humidity, carbon dioxide, water, temperature and shading. Loach (1992) reported that it is important to minimize transpiration from cuttings, which lack roots because transpired water cannot be easily replaced. It is also important to develop conditions that promote photosynthetic production of carbohydrates, especially when primordium is well advanced.

Light

Radiation plays an important role in rooting response, in adventitious root development and survival ability of cuttings (Al-Saqri & Alderson, 1996; Palanisamy & Kumar, 1997). Light influences the level and translocation of growth hormones as well as assimilate production. Cuttings of neem (*Azadirachta indica*) were planted in the shade house and natural conditions and given similar watering, temperature and humidity except radiation. The percentage of rooting was higher in the shade house than in natural conditions. This indicated that low radiation might have a positive role on enhancement of rooting. Eliasson & Brunes (1980) also reported similar results in rooting of aspen and willow. Hansen (1988) stated that higher radiation enhanced rooting in *Pinus bankisiana* and *P. sylvestris* than low radiation.

Most researchers reported that shading of cuttings was once thought not to be advantageous for root development. It was believed that shading hindered the photosynthesis process, which would reduce the rooting activity of cuttings. But shading prevents leaf scorch, and aids in the prevention of excess build up of carbohydrates, which will actually hinder the development of rooting systems (Wiessman-Ben & Tchoundjeu, 2000). It also limits transpiration by reducing the energy load on the leaves (Harrison-Murray, Thompson & Knight, 1992).

Temperature

Temperature is one of the most important aspects of successful propagation. As temperature rises, the respiration of a plant will also rise. Hartmann *et al*. (1997) reported that in common terms higher temperatures, to some point, will generate higher activity levels in the plant. According to Alegre, Toledo, Martinez, Mora & De Anderes (1998), in the propagation of *Dorycnium pentaphyllum* and *D. hirstum* cuttings the temperature of the rooting environment significantly affected each variable. According to Hartmann *et al*. (1997) temperatures between 18 ºC to 25 ºC and 25 ºC to 32 ºC are considered optimum for rooting in most temperate and tropical species, respectively.

Water

Water potential is extremely important with any type of cutting propagation. Cuttings do not have the root system and, therefore, they cannot maintain turgor pressure in the absence of water, even for short periods of time. This makes the availability of water critical to survival of cuttings (Loach, 1992). Not only the availability of water is important but also the quality of water. The presence of soluble salts in the water can create a hostile environment for a cutting trying to root. Soluble salts in excess of 1400 ppm could inhibit growth (Hartmann *et al.,* 1990).

Season

Season is one of the major factors that affect rooting success of cuttings (Klein, Cohen & Hebbe, 2000). Seasonal variation in rooting efficiency is very common in woody plants and there is optimal time for root establishment for each species (Howard, 1996). Rooting of pistachio cuttings from mature trees has been unsuccessful regardless of season (Al-Barazi & Schwabe, 1982). Puri & Vermat (1996) stated that cuttings of *Dalbergia sissoo* could be rooted in spring and monsoon seasons, while winter cuttings did not root at all. According to Hartmann *et al*. (1990) and Wilson (1993) softwood cuttings taken in summer usually tend to root more easily than cuttings taken in the winter. In contrast, the percentage and number of roots of Eastern red cedar cuttings increased during winter (Henry, Blazich, & Hinesey, 1992). On the other hand, rooting of cuttings of Mytaceae family (*Chmaelaucium sp*) is unaffected by season (Curir, Sulis, Mariani, Van Sumere, Marchesini & Dolci, 1993).

In most vegetatively propagated plants, growth activity is low during winter. However, as the temperature rises, carbohydrates and growth promoters are mobilized and help root development. A number of studies suggested that rooting of cuttings is facilitated when carbohydrates and growth promoters are in abundance (Puri & Vermat, 1996).

1.3 **Seed germination**

In the germination process, the seed's role is that of a reproductive unit; it is a thread of life that assures the survival of all plant species. Germination is defined as the protrusion of the radicle through the surrounding seed covering. The major event that occurs in germination includes water imbibition, enzyme activation, storage tissue breakdown, initiation of embryo growth, rupture of the seed covering and establishment of the seedling (Copeland & McDonald, 1985).

1.3.1 **Effect of temperature and light on seed germination**

Temperature

Plant growth is very sensitive to temperature. Each species has at any given stage in its lifecycle and in any given set of conditions, a base temperature below which it will not grow, and an optimum temperature (or range of temperatures) at which it grows well (Salisbury & Ross, 1992). Ikuma & Thimann (1964) reported that temperature affects both germination percentage and germination rate. The effect on germination can be expressed in terms of cardinal temperatures, that is the minimum, optimum and maximum temperatures at which germination occurs. The minimum temperature is sometimes difficult to define since germination may actually be proceeding but at such a slow rate that denaturation of germination is often made before actual germination is completed. The optimum temperature may be defined as the temperature giving the greatest percentage of germination within the shortest time. The maximum temperature is governed by the temperature at which denaturation of proteins essential for germination occurs (Copeland & McDonald, 1985).

The response to temperature depends on a number of factors including the species, variety, growing region, quality of the seeds and duration of time from harvest. As a general rule,

temperate region seeds require lower temperatures than do tropical region seeds and wild species have lower temperature requirements than do domesticated plants (Copeland & McDonald, 1985).

Godwin, Aflakpui, Gregory & Froud-Williams (1998) experimented on the effect of temperature on seed germination rate of *Striga hermonthica.* The seeds were conditioned on a moist filter paper either at 30 ºC for 14 days or 20 ºC for 15 days in an incubator. A synthetic stimulant, GR-7 was applied to conditioned seeds prior to exposure to various temperatures. No seeds conditioned at 30 ºC germinated at < 0 ºC or > 42.5 ºC within 312 h. For seeds conditioned at 20 ºC, germination occurred at temperatures between 25 and 42.5 ºC within 312 h. A positive linear relationship was established between the rate of germination of 90% of the final germination percentage and temperature up to the respective optimal temperature. The rate of germination at 20 ºC was found to be lower than for seeds conditioned at 30 ºC. These results correspond with those of Hsiao, Worsham & Moreland (1979) and Reid & Parker (1979) that temperatures between 30 and 35 ºC have normally been regarded as optimal for the germination of *Striga* seeds.

Cantliffe, Sung & Nascimento (2000) experimented on lettuce seed germination. The optimum temperature for lettuce seed germination varied with the genotype. It was found that the optimum temperature of most lettuce genotypes was between 15 ºC and 22 ºC.

An experiment was conducted in Argentina on the effect of temperature on germination of *Prosopis argentina* and *Prosopis alpataco*. The objective of the study was to determine the germination response to different temperatures for seeds of both species and other seed characteristics affecting the germination process. Under controlled conditions, daily and final germination percentages, germination rates and seedling vigour were determined. The following temperatures were tested: 10, 15, 20, 25, 30, 35, 40 and 45 ºC. The mean seed weight and the two greatest seed diameters were registered. *P. argentina* seeds showed 100% germination between 20 ºC and 40 ºC on the seventh day of incubation. Those incubated at 15 ºC only reached 66% achieving 100% on the twelfth day. At 45 ºC there was practically no germination. On the seventh day of incubation, *P. alpataco* showed 100% germination between 15 ºC and 40 ºC. At 45 ºC, 22% of seeds germinated although radicles did not extent afterwards. No seeds of either species germinated at 10 ºC. There were significant differences in germination rates at different temperatures for both species (Villagro, 1995).

The germination requirements of *Cladium jamaicense* and *Typha domingenis* were studied under controlled conditions in the laboratory. Treatments included six temperature regimes (constant temperatures of 15, 20, 25, 30 and 2 fluctuating day: night temperature regimes of 25:10 ºC and 30:20 ºC), light levels (14:10h light: dark photoperiod) and 24h dark environment. The average incubation period needed for seeds to germinate was shorter for *T. domingensis* (1.1 to 19.5 days) than for *C. jamaicense* (26 to 46 days) and the final germination percentage was higher for *T. domingensis* than for *C. jamaicense* (85% vs. 42%). *C. jamaicense* only germinated with fluctuating temperatures whereas *T. domingensis* germinated at all temperatures (Lorenz, Brix, Karen, McKee, Irving, Mendelssohn & Shilimiao, 2000).

Ramin (1997) worked on the influence of temperature on germination of *Taree irani*. The experiment was carried out to determine the effect of various constant (6, 10, 15, 20, 25, 30 and 35 ºC) and alternating (15/5, 20/10 and 30/20 ºC) temperatures on seed germination. Results obtained from these experiments showed that in the absence of other limiting factors (e.g. water) the germination of taree seed cultivars Shadegani and Isfahani was highly controlled by temperature. This finding is in general agreement with the report by Garcia-Huidobro, Moteith & Squire (1982a) in pearl millet; Covell, Ellis, Roberts & Summerfield (1986) in grain legumes and Mwale, Azam Ali, Clark, Bradley & Gatha (1994) in sunflower. The rate of germination in both cultivars was higher between 15 to 20 ºC and it was lower at both sub- and supra-optimal temperatures.

Temperature plays an important role in controlling the growth and development of plants. Jacobsen & Back (1998) experimented on the influence of temperature on seed germination rate in quinoa (*Chenopodium quinoa* Willd.). The relationship between germination and constant temperature in the range 8, 10, 15, 20, 25, 30, 35 ^{o}C were tested for quinoa. The results indicated that the optimal constant temperature for maximal germination percentage was between 15 and 20 ºC. The optimal temperature for germination rate estimated for the germination curves seem to be 30 to 35ºC for up to 80% germination, and 25 to 30 ºC for the higher level of cumulative germination.

Light

Light is one of the most important environmental factors regulating growth and development of plants. When absorbed by chlorophyll, light represents a source of energy. However, when absorbed by phytochrome, light controls plant photomorphogenesis. Phytochrome exists under a red (Pr) and a far-red (Pfr) absorbing distinct forms. During exposure to red light, Pr undergoes photochemical conversion to a physiologically active far-red absorbing form, Pfr, which triggers a variety of complex physiological responses such as flowering and seed germination (Mancinelli, 1994).

Among cultivated species there is very little evidence for light as a factor influencing germination. The seeds of most cultivated plants usually germinate equally well in the dark and in the light. Seeds may be divided into those which germinate only in the dark, those which germinate only in continuous light, those which germinate after being given a brief illumination and those which are indifferent to the presence or absence of light during germination (Mayer & Poljakoff-Mayber, 1975). Several experiments have been conducted and the response of seeds of different species to light are classified in Table 1.1.

In many cases light controlled seed germination is a phytochrome-mediated response (Frankland, 1976). The promotion of seed germination takes place after exposure to white or red light which results in a high equilibrium ratio of Pfr versus Pr. Giba, Grubisic & Konjevic (1995) studied the involvement of phytochrome in light induced germination of blue berry (*Vaccinium myrtillus L.*) seeds. The results showed that the germination of *V. myrtillus* seeds depended on the red light irradiation time. The escape time for blue berry seeds was found to be about 100 hrs.

Bewley & Black (1994) experimented on the effect of light on seed germination of *Caesulia axillaris*. The seeds were kept at 28 ± 1 ºC for germination in light-proof polythene bags lined with black cloth. Seeds of *C. axillaris* showed absolute dependence on light for germination after harvest. A 10-minute exposure to red light caused 59% seed germination. Similar results were observed earlier by a number of workers. Far-red light could also induce 43% germination of seeds in *C. axillaris*. Far-red light is known to promote germination in a few species. A brief 10 minutes blue light exposure induced 50% germination.

The effect of light on germination of Begonia (*Semperflorens cultorum*) seeds was tested. The effect of irradiance was investigated by subjecting the seeds to continuous irradiance, continuous darkness and ambient irradiance. Continuous irradiance was tested in a controlled environment growth chamber while continuous darkness and ambient irradiance were tested in a controlled environment glass greenhouse. Results showed that light was necessary for germination. However, there was no difference in the percentage of germination between seeds exposed to continuous irradiance and ambient conditions (87% and 91% respectively). At 5 days after sowing, seeds in one petri-dish from the dark treatment were exposed to ambient irradiance in the greenhouse for 10 seconds then refoiled. Seeds that were in darkness for the entire 21 days did not germinate while those in the petri-dish that had been briefly exposed to light did germinate (radicle emerged), thus indicating a very low total irradiance requirement for germination (Jacobsen & Back, 1998).

Seeds of many plant species, mostly those that inhabit open, or frequently disturbed habitats, require light to germinate. Environmentally induced photosensitivity of seeds is often interpreted as an adaptation ensuring that seeds will germinate in sites in which the probability of seedling establishment is high. Such mechanism will be particularly advantageous for species with small seeds whose seedlings cannot survive in established vegetation (Gross, 1985).

Table 1.1 Response of seeds of different species to light, A-seeds whose germination is favoured by light; B-seeds whose germination is favoured by dark and C, seeds indifferent to light or dark (Mayer & Poljakoff-Mayber, 1975)

A	B	C
Adonis vernalis	*Ailanthus glandulosa*	*Anemone nemorosa*
Alisma plantago	*Aloe variegata*	*Bryonia alba*
Bellis perennis	*Cistus radiatus*	*Cystisus nigricans*
Capparis spinosa	*Delphinium elatum*	*Datura stramonium*
Colchicum autumnale	*Ephedera helvetica*	*Hyacinthus candicans*
Erodium cicutarium	*Evonymus japonica*	*Juncus tenagea*
Fagus silvatica	*Forsynthia suspense*	*Linaria cymbalaria*
Genista tinctoria	*Gladiolus communis*	*Origanum majorana*
Helianthemum chamaecistus	*Hedera helix*	*Pelargonium zonale*
Iris pseudacorus	*Linnaea borealis*	*Sorghum halepense*
Juncus tenuis	*Mirabilis jalapa*	*Theobroma cacao*
Lactuca scariola	*Nigella damascena*	*Tragopo pratensis*
Magnolia grandiflora	*Phacelia tanacetifolia*	*Vesicaria viscosa*
Nasturtium officinale	*Silene conica*	
Oenothera biennis	*Tamus communis*	
Panicum capillare	*Tulipa gesneriana*	
Resedea lutea	*Yucca aloipholia*	
Salvia pratense		
Suaeda maritime		
Tamarix germanica		
Taraxacum officinale		
Veronica arvensis		

1.4 **Fertigation**

Irrigation and fertilization are the most important management factors through which farmers control plant development, fruit quality and yield. The maintenance of nutrients and water at optimum levels within the rhizosphere of plants is a primary factor to achieve high yield, to improve quality and increase fertilizer and water use efficiencies. Because of this, the application of fertilizers through the irrigation system became a modern irrigation practice in agriculture (Phene & Beale, 1976; Phene & Sanders 1976; Hairstone, Scheepers & Conville, 1981; Elfving, 1982; Papadopoulos 1985; 1986a; 1986b; 1987a; 1988b). Fertigation is defined as the application of nutrients using an irrigation system by introducing the nutrients into the water flowing through the system. Papadopoulos & Eliades (1987) reported that application of fertilizers with the irrigation water (fertigation) is a regular and widely accepted fertilizing practice under greenhouse production.

According to Goldberg & Shmueli (1969; 1970) fertilizers were probably the first chemicals to be injected in the modern irrigation systems. Many types of chemicals have been injected into irrigation systems, including herbicides, fungicides, insecticides, nematicides, growth regulators and fumigants (Goldberg & Uzad, 1976). This process of adding agrichemicals to the soil with irrigation water is called "chemigation" and when the chemicals are fertilizers is "fertigation". Fertigation has proved to be a more efficient means for the application of fertilizers than other conventional practices (Phene & Beale, 1976; Ingestad & Lund 1979; Haynes, 1985). Some potential advantages of fertigation are: improved efficiency of fertilizer recovery (Phene & Beale 1976; Miller, Rolston, Rauschkolb & Wolfe, 1981), minimal fertilizer losses due to leaching (Bresler, 1977; Papadopoulos, 1985), control of nutrient concentration in soil solution (Bar-Yosef, 1977; Papadopoulos, 1986a; 1986b; 1987c), control of N-fertilizers and flexibility in timing of fertilizer application in relation to crop demand based on the development and physiological stage of crops (Snyder & Burt 1976; Bresler, 1977; Kovach, 1983). Fertigation reduces fluctuation of soil solution salinity due to fertilizers (Papadopoulos, 1985), thereby improving soil solution conditions particularly for salt sensitive crops (Papadopoulos, 1986b; 1987c), and conserve labour and energy. In areas with insufficient rainfall, fertigation offers the best and sometimes the only way of ensuring that nutrients enter the root zone. Fertigation through drip irrigation further allows crops to be grown on marginal lands, such as sandy and rocky soils, where accurate control of water and nutrients in the plant's root environment is critical (Bar-Yosef, 1977; Papadopoulos, 1988b; 1989; 1990a; 1990b).

Possible disadvantages include unequal chemical distribution when irrigation system design or operation is faulty, over fertilization in places where irrigation is not based on actual water requirements or leaching if rainfall occurs at the time of fertilizer application, and chemical reaction in the irrigation system leading to corrosion, precipitation of chemical materials and/or clogging of outlets (Bar-Yosef, 1977; Papadopoulos, 1988b; 1989; 1990a; 1990b).

1.4.1 **Crop response to fertigation**

Fertigation has increased dramatically in the past 15 years, particularly for sprinkler and drip systems. For drip systems, the expansion is mostly in horticultural and high value crops. Bhella (1986) and Karlen & Robbins (1983) reported that drip irrigation is an effective method of supplying water and soluble nutrients to plant growing on a polyethylene mulch-covered bed. Pier & Doergo (1995) and Thompson & Doergo (1996) reported that surface drip irrigation and fertigation with fluid N can result in optimum crop yield, quality and economic returns. Locascio (1977) reported highest yields of strawberries with 50% of the N and K applied preplant and 50% applied through drip irrigation tubes. Isobe (1974) working with sugarcane, found greater efficiency and greater dry matter accumulation with daily vs. biweekly applications of nitrogen through drippers. Bhella (1986) reported yield increases with muskmelons when N was added postplant through the drip irrigation tube. A potential problem associated with drip irrigation is leaching of nutrients. Bar-Yosef (1977) and Berstein & Francois (1973) demonstrated that ions move with the irrigation water away from the injection site. This movement could result in nutrient deficiencies in the root zone.

Studies were conducted to determine the effect of N application frequency through drip irrigation tubing on NO_3-N movement and accumulation in the bed profile at two locations: Charleston, S.C. and Clayton, N.C. Results showed that increasing N application frequency resulted in increased yield (Cook, 1980). The number of tomatoes produced was not affected by N treatment at either location, but fruit size increased with increasing N application frequency. Orange and grapefruit trees in Israel and Florida were found to have higher yield and N fertilization use efficiency under microfertigation than under split N broadcast application (Dasberg, Bar-Akiva, Spaziskys & Cohen, 1988; Boman, 1996). Alva & Mozaffari (1995) reported that the main advantage of N microfertigation over broadcast N fertilization in orange trees was the reduced nitrate leaching below the soil root volume. Reduced NO_3 leaching under microfertigation, without a decline in fruit yield or quality has also been reported for annual crops like tomatoes (Miller, Rolston, Rauschkolh & Wolfe, 1976) and celery (Feigin, 1982). Several studies have shown that crop yield response to N was stronger under microfertigation than under micro-irrigation plus broadcast N application in apples (Assaf, 1983), tomatoes (Bar-Yosef, 1977; Clough, Locascio & Olson, 1992) and in lettuce (Bar-Yosef & Sagiv, 1982b).

Rauschkolb, Rolston, Miller, Carlton & Burau (1979) showed that P drip fertigation resulted in higher P content in tomato plants than P banding with an identical P rate. Bar-Yosef (1989) found that P drip fertigated sweet corn gave a significantly higher yield than drip irrigated sweet corn that also received preplant P fertilization. Enhanced response to micro-fertigated K has also been reported for citrus (Lavon, Erner, Shapziski & Mohel, 1995), grapefruit (Boman, 1996), grapevine (Christensen, Peacock & Bianchi, 1991) and sugarcane (Ingram & Hilton, 1986). The response of P and K fertigation became more pronounced as N fertigation improved and became a non-limiting growth factor.

Bhella & Wilcox (1985) advocated continuous fertigation of surface drip irrigated muskmelon with 150 mg/L N and 50 mg/L N during vegetative and reproductive stages respectively. Lahav & Kalmar (1988) reported that banana (*Musa cavendish*) yields with surface drip irrigation on a clay soil were not affected by continuous versus weekly fertigation events. Cook & Sanders (1991) examined the effect of fertigation frequency on tomato yield in a loamy sand soil and the result showed that daily or weekly fertigation significantly increased yield as compared to less frequent fertigation. Stark (1993) reported that 75 mg/L N was the optimum concentration for continuous fertigation of tomatoes with surface drip irrigation.

The impact of fertigation via sprinkler irrigation on nitrate leaching and corn yield in Thailand was examined. The results showed that when 150 kg/ha N or higher was applied, the grain yield was maximum and there was no significant difference between 150 and 200 kg/ha N. It was concluded that N fertigation could be used to get high corn yield production with minimum rate of leaching (Mohammad, Clemente, Gupta, Loof & Hansen, 2002).

Hartz, LeStrange & May (1993) investigated the response of bell pepper (*Capsicum annuum L.)* to five rates of N fertigation between 0 and 336 kg/ha N at two drip-irrigated sites. The crop response to N application rate was similar at both sites, with maximum fruit yield and mean fruit size peaking at the 168 to 252 kg/ha rates. This response agreed with those of Locascio (1981) and Payero (1990) who found that maximum pepper yields peaked at 224 and 240 kg/ha N respectively.

Thompson, Walworth & Sower (1999) experimented on the effect of fertigation frequency on yield and quality of subsurface drip-irrigated broccoli. Subsurface drip-irrigated broccoli received experimental combinations of N rate of 176 and 268 lbN/acre (195.6 and 297.8 kg N/ha) and fertigation frequencies of daily, weekly, bi-weekly and monthly during winter in 1998-1999. The results showed that marketable broccoli yields were increased slightly with N application of 268 lbN/acre (297.8 kg N/ha) compared to 176 lbN/acre (195.6 kg N/ha). It was concluded that nitrogen fertigation frequency does not seem to be a critical management variable for subsurface drip irrigated broccoli grown on medium textured soils in Arizona.

Orphanos & Eliades (1992) examined nitrogen fertigation of Valencia oranges irrigated by drip or minisprinklers. The results showed that fruit quality was slightly impaired by increasing N supply. The most important effect was increased peel thickness but such increase was only 10% at the most. Apparently, the favourable weather conditions enabled the fruit to acquire high juice percentage, and high soluble solids and acid contents even under high N supply.

CHAPTER 2

INFLUENCE OF CUTTING POSITION, ROOTING HORMONE AND ROOTING MEDIA ON STEM CUTTINGS OF FEVER TEA

2.1 **Introduction**

Rooting hormones and rooting media play an important role in rooting of stem cuttings and this could either be through their direct effect on the cuttings or through their interactions (Leakey, 1990; Ofori *et al*., 1996). A number of studies have demonstrated that exogenous application of auxin hormone accelerates the rate of rooting, increases final rooting percentage and increases the number of roots of leafy cuttings (Leakey, 1982; Leakey, 1990). According to Bradley (2001) there are many auxins that can be used to stimulate adventitious roots but the two that are used most often commercially are indoleacetic acid (IAA) and naphthalene acetic acid (NAA). Both these auxins, either alone or in combination, are the active ingredients in most commercial rooting formulations although indolebutyric acid (IBA) is more effective for a wider range of species. Bradley, (2001) reported that auxin (IAA) regulates many important aspects of plant growth and development including apical dominance, tropic response, lateral root and root hair formation and vascular tissue differentiation.

Auxin effects are mediated by two different pathways, i.e. immediate or direct effects on the cell and the turning on of new patterns of gene expression. It was reported that when auxins arrive at the surface of the cell, they initiate such immediate responses such as change in movement of ions in and out of the cell through the plasma membrane, extension of the cell wall and elongation of the cell. Even though auxins promote adventitious roots and development of stem cuttings the use of auxins at appropriate concentration is essential because the wrong concentration can inhibit rooting or it can act as a growth retardant when applied in higher concentrations (Wiessman-Ben & Tchoundjeu, 2000).

Macdonald (1986) reported that one of the important criteria for the successful rooting of cuttings is a reliable rooting media. The appropriate propagation medium depends on species, cutting type, season, propagation system, and the cost and availability of the medium (Hartmann *et al.,* 1990). Rooting medium has four functions, i.e. to hold the cutting in place

during the rooting period, to provide moisture for the cutting, to permit penetration and exchange of air at the base of the cutting and to create a dark or opaque environment by reducing light penetration to the cutting base (Hartmann *et al.,* 1990). Grange & Loach (1983) reported that water uptake by cuttings is indirectly proportional to the water content of the medium as determined by its water retention and aeration properties.

The influence of cutting position is sometimes referred to as the influence of topophysis (Hartmann & Kester, 1983). The ability has been known to vary between cuttings from different parts of the same plant, especially in woody species (Leakey & Mohammed, 1985). Apical cuttings are mostly softwood or semi-hardwood with developing apex and associated with young leaves, which may produce rooting promoters but could be inhibitors if present in excess. Poor rooting of apical cuttings could be due to the softness of leaves and stems (Hansen, 1986; Leakey & Coutts, 1989).

In many vegetatively propagated plants, highest rooting was found when cuttings were taken from the basal portion of the shoot (Hansen, 1986; Hartmann *et al.,* 1997). The good rooting ability of basal cuttings could be due to somewhat higher food reserves as total sugars or it could have been due to the accumulation of natural auxins in the shoot bases or other root promoting factors with relatively low levels of rooting inhibitors (Al-Saqri & Alderson, 1996).

There is no information available on propagation of *Lippia javanica* using stem cuttings. Therefore, the aim of this study was to determine the effect of cutting position, rooting hormone and rooting media on stem cuttings of fever tea. These findings will help growers on how to produce *L. javanica* cuttings.

2.2 **Materials and Methods**

2.2.1 **Experimental site**

The experiment was conducted at the University of Pretoria's Hatfield Experimental Farm in a mist bed from January to February 2003. Temperatures in the mist bed ranged between 27 ºC and 30 ºC. The mist nozzles sprayed water for 8 seconds every 2 minutes and the relative humidity was kept constant by a fogging system.

2.2.2 **Experimental design and treatments**

The experiment was a 2^3 factorial laid out as a randomised complete block design (RCBD) and replicated 10 times. The treatments were as follows:

- Media: Sand or pine bark
- Hormone: With or without Seradix No. 2 (0.3% IBA)
- Position: Apical or basal

2.2.3 **Methodology**

Stem cuttings were taken from the mother plant of *Lippia javanica* (MR-III-029) received from the CSIR. The cuttings were prepared using sterile pruning scissors. Semi hardwood cuttings (320) with 2 leaves each were made. One hundred and sixty were apical cuttings and the other 160 cuttings were basal cuttings, all having a length of 8 cm. Forty sections of seedling trays were cut from the 200 cavity seedling trays and each tray consisted of 8 cavities. The 8 cavities were randomly filled with either sand or pine bark. Half of the cuttings were dipped in a powder of Seradix No.2 (0.3% IBA) hormone whereas the other half were planted without application of the hormone. Sampling was done after every 5, 10, 15 and 20 days after planting.

2.2.4 **Measurements and statistical analysis**

After every 5 days, eighty cuttings were harvested from the trays. The fresh mass of each cutting was determined, the stem circumference was measured using a vernier calliper, the number of leaves and roots were counted and the root length was measured using a vernier calliper. The data was analysed using the SAS programme (statistical package version 8.2) and the analysis of variance was done to determine the effect of cutting position, rooting media and rooting hormone on the dependent variables.

2.3 **Results**

2.3.1 **Fresh mass of cuttings**

Fresh mass was affected by the interaction of cutting position and growth hormone at 20 days after establishment. Apical cuttings treated with hormone had similar fresh mass as compared to apical cuttings without the application of hormone. However, basal cuttings treated with hormone had higher mass than basal cuttings without hormone. Hormone application increased fresh mass of basal cuttings as compared to apical cuttings, which showed no effect to hormone application. Generally, basal cuttings with hormone application had the greatest fresh mass (Table 2.1).

Table 2.1 Interactive effect of hormone and cutting position on fresh mass of fever tea cuttings at 20 days after establishment

Cutting position	Hormone	Fresh mass (mg)
Apical	With	1.27[a]
	Without	1.25[a]
Basal	With	1.67[b]
	Without	1.18[c]

Means within a column followed by the same letter are not significantly different at 5% level

It was found that the propagation media used did not affect fresh mass of fever tea cuttings. Even though there were no significant differences on the fresh mass for both apical and basal cuttings of fever tea in different media, cuttings performed slightly better when propagated in pine bark than in sand.

In terms of rooting hormone, there were significant differences on fresh mass of cuttings at 5 and 15 days after planting. Cuttings treated with hormones at 5 and 15 days after planting showed greater fresh mass than cuttings treated without hormones. Even though at 10 and 20 days after planting there were no statistical differences cuttings treated with hormone tended to have more fresh mass (Fig. 2.1).

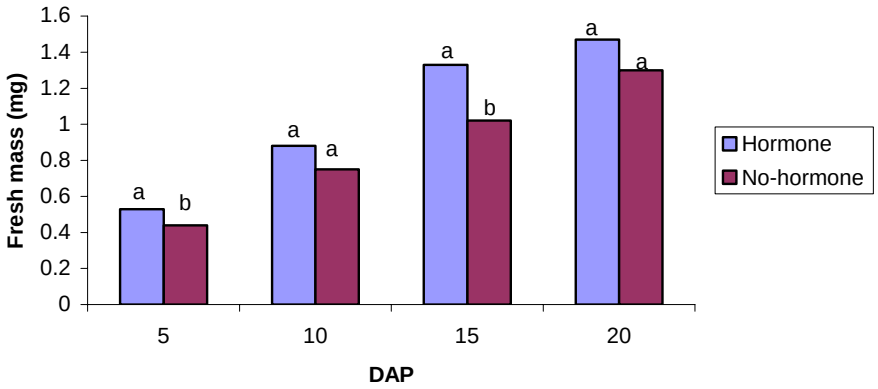


Fig. 2.1 Effect of rooting hormone on fresh mass of fever tea stem cuttings at 5, 10, 15 and 20 days after planting

*Statistical comparison is between histogram of different colours per days after planting

2.3.2 **Cutting stem circumference**

Through visual observations, there were differences between the stem circumferences of apical and basal cuttings. Basal cuttings had thicker stems as compared to apical cuttings. Generally stem cuttings grown in pine bark media had thicker stems as compared to those in sand.

2.3.3 **Cutting root number**

There were interactions between cutting position and media on the number of roots of fever tea cuttings at 20 days after establishment. Apical cuttings grown in sand had more roots than apical cuttings grown in pine bark media. In contrast, basal cuttings grown in pine bark had more roots than basal cuttings grown in sand (Table. 2.2).

Table. 2.2 Interactive effect of cutting position and growth media on root number of fever tea cuttings at 20 days after establishment

Cutting Position	Media	Root number
Apical	Sand	25.94[a]
	Pine bark	16.45[b]
Basal	Sand	14.39[c]
	Pine bark	23.29[ab]

Means within a column followed by the same letter are not significantly different at 5% level

Cutting root number was significantly affected by application of hormone at different days after planting. Rooting hormone had a strong positive effect on the number of roots of fever tea. Regardless of sampling date, number of roots produced were improved when rooting hormone was applied (Fig. 2.2).

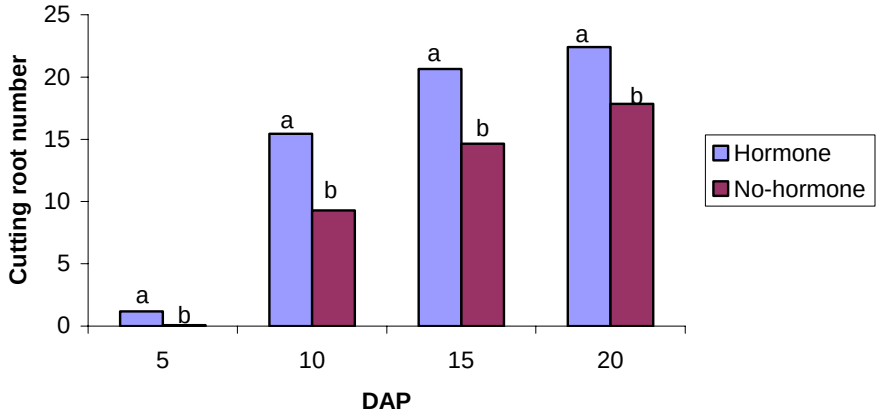


Fig. 2.2 Effect of rooting hormone on number of roots of fever tea cuttings

*Statistical comparison is between histogram of different colours per days after planting

2.3.4 **Rate of rooting of cuttings**

At 5 days after planting, apical cuttings showed callus development on the stem whereas on basal cuttings nothing had occurred. At 10 days after planting, most of the apical cuttings showed more roots than basal cuttings. However, at 15 to 20 days after planting, basal cuttings rooted similarly to apical cuttings (Fig. 2.3 a, b and c).

Fig. 2.3a Rooted apical cuttings at 15 days after establishment

Fig. 2.3b Rooted basal cuttings at 15 days after establishment

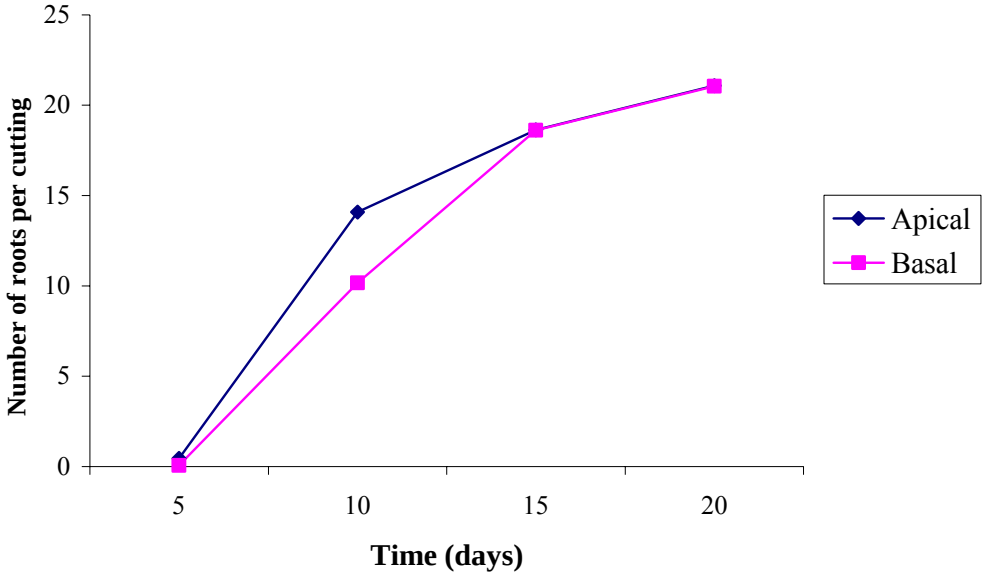


Fig. 2.3c Rate of rooting of fever tea stem cuttings

2.3.5 **Cutting root length**

There were no interaction effects on the root length of fever tea from 5 to 20 days after establishment. However, there was a significant effect of growing media on root length. Plants grown in pine bark had longer roots than plants grown in sand (Fig. 2.4).

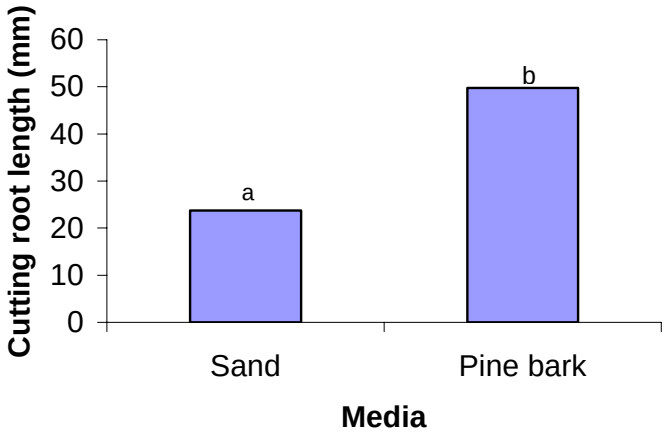


Fig. 2.4 Effect of growing media on root length of fever tea stem cuttings

2.3.6 **Cutting leaf number**

There were no interactions on the number of leaves of fever tea cuttings from 10 to 20 days after establishment. There was, however a significant effect at day 15 on the number of leaves due to cutting position and growing media (Table 2.3). Basal cuttings had significantly more leaves than apical cuttings. Cuttings planted in pine bark media had significantly more leaves than cuttings planted in sand media at 15 days after establishment. Hormone treatment had no significant effect on the number of leaves of fever tea cuttings.

Table 2.3 Effect of cutting position and growing media on leaf number of fever tea cuttings

DAP	Cutting position		Growing media	
	Apical	Basal	Sand	Pine bark
10	6.49[a]	5.43[a]	5.84[a]	6.17[a]
15	7.39[a]	9.61[b]	7.38[b]	9.27[b]
20	11.10[a]	11.97[a]	11.11[a]	11.87[a]

Means within a column followed by the same letter are not significantly different at 5% level

2.4 **Discussion and Conclusions**

Results indicated that the establishment of fever tea (*Lippia javanica*) stem cuttings on a mist bed can be successfully propagated. Vegetative propagation of fever tea was possible and successful in this study. There are certain factors that should be considered which affect rooting of stem cuttings. These factors are position of cuttings (apical or basal) on a plant shoot, type of media used and the application of hormone or not.

The root development of fever tea depended on the number of days after planting. Similar results were found with *Milicia excelsa* where by rooting increased with time after planting. Apical cuttings are mostly soft wood or semi-hard woodcuttings with developing apex and associated young leaves, which produce rooting promoters (Leakey & Coutts, 1989). This characteristic helps the apical cuttings to produce roots at an earlier stage as compared to other types of cuttings. In this study apical and basal cuttings showed maximum rooting at 15 days after planting.

Through visual observations basal cuttings showed thicker stems than apical cuttings. Leakey and Mohammed (1985) reported that thicker and longer cuttings rooted well as compared to thinner and shorter ones perhaps because the larger cuttings contained more starch in the stem. Wilson (1993) experimented on the effect of rooting caused by stem variations and sizes on stem cuttings. It was reported that the size of the stem cutting might contribute variation in the rooting ability since diameter of the stem varies along the shoots. Leakey (1990) confirmed that stem diameter might have an influence on rooting by determining the storage capacity of carbohydrates produced pre- and post-severance. Similar results were observed with the stem circumferences of apical and basal cuttings of fever tea.

Like many other species the rooting ability of fever tea was sensitive to rooting hormone and rooting media. In this study fever tea was influenced by rooting hormone when seradix no.2 was applied, it enhanced rooting of both apical and basal cuttings. Al-Barazi & Schwabe (1982) confirmed that the purpose of treating cuttings with auxins was to increase the percentage of rooting, root initiation, root number and uniformity of roots. Many studies reported that the rooting hormones such as auxins have an important role in the development of adventitious roots, increasing rooting percentage, improving quality of roots and uniformity in rooting of cuttings. Number of roots per rooted cutting of fever tea were found to respond well to the application of rooting hormone seradix no. 2. Cuttings treated with hormone produced more roots as well as longer roots than cuttings treated without hormone application. Similar results were reported by Ofori *et al.* (1996) where the mean number of roots per rooted cutting was higher (80%) when the cuttings of *Milisia excelsa* were treated with IBA. Rooting media is one of the most important factor, which affect the rooting success of cuttings (Leakey, 1990; Berhe & Negash, 1998). But in this study fever tea showed longer and more roots in pine bark media than sand. Hartmann *et al.* (1997) reported that an ideal propagation medium is known to provide sufficient porosity to allow good aeration and this ensures adequate oxygen availability for the developing root system.

The presence of leaves on the cuttings also plays an important role because plants need nutrients and metabolites for growth and development. In stem cuttings this metabolic activity takes place in the leaves that remain on the cuttings (Wiesman-Ben & Tchoundjeu, 2000). Hartman *et al*. (1990) reported that the presence of leaves on cuttings exerts a strong stimulatory influence on root initiation. In this study more leaves were found on basal cuttings as compared to apical cuttings. The rooting media also had an effect on the number of

leaves. The presence of leaves on cuttings has been found to have a considerable influence on rooting of cuttings, because of their ability to produce endogenous auxins and carbohydrates by means of photosynthesis (Leakey & Coutts, 1989; Hartman *et al*., 1990; 1989; Smalley *et al*., 1991 Ofori, *et al*., 1996;). It also influences the water status on cuttings. In this study, pine bark media increased the number of leaves due to its properties of having a high water holding capacity whereas in sand the cuttings were drying out easily and number of leaves was less. Furthermore, most of the cuttings grown in sand lost some of their leaves as compared to cuttings grown in pine bark. It was reported that root length and rooting percentage showed some dependence on the type of medium used in *Rosa centifolia* cuttings. When peat perlite was used, it resulted in significantly higher rooting percentage and length than vermiculite. With fever tea, cuttings grown in pine bark media had significantly longer roots as compared to those grown in sand.

2.5 **Summary**

Stem cutting is one means of vegetative propagation. It has been used to propagate many plants such as ornamentals, forestry and agro-forestry plants. There are certain limiting factors, which affect the successful root development of stem cuttings, and these factors are position of cuttings, environmental and physical factors, rooting media and rooting hormones. In this study, apical and basal stem cuttings of fever tea were grown in either pine bark or sand growing media. The cuttings were treated with either Seradix No. 2 hormone (0.3% IBA) or without hormone. The aim was to determine the best cutting position, ideal propagation media and the effect of hormone on rooting of fever tea stem cuttings.

Apical cuttings rooted earlier than basal cuttings, but at 15 to 20 days after establishment both cuttings rooted similarly. Pine bark growing medium gave good results as compared to sand medium on both apical and basal cuttings in terms of the root length of the cuttings. Basal cuttings resulted in thicker stem circumferences and more number of leaves as compared to apical cuttings. Seradix No. 2 hormone increased the fresh mass, stem circumference, number of roots and number of leaves for both apical and basal cuttings. The results suggested that for the establishment of fever tea stem cuttings both apical and basal cuttings can be used and pine bark is the ideal propagation medium. Cuttings can be ready for transplanting at 15 to 20 days after establishment and Seradix No. 2 hormone (0.3% IBA) is recommended to promote rooting of the cuttings.

CHAPTER 3

LIGHT AND TEMPERATURE EFFECTS ON SEED GERMINATION OF FEVER TEA (*LIPPIA JAVANICA)*

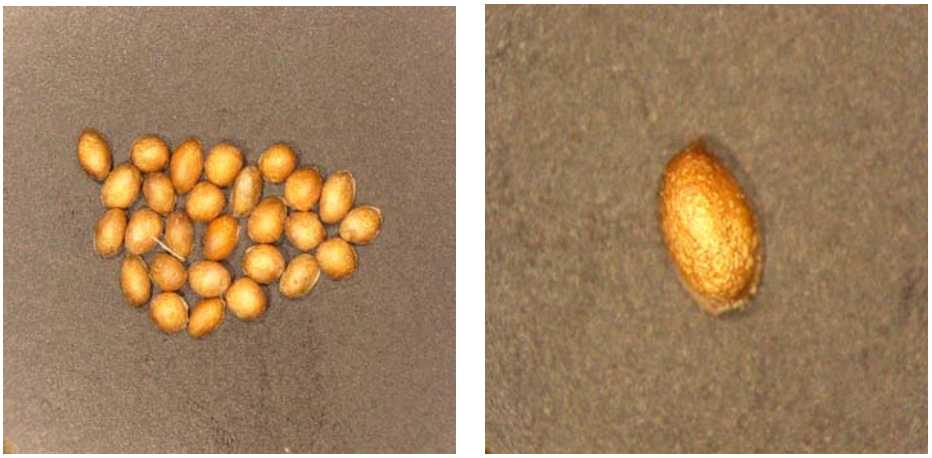
3.1 **Introduction**

Seeds germinate when placed in environmental conditions favourable to the process of germination. Among the conditions required are an adequate supply of water, suitable temperature, composition of gases in the atmosphere, as well as light for certain seeds (Mayer & Poljakoff-Mayber, 1975). Light quality and quantity play an important role in germination of photosensitive seeds (Casal & Smith, 1989). When absorbed by chlorophyll, light represents a source of energy. However, when absorbed by phytochrome, light controls plant morphogenesis. Phytochrome exists under a red (Pr) and a far-red (Pfr) absorbing distinct forms. Upon exposure to red light, Pr undergoes photochemical conversion to a physiologically active far-red absorbing form Pfr, which triggers a variety of complex physiological responses such as flowering and seed germination (Mancinelli, 1994).

According to Salisbury and Ross (1992) plant growth is very sensitive to temperature. Each species at any stage in its life cycle have a base temperature below which it will not grow, and an optimum temperature at which it grows. There is no information available on the seed germination of *Lippia javanica.* The study was undertaken to determine the effect of light and temperature on seed germination of fever tea.

3.2 **Materials and Methods**

The seeds of fever tea (Fig. 3.1) were received from the Council for Scientific and Industrial Research (CSIR). Most of the seeds were closed within a thin layer covering. The thin layer covering was removed and the seeds were tested for germination before sowing. A tetrazolium test indicated that 95% of the seeds were viable.



a **b**

Fig. 3.1 Fever tea (*L. javanica*) seeds (a) 10 x and (b) 100x magnification

The experiment was conducted in a laboratory in the Department of Botany at University of Pretoria in growth chambers (Labcon model L.T.G.C with ± 0.3ºC, South Africa). Germination of fever tea was tested at constant temperature regimes (15, 20, 25 and 30ºC) with a continuous light or dark period and alternate temperatures of 20:30 and 16L:8D (light: dark combination respectively. The selection of the temperatures was done based on the preliminary studies where better germination was recorded. Four hundred viable seeds were sterilized for 1 minute in 70% ethanol then rinsed for 1 min with sterilized water. The seeds were sterilized again for 5 minutes in 1% sodium hypochloride then rinsed thoroughly with sterile water. The seeds were sowed in 9 cm clear plastic petri dishes and 2 filter papers of Whatman no. 1 were moistened with 5 ml of distilled water. Four petri dishes were used per incubator and in each dish 20 seeds were sown. The petri dishes were properly closed with parafilm ‘‘M’’ to reduce evaporation of moisture and placed in the incubators. When the moisture was low distilled water was used for moistening the seeds. The experiment was carried out with four replicates. Germinated seeds were removed every day or after every second day. Germination tests were stopped when the final germination percentages were reached in four weeks. The data was analysed using SAS system for windows (8ed) (1999-2000). The data analysed was seed germination percentage and mean germination time (germination rate). Mean germination time was calculated based on the formula of Hartmann *et al*. (1997).

$$MGT = \frac{N1T1 + N2T2...NxTx}{NT}$$

MGT = Mean germination time

N = Number of seeds germinated within consecutive intervals of time

T = Time between the beginning of the test and end of the particular measurement

NT = Total germinated seeds

3.3 **Results**

The seeds of fever tea showed highly significant differences in germination percentage ($P<0.05$) due to the difference in temperature treatments (Fig. 3.2). Seeds germinated to lower percentage at 15 ^{o}C constant temperature. Germination percentage of fever tea increased at 20 to 30 ^{o}C constant temperatures and 20/30 ^{o}C alternate temperatures but the difference among them was not significant. Though the difference among them was not significant, slightly better germination of fever tea was recorded when the temperature was alternate than constant. On the other hand, small size of the radicle was observed when the seeds were incubated in lower temperature (15 ^{o}C).

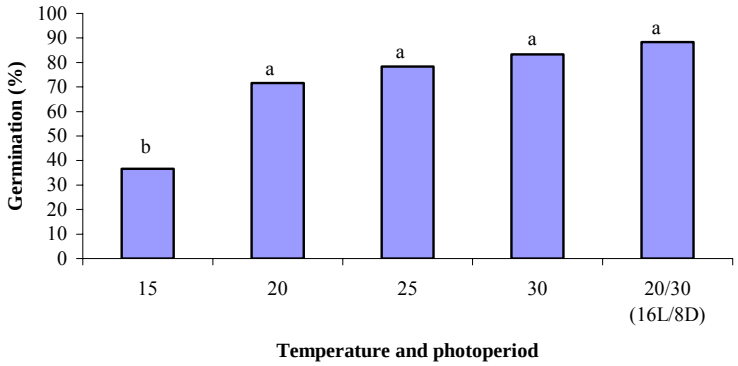


Fig. 3.2 Germination percentage of fever tea (*L. javanica*) at various photoperiod and temperatures

Fever tea seeds started to germinate after 8 days from incubation and last germination was observed after 30 days from sowing. However, mean germination time or germination rate of fever tea did not show significant differences due to the temperature differences (Fig. 3.3).

Even though the differences in mean germination time (MGT) among the temperature treatments was not significant, seeds incubated at 25 ^{o}C constant temperature required longer time (21 days) for the radicle to emerge compared to 15 days at 30 ^{o}C constant temperature.

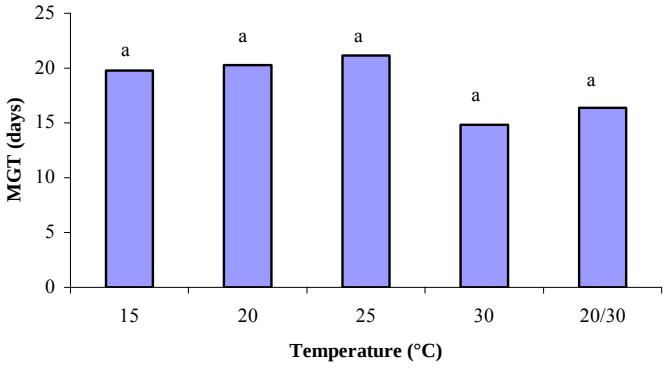


Fig. 3.3 Mean germination time of fever tea seeds as affected by various temperatures

Germination percentage of fever tea also showed significant differences ($P<0.05$) due to differences in photoperiod exposure (Fig. 3.4). Seeds of fever tea prefer to germinate to higher percentage (86%) when exposed to continuous light than alternate light and dark (62%). That is, the longer the time of exposure to light the higher the germination percentage of fever tea seeds.

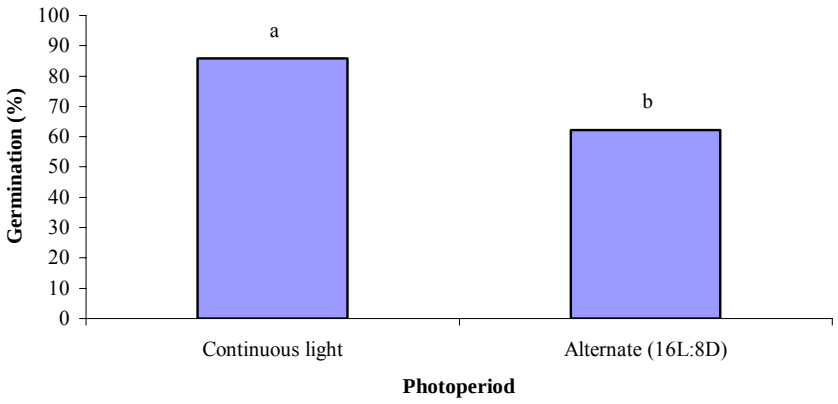


Fig. 3.4 Germination percentage of fever tea seeds as affected by photoperiod

Similarly to germination percentage, mean germination time of fever tea seeds was also affected by photoperiod. There was a highly significant difference ($P<0.01$) between continuous light and alternate light and dark periods (Fig. 3.5). The radicle required longer time to emerge when the seeds were incubated in continuous light than alternate light and dark. In other words, alternating light and dark improved emergence of the radicle than continuous light.

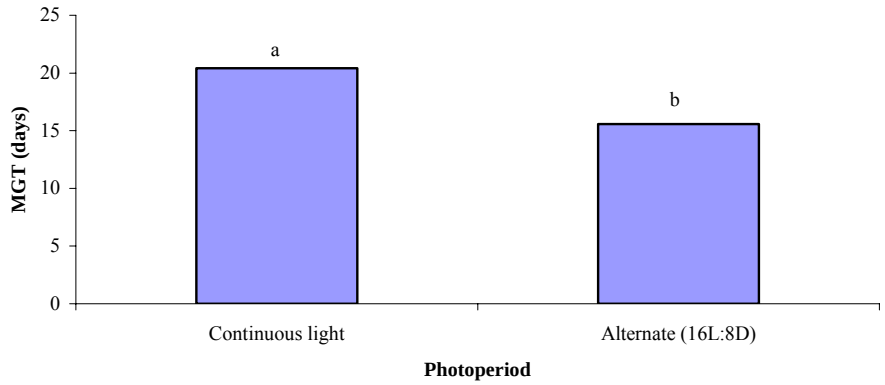


Fig. 3.5 Mean germination time of fever tea seeds as affected by photoperiod

3.4 **Discussion and Conclusions**

In a seed germination test, temperature was found to be the most important environmental factor that regulates the timing of germination. It can affect germination when applied in either alternating or in constant pattern (Hartmann *et al*., 1997; Copeland & McDonald, 1985). The optimum temperature of non-dormant seeds of most plants is between 25 and 30 ^{o}C. The germination percentage of fever tea was affected by temperature. In this study seeds of fever tea germinated better at constant 20, 25, 30 ^{o}C and alternating 20/30. Similar results were reported by Rojas-Archiga, Orozco-Segovia & Vazquez-Yanes (1997) where at both constant and alternating temperatures the four-barrel cacti, *Echinocactus platyacanthus*, *Ferocactus recurvus*, *F. robustus* and *F. flavovirens*, were positively photoblastic (i.e. their seeds did not germinate in darkness). Zaia & Takaki (1998) reported the same where germination of seeds of *Tibouchina pulchra* and *T. granulosa* presented a strong light dependence where seeds never germinated when exposed to continous darkness. Fever tea seeds failed to germinate in continuous darkness.

Light requirements in seed germination differ from species to species. Seeds might germinate to higher percentage in light whereas others in the dark (Baskin & Baskin, 2001). Seeds of some species have an initial light requirement only after burial in the soil. The seeds of fever tea responded positively to light and seeds incubated to continuous light recorded higher germination percentage and germination rate. Some flower crops like allssum, begonia, and calceolaria, coleus, *Kalanchoe,* primrose and *Saintpaulia* require light for germination whereas flower crops like calendula, delphinium, pansy, annual phlox and annual verbena require darkness for germination. Light sensitive seeds are characterised by being small in size, in which shallow depth of planting would be an important factor favouring survival, otherwise if covered too deeply, the epicotyl may not penetrate the soil surface (Hartmann *et al.*, 1997). The same could be true with fever tea since the seeds are small in size. Species whose seeds can accumulate in the soil, small seeded species are expected to have a light requirement for germination, while germination in large seeded ones might be expected to be indifferent to light (Kasperbauer & Hunt, 1988). Studies showed that light represents a source of energy when absorbed by phytochrome where it controls plant morphogenesis (Mancinelli, 1994).

Some studies showed that light indifferent species such as columnar may germinate even if they are buried deeper than those of other species. The thicker layer of the soil provides more moisture for germination; moreover these species have larger and heavier seeds than the positive photoblastic barrel cacti (Leishman & Westoby, 1994; Fehner, 1985). The same could have occurred with fever tea seeds if they were planted directly in the soil. Because of its smaller seeds the lower depth of planting covered by the thin layer of soil will be more advantageous. It was also found that sandy soils tend to have a higher light transmission than silty loams (Pons, 1992). The same hypothesis could be applicable to fever tea germination since they showed preferences to germination with light than with darkness.

Mean germination time of fever tea was also affected by photoperiod. The radicle required longer time to emerge when incubated in continuous light than alternate light and dark. The seeds preferred to germinate to higher percentages when exposed to continuous light than alternate light and dark. This showed that the seeds are more sensitive to differences in light than differences in temperature. Based on this results better germination of this species can be obtained by germinating seeds under continuous light at 20-30 ^{o}C constant temperatures.

3.5 **Summary**

Seed germination is the emergence of the radicle through the seed coat when placed under favourable conditions. The germination requirement of fever tea is not known. The aim of this study was to investigate the ideal germination temperature and light combinations of fever tea seeds. Fever tea was tested at constant temperature regimes (15, 20, 25 and 30 ^{o}C with continuous light or dark period) and alternating temperatures of 20:30 and 16L: 8D (light: dark) combinations respectively.

Germination of fever tea seeds started after 8 days and seeds germinated up to 30 days from sowing. Seeds germinated in constant and alternating temperatures ranging from 15 to 30 ^{o}C. The germination percentage was improved at continuous or alternating temperatures above 20 ^{o}C. The highest germination percentage was 86% at continuous light. Seeds responded well at constant and alternating temperatures. The mean germination time was high in continuous light than alternating light and dark. According to these investigations, fever tea seeds where found to be positively photoblastic. Regardless of temperature, seeds failed to germinate in continuous darkness. For better germination of this species germination can be obtained under continuous light of 20-30 ^{o}C.

CHAPTER 4

EFFECT OF FERTIGATION FREQUENCY AND GROWTH MEDIUM ON GROWTH, YIELD AND QUALITY OF DRIP IRRIGATED FEVER TEA

4.1 **Introduction**

The application of fertilizers through the irrigation system is referred to as fertigation. The increase in yield and its quality together with the improved water and fertilizer efficiency, make fertigation an attractive technology in modern irrigated agriculture and particularly in greenhouse conditions. The acceptance of fertigation approach by farmers is directly related to the main factors such as efficient use of inputs, saving in energy and labour and improved quality produce (Papadopoulos, 1992). Moreover, in areas with insufficient rainfall, fertigation offers the best and sometimes the only way of ensuring that nutrients enter the root zone (Papadopoulos, 1992).

Drip irrigation is one of the most effective methods to supply water to crops (Harz, 1993). It can result in water saving if the correct management procedures are applied. Pier & Doergo (1995) and Thompson & Doergo (1996) reported that in general, at least 10 to 30% water saving can be realized in drip irrigation as compared to conventional surface irrigation systems. According to Hillel (1987) the wetted portion of the soil is maintained in a continuously moist state and never allowed to be depleted or approach the so-called wilting point. This creates a uniquely favourable soil moisture regime and gives drip irrigation a distinct advantage over sprinkler and surface irrigation. One of the most common problems encountered with drip irrigation amongst producers is that irrigation is applied in excess of crop requirements. The advantage of water savings by drip is forfeited if crops are over irrigated (Hillel, 1987). According to Bravdo & Proebsting (1993) the water distribution under each dripper forms a bulb shaped or onion shaped wetted zone that gradually becomes carrot shaped and eventually form a "chimney''leading the excess water downwards towards the water table (Hillel, 1987). There is no information available on growth, yield and quality of fever tea. Therefore, the aim of this study was to determine the effect of fertigation frequency and growth media on growth, yield and quality of drip irrigated fever tea.

4.2 **Materials and Methods**

4.2.1 **Location**

The experiment was established on 16/09/2002 at the University of Pretoria's Experimental Farm in a tunnel. The area is situated approximately 25º45'S and 28º16'E latitude and longitude respectively and at an altitude of 1327 meters above sea level. An average light intensity of 751 $d^{-1}{}_{S}{}^{-1}$ in the tunnel was measured with sun fleck ceptometer (Decagon Pullman, WA). The tunnel consisted of two fans and a wet wall inside for controlling temperature.

4.2.2 **Treatments**

The experiment was laid out in a split plot factorial design with five fertigation frequencies (0.4 L/day, 1L/day, 2L/day, 2L/day, 2L/2nd day and 2L/week) and two growing media (pine bark and sand) replicated twice. Fever tea plants were planted together with other four plant species (viz. African potato, bush tea, wild ginger and pineapple flower).

Fertigation frequencies were applied through a drip irrigation system. In every fertilization through the irrigation system one (1) g of the fertilizer mixture (Feed All) was dissolved in one (1) g of water (Table. 4.1). Each of the five fertigation frequencies consisted of twenty plants giving a total population of 200 plants for the experiment. Each planting material planted in a bag was regarded as a replicate which gave the order of 5x2x20.

4.2.3 **Planting materials**

Planting materials used were young plants established from cuttings grown in the greenhouse at University of Pretoria's experimental farm. One hundred plants were planted in 10 litre plastic bags filled with sand and the other 100 planted in 10 litre plastic bags filled with pine bark. The tunnel consisted of five rows in which the plastic bags were placed. Each row had two hundred plastic bags of fever tea with other 4 plant species.

Table 4.1 Aqua sol nutriblends water soluble plant food for fertigation of fever tea during 2002/2003

Elements	Quantity
Nitrogen (N)	160g/kg
Phosphorus (P)	50 g/kg
Potassium (K)	220 g/kg
Calcium (Ca)	11 g/kg
Magnesium (Mg)	3 g/kg
Boron (Bo)	335 mg
Iron (Fe)	356 mg
Zinc (Zn)	100 mg
Manganese (Mn)	125 mg
Molybdenum (Mo)	12.5 mg
Copper (Cu)	12.5 mg

4.2.4 **Data collected**

The stem circumference, number of branches and plant height were recorded at 8, 16 and 32 weeks after planting. At week 32 the plants were harvested and the fresh mass was determined. Forty samples of fever tea leaves each with the fresh mass of 200 g were taken to Wits University at the Department of Pharmacy and Pharmacology for hydro-distillation of essential oil. Twenty leaf samples were taken to the microscopic laboratory for the determination of oil glands and trichomes on the abaxial and adaxial surfaces of the leaves as affected by fertigation frequencies and growing media.

4.2.5 **Method used for oil extraction and analysis**

Two hundred grams of fever tea fresh leaves harvested from a tunnel at 32 weeks after planting were pooled together for oil yield determination by hydro-distillation. The essential oil of fever tea was determined through hydro distillation for 3 hours using all-glass Clevenger-type apparatus at Wits University Pharmacology Laboratory. The essential oil isolation of the chemical compounds was performed at the Council for Scientific and Industrial Research (CSIR). The analysis was carried out on a Hewlet Packard 5890 gas

chromatography (GC) series II fitted with HP-5 mass spectrometric (MS), Cross-linked 5%. [PH ME Siloxane (Agilent Technologies, Palo Alto, CA) column (30m x 0.25mm x 0.25µm film thickness)]. The column head pressure was 55 kPa. The chromatograph was coupled to a Hewlett Packard 2971 (agilent Technologies, Palo Alto, CA) series mass selective detector. The temperature programme was as follows: Split and splitless injection –5 min solvent delay, initial temperature: 50ºC--50ºC→240ºC @ 3ºC/min. Helium was used as carrier gas at a flow rate of 35cm/s split flow 30-40:1. The software used was chemistation (Agilent Technologies, Palo Alto, CA). The procedure used for isolation of chemical compounds of fever tea is summarised in Table 4.2.

4.2.6 **Preparation of leaves for the scanning electron microscope**

Newly matured leaves were taken from each of the five fertigation frequencies i.e. 0.4L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week grown in sand or pine bark media for determination of oil glands and trichomes developed on the leaves. Two 10 mm sections were cut from each leaf and fixed overnight in 2.5% gluteraldehyde in 0.075 M phosphate (pH 7.4) at 21ºC. After fixation, the leaves were rinsed in 3 changes of 15 minutes each of 0.075 M phosphate buffer. Dehydration was done by rinsing the materials for 15 min in each of an ascending series of ethanol 50%, 70%, 90%, 100% and 3 times in 100% ethanol. The leaves were dried with CO_2 in a critical point drier (Bio-Rad E 3000; Watford, England) and mounted on aluminium stubs, sputter coated with gold (Polaron E5 200 C; Watford, England) and viewed at 5 KV in a JSM-840 scanning electron microscope (JEOL; Tokyo, Japan).

4.2.7 **Data analysis**

The data was analysed using SAS system (statistical package version 8.2). The analysis of variance was done to determine the effect of fertigation frequency and growing media on the dependent variables.

Table 4.2. Procedure used for isolation of chemical compounds from fever tea essential oil

Sample description	Essential oils
Concentration	1% solution
Solvent	Hexane
Volume	1 ml
Column type	N_2
Carrier gas flow	7.8 psi
Inlet pressure	2.5 ml/min
Make up gas	N_2
Oven temp. program	Yes
Initial temp	50ºC
Initial hold	1 min
Program 1 rate	5ºC
Program 1 final	200ºC
Program 1 hold	5 min
Program 2 rate	-
Program 2 final	-
Program 2 hold	-
Program 3 rate	-
Program 3 final	-
Program 3 hold	-
Detector auxillary (b)	FID
Temperature	300ºC
FID (b) initial range	12
FID (b) initial range	8 ml
Hydrogen flow	33.8 ml
Synthetic air flow	337.5 ml
Injection mode	Split
Initial relays	1
Split flow	49 ml
Injector temperature	220ºC
PC attenuation	1
PC run time	36 min

4.3 **Results**

4.3.1 **Growth and development of fever tea plants**

Plant height

There were no interactions between fertigation frequency and the growing medium for plant height of fever tea at 8 weeks after planting. The only significant effects were due to growing media. Plants grown in pine bark media were significantly taller than plants grown in sand. Plant height for plants grown in pine bark media increased from 14 cm at transplanting to 28 cm whereas in sand, plant height increased from 14 cm to 24 cm. At 32 weeks after planting there was a significant effect from the main effects of fertigation frequency and growing medium. Plants fertigated with 2L/day were significantly the tallest followed by plants fertigated with 0.4L/day, 1L/day, 2L/2nd day and 2L/week. Amongst all the fertigation frequencies plants fertigated with 2L/week were the shortest (Fig. 4.1). At 32 weeks plants grown in sand media were taller than plants grown in pine bark. Plant height in sand media increased from 14 cm to 32 cm and in pine bark media the increment was from 14 cm to 29 cm. This is in contrast to when the plants were younger, where better plant growth was obtained in pine bark media (Fig. 4.2).

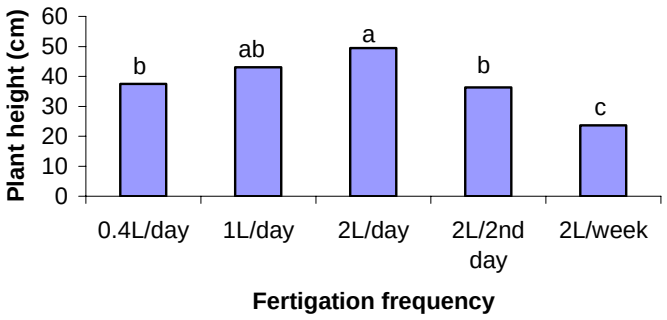


Fig. 4.1 Effect of fertigation frequency on plant height of fever tea at 32 weeks after planting

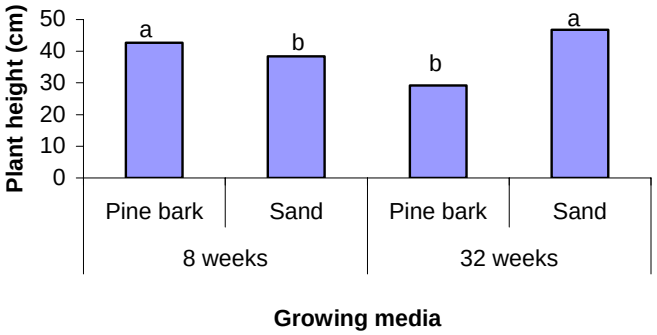


Fig. 4.2 Growing media effect on plant height of fever tea at 8 and 32 weeks after planting

At 16 weeks after planting, there were interactions between fertigation frequency and the growing medium for plant height of fever tea. Plants fertigated with 2L/day and 1L/day grown in sand were significantly the tallest than plants from other fertigation frequencies in both sand and pine bark media. Plants fertigated with 0.4L/day, 1L/day, 2L/2nd day and 2L/week grown in pine bark media had similar plant height. Across all the fertigation frequencies, plants grown in sand were significantly the tallest when compared to plants grown in pine bark media (Fig. 4.3).

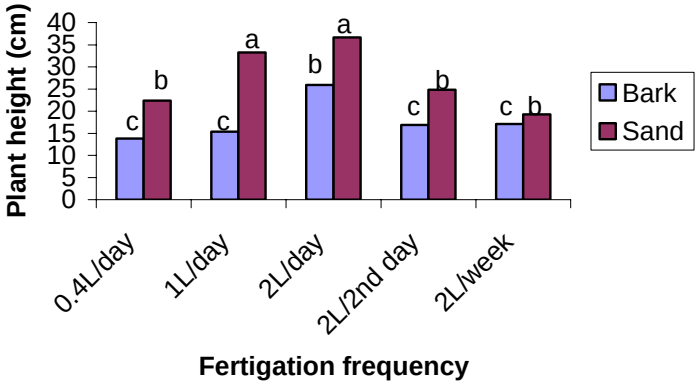


Fig. 4.3 Interactive effect between fertigation frequency and growth medium on plant height of fever tea at 16 weeks after planting

Stem circumference

There were interaction effects between fertigation frequency and growing medium for stem circumference of fever tea at 8 weeks after planting. The results showed that plants fertigated with 2L/day grown in pine bark media were significantly thicker than plants fertigated with 0.4L/day, 1L/day, 2L/2nd day and 2L/week. Across all the fertigation frequencies plants grown in pine bark resulted in thicker stems than plants grown in sand. Plants fertigated less, i.e. with 2L/week were the thinnest when grown in sand as compared to other treatments (Table 4.3).

Table 4.3 Interactive effect of fertigation frequency and growth media on the stem circumference of fever tea at 8 weeks after planting

Fertigation frequency	Growth media	Stem circumference (cm)
0.4L/day	Pine bark	6.35^{c}
	Sand	7.86^{ab}
1L/day	Pine bark	6.99^{abc}
	Sand	6.10^{c}
2L/day	Pine bark	8.94^{a}
	Sand	6.79^{c}
2L/2nd day	Pine bark	6.73^{c}
	Sand	7.48^{b}
2L/week	Pine bark	6.95^{c}
	Sand	5.14^{d}

Means within the columns followed by the same letter are not significantly different at 5% level

At 16 and 32 weeks after planting there were no interactions between fertigation frequency and growth medium for stem circumference of fever tea. There was a significant effect on stem circumference from the main effects of fertigation frequency and growing media. In terms of the growing media, at 16 and 32 weeks after planting plants showed thicker stems when grown in sand media than in pine bark (Fig. 4.4). Contrastingly, when plants were younger they showed thicker stems in pine bark than in sand. Plants grown in sand were

observed to grow stronger with thicker stems and standing upright as compared to pine bark where, plants were bending and had thinner stems.

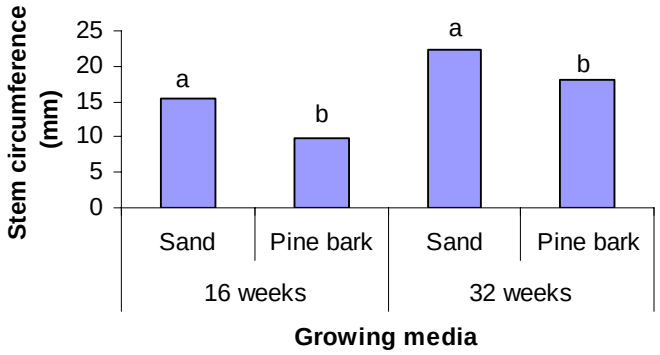


Fig. 4.4 Growing media effect on stem circumference of fever tea at 16 and 32 weeks after planting

At 16 weeks after planting plants fertigated with 1L/ day had thicker stems. However, plants receiving 0.4L/day, 2L/day and 2L/2nd day had similar thickness of stems. But when plants received 2L/week the stems were the thinnest (Fig. 4.5). Similar results occurred when the plants were 32 weeks. They gave thicker stems when fertigated with 1L/day and thinner stems when fertigated with 2L/week (Fig. 4.6).

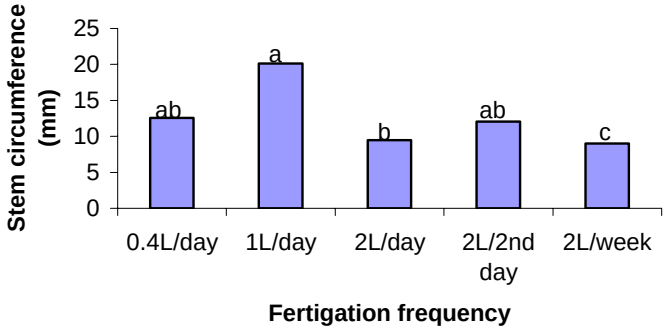


Fig. 4.5 Effect of fertigation frequency on stem circumference of fever tea at 16 weeks after planting

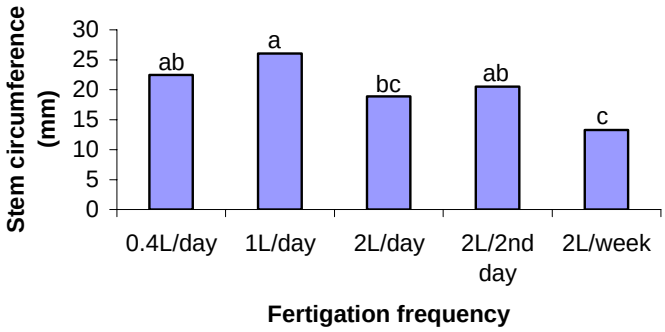


Fig. 4.6 Effect of fertigation frequency on stem circumference of fever tea at 32 weeks after planting

Number of branches

The results showed no interactions between fertigation frequency and growing media on number of branches of fever tea. At 32 weeks after planting there was a significant effect on number of branches from the main effects of fertigation frequency and growing media. The number of branches were significantly more in plants fertigated with 0.4L/day, 1L/day, 2L/day and 2L/2nd day compared to plants fertigated with 2L/week (Fig. 4.7). Plants grown in sand media had significantly more branches than plants grown in pine bark media (Fig. 4.8).

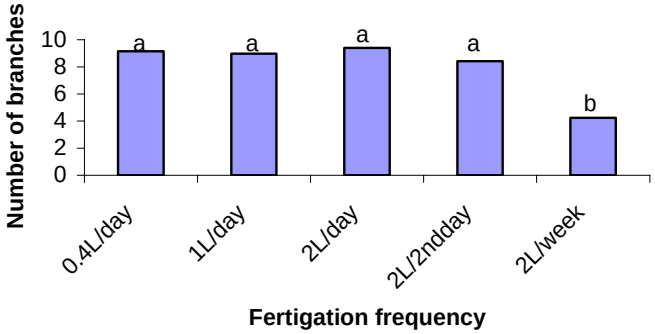


Fig. 4.7 Effect of fertigation frequency on number of branches of fever tea at 32 weeks after planting

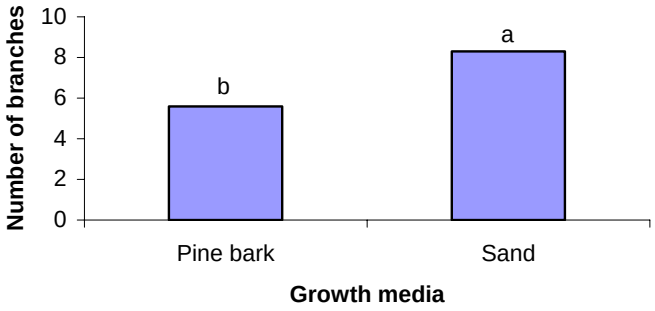


Fig. 4.8 Effect of growth medium on number of branches of fever tea at 32 weeks after planting

Fresh mass

There were interactions between medium and fertigation frequency for the fresh mass of fever tea at 32 weeks after planting. Plants fertigated with 0.4L/day and 1L/day and grown in sand yielded significantly more fresh mass followed by plants fertigated with 2L/day and 2L/2nd day. However, plants grown in pine bark media gave more fresh mass when fertigated with 0.4L/day and 1L/day (Table. 4.4). Fresh mass was drastically reduced when plants received 2L/day, 2L/2nd day and 2L/week when grown in pine bark media. Furthermore, plants grown in sand irrespective of fertigation frequency produced more mass compared to plants grown in pine bark.

Table 4.4 Interactive effect of fertigation frequency and growth medium on the fresh mass of fever tea at 32 weeks after planting

Fertigation frequency	Growth media	Fresh mass (g)
0.4L/day	Pine bark	88.70^{c}
	Sand	246.09^{ab}
1L/day	Pine bark	90.45^{c}
	Sand	254.19^{a}
2L/day	Pine bark	64.17^{d}
	Sand	182.13^{b}
2L/2nd day	Pine bark	52.34^{de}
	Sand	162.67^{b}
2L/week	Pine bark	33.91^{de}
	Sand	44.39^{e}

Means within the columns followed by the same letter are not significantly different at 5% level

4.3.2 **Effect of fertigation frequency and growth media on oil yield of fever tea**

There were no interactions between fertigation frequency and growing medium in terms of fever tea oil yield. Oil yield was also not significantly affected by fertigation frequency (Fig. 4.9). However, plants grown in pine bark gave significantly higher oil yield (0.33%) compared to plants grown in sand (0.25%) (Fig. 4.10).

4.3.3 **Growing medium effect on development of oil glands and trichomes of fever tea leaves**

There were no significant differences between oil glands and trichomes on the upper (adaxial) and lower (abaxial) surfaces of the leaves as affected by growing medium. Though there were no significant differences on the development of oil glands and trichomes on both leaf surfaces, trichomes and oil glands were more on the adaxial surfaces than on the abaxial surfaces regardless of the treatments (Fig. 4.11).

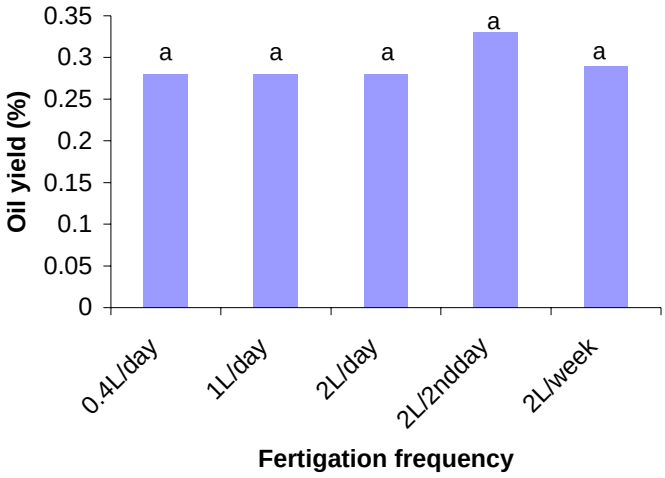


Fig. 4.9 Fertigation frequency effect on oil yield of fever tea at 32 weeks after planting

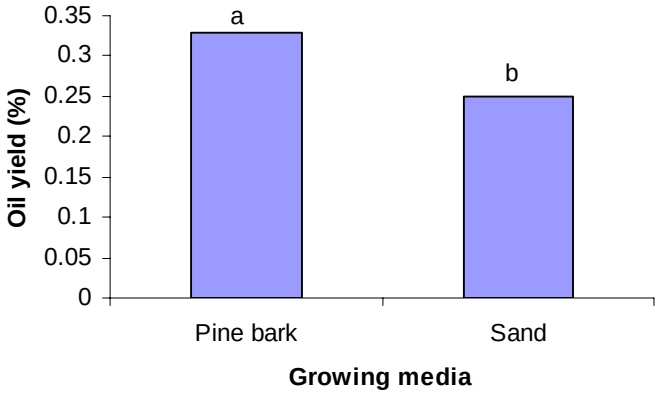
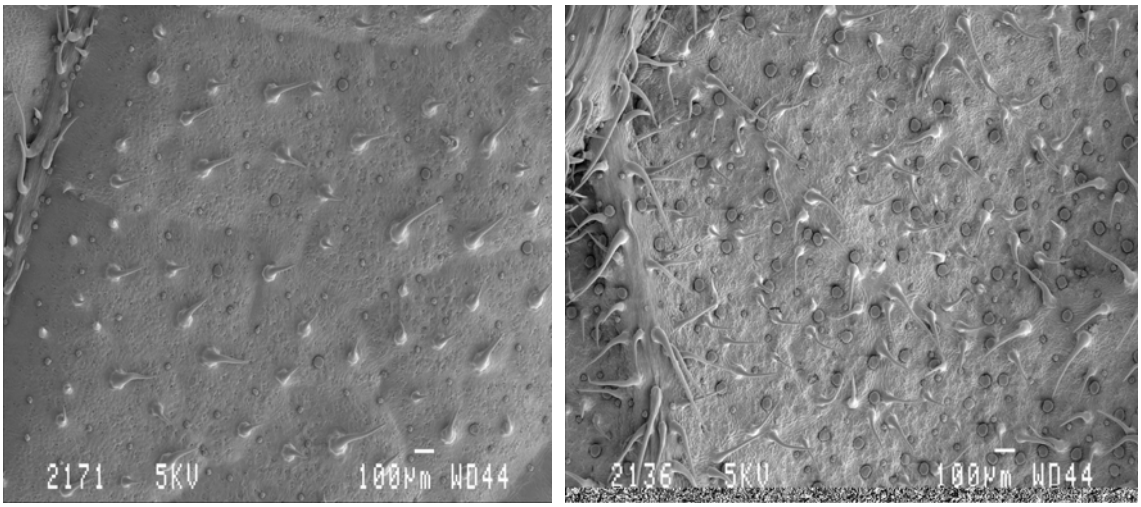


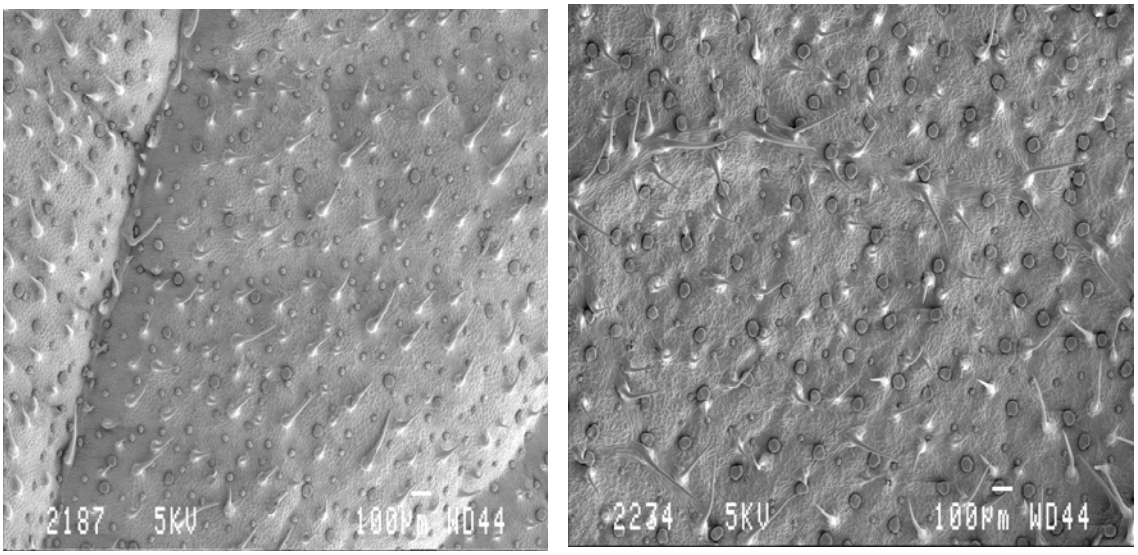
Fig. 4.10 Effect of growing media on oil yield of fever tea after extraction



Abaxial surface **Adaxial surface**

Fig. 4.11 Development of oil glands and trichomes on abaxial and adaxial surfaces of fever tea leaves as affected by growing medium

Pine bark medium resulted in larger oil glands than sand medium regardless of the treatments. Larger oil glands developed in pine bark media gave higher oil yield percentages during hydro-distillation as compared to sand media (Fig. 4.12).

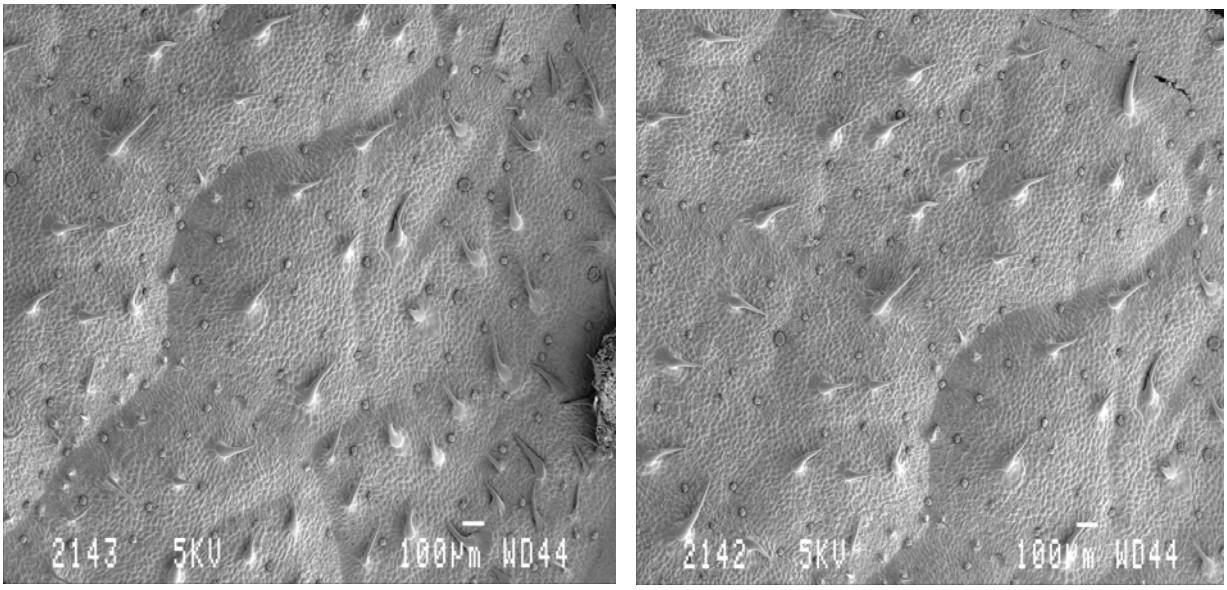


Sand media **Pine bark media**

Fig. 4.12 Development of oil glands and trichomes as affected by sand and pine bark media

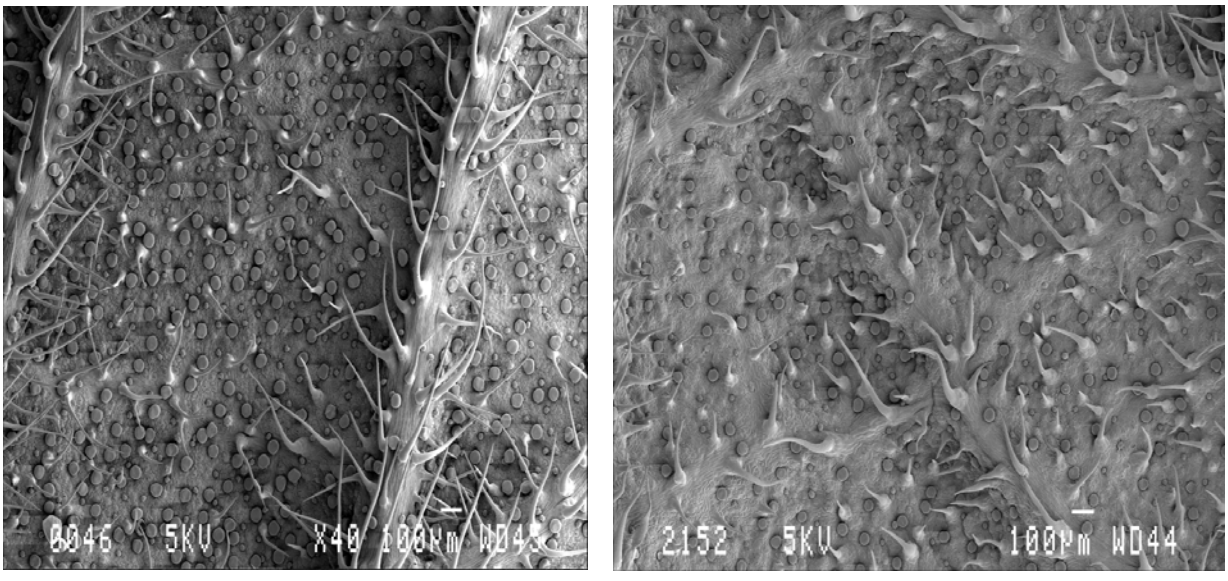
4.3.4 **Fertigation frequency effect on the development of trichomes and oil glands of fever tea leaves**

Development of oil glands and trichomes was not affected by the fertigation frequency. Plants fertigated with 2L/day gave similar trichomes and oil glands like plants fertigated with 2L/week on both leaf surfaces. (Fig. 4.13 and Fig. 4.14).



2L/week **2L/day**

Fig. 4.13 Effect of fertigation frequency on the development of oil glands and trichomes on the upper surfaces of the leaves



2L/week **2L/day**

Fig. 4.14 Effect of fertigation frequency on lower surfaces of fever tea leaves

4.3.5 **Effect of fertigation frequency and growth medium on essential oil compounds extracted from fever tea leaves**

There were no significant differences between fertigation frequency and growing medium on the essential oil compounds of fever tea. There was a significant effect between the percentage oil compounds detected from fever tea essential oils. The compounds identified were monoterpenes i.e. α-pinene, sebinen, 1.8 cinede, myrcene, ipsenone and ipsedienone. The sesquiterpenes were β-caryophyllene and germacrene-D. In this study compounds which gave the smallest percentages were α-pinene, sebinen, 1.8 cinede, myrcene, ipsenone and ipsedienone. Compounds with the highest percentages were β-caryophyllene and germacrene-D (Fig. 4.15).

Fertigation frequency didn't affect the chemical compounds of fever tea. Plants which received water frequently and plants which were stressed gave similar compounds after extraction. The same occurred with growing media used. Pine bark and sand media didn't influence the chemical compounds of fever tea. Monoterpenes and Sesquiterpenes were detected from both growing media.

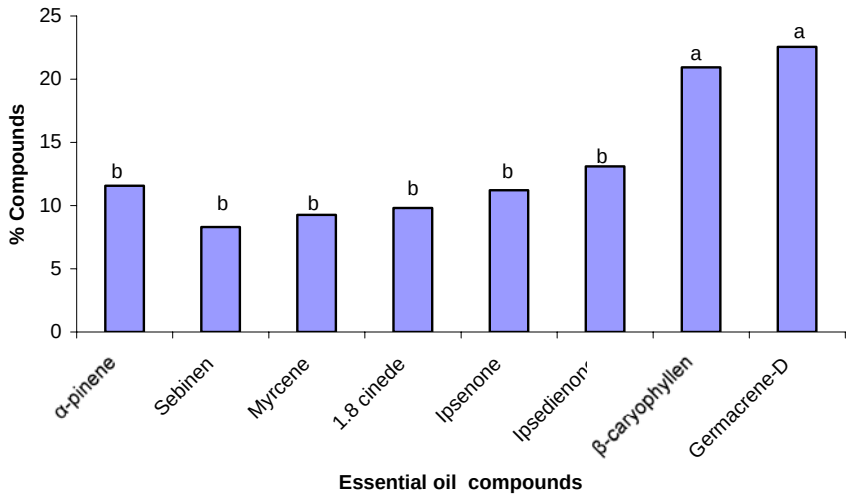


Fig. 4.15 Percentage (%) compounds and essential oil compounds of fever tea as affected by fertigation frequency and growth medium

4.4 **Discussion and Conclusions**

This study was designed to investigate the effect of fertigation frequency and growth medium on growth, yield and quality of fever tea. Fertigation frequency and growth medium are known to improve growth and development of many plant species by increasing yields and qualities. There are however, certain growth parameters that could be affected during growth and development stages of fever tea plant. These parameters are plant height, stem circumference, number of branches, fresh mass, essential oil yield and chemical compounds of essential oils.

In this study fertigation frequencies and the growing media affected the plant height of fever tea. Plants that received water more frequent had the tallest plant height when compared to stressed plants. Fever tea was very sensitive to water deficit. Similar responses occurred in mango trees where trees that had deficit irrigation had smaller plant height when compared to the well-irrigated ones (de Villiers, 2001). Thompson et al. (1989) reported that the more frequent the fertigation, the more the plant height increases. Furthermore, it was reported that daily or weekly fertigation increases plant growth when compared to less frequent. To achieve maximum plant height with fever tea it is advisable to give the plant more water than stressing it.

The growing media is another important factor that affects the plant height of some plants. Fever tea responded positively to sand medium in terms of the plant height than pine bark. This positive response of fever tea to sand could be related to its characteristics of allowing water to move easily through it and also allowing oxygen to pass through it. Another reason for fever tea plant to do well in sand medium might be its natural habitat, because it likes rocky sandy soil and dry woodlands maybe the sand medium used was similar to the soil it preferred when growing naturally. Hartman & Kester (1983) also supported this statement by reporting that sand medium must be sufficiently porous so that excess water drains away permitting adequate penetration of oxygen to the roots. Fever tea resulted in smaller plant heights when grown in pine bark and this might be caused by water logging, and more of carbon dioxide in the medium. It was also reported that pine bark medium is known to contain certain compounds such as phenols, resins, terpenes and tannins which might be toxic or harmful to other plants (Hartmann & Kester, 1983). To obtain optimum plant heights with fever tea it is advisable to use sand medium.

Stem circumference and number of branches were significantly affected by fertigation frequency and growth medium. Fever tea plants had thicker stems when grown in sand than pine bark. But contrastingly, when plants were younger they had thicker stems in pine bark. Number of branches were also more in sand. When fever tea plants were stressed they had thinner stems and fewer branches irrespective of the growing medium. De Villiers (2001) reported that in some plants if the water supply is 50% of field capacity longitudinal shoot growth was reduced by 55% while at a water supply of 25% field capacity the reduction was about 79% with both water regimes compared to 100% field capacity. Plants that received 100 % field capacity had an increase in longitudinal shoot growth. It was also reported that water deficits decrease tree trunks. Similar results were found during the growth stages of fever tea where the stem circumference and number of branches increased when plants received water daily. When plants received less water number of branches and stem circumference decreased.

Essential oil yield of fever tea was not affected by fertigation frequency. The significant effect was due to growth medium. Plants grown in pine bark gave more essential oil yield than plants grown in sand. Essential oils are mostly produced and stored in specialised structures on the surface of the plant or in plant tissues (Svoboda, Hampson & Hunter, 1989). In this study identification was made of oil glands which contain essential oils, and trichomes which protect the leaves against some external factors. The number of oil glands and trichomes were not affected by fertigation frequency. The oil glands of plants that grew in pine bark appeared larger than the ones from plants that grew in sand.

Chemical compounds of fever tea were not affected by fertigation frequency or growth medium. In this study the chemical compounds detected from essential oils of fever tea were monoterpenes (i.e. α-pinene, sebinen, 1.8 cinede, myrcene, ipsenone and ipsedienone) and the sesquiterpenes (i.e. β-caryophyllene and germacrene-D). Compounds that gave the smallest chemical percentages and the shortest time to be detected were α-pinene, sebinen, 1.8 cinede, myrcene, ipsenone and ipsedienone. Compounds found with the highest chemical percentages with highest peaks were β-caryophyllene and germacrene-D. Some authors reported similar results whereby chemical compounds of fever tea with the smallest peak and the shortest retention time were the monoterpenes (Mwangi *et al.,* 1997). Similar findings occurred with fever tea chemical compounds, monoterpenes were detected within the shortest time. Myrcene in fever tea was found to be one of the important chemical compounds, which is

used in aromatherapy. Kuney (1994) reported that the greatest use of myrcene was an intermediate in the commercial production of terpene alcohols, geraniol, nerol and linalool, which served as intermediates for the production of large volume aroma and flavour chemicals. It was also used in large quantities of aroma compounds myrcenol and its derivatives. . For the production of fever tea essential oil, plants should be grown in pine bark media but for the production of tea more vegetative growth was produced in sand medium. The recommended fertigation frequency that improved plant height, stem circumference, number of branches and fresh mass of fever tea was either 1L/day or 2L/day.

4.5 **Summary**

Fever tea is one of the most important medicinal plants in the family Verbenaceae. Due to these important aspects of fever tea, the response of fever tea to drip fertigation frequency and growing medium produced in a tunnel has not been studied. The objective of this investigation was to determine the effect of fertigation frequency and the growing medium on the growth, yield and quality of fever tea. The experiment was carried out in a tunnel at University of Pretoria's Experimental farm. The main variables studied were five fertigation frequencies (0.5L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and two growing medium (pine bark vs. sand). Measurements were made of plant growth i.e. plant height, stem circumference, number of branches at 8, 16 and 32 weeks after planting and the fresh mass was determined at harvesting. The oil yield was determined at 32 weeks immediately after harvesting through hydro-distillation. The chemical compounds were identified from the essential oils from hydro-distillation.

At 8 weeks after planting plants grown in pine bark media had better growth and increased the plant height, stem circumference as compared to plants grown in sand media. At 16 and 32 weeks after planting plants grown in sand increased plant height, stem circumference and number of branches as compared to pine bark media. In other words, fever tea plants grew better in pine bark at early growth stages and at later stages plants grew well in sand.

Fever tea plants that received highest fertigation frequency (2L/day) had significantly higher plant height, stem circumference and number of branches as compared to plants that received the lowest fertigation frequency (2L/week).

The oil yield percentages of fever tea were significantly higher when grown in pine bark than in sand. Oil glands of fever tea appeared larger when produced in pine bark media as compared to sand media. Fertigation frequency and growing medium did not affect number of oil glands and trichomes produced. Chemical compounds of fever tea were also not affected by fertigation frequency or growth medium. Monoterpenes and sesquiterpenes were identified from the essential oil of fever tea. Compounds that gave the smallest chemical percentages and the shortest time to be detected were α-pinene, sebinen, 1.8 cinede, myrcene, ipsenone and ipsedienone. Compounds found with the highest chemical percentages with highest peaks were β-caryophyllene and germacrene-D.

For the production of fever tea essential oil, plants should be grown in pine bark media but for the production of tea more vegetative growth was produced in sand medium. The recommended fertigation frequency that improved the plant height, stem circumference, number of branches and fresh mass of fever tea was either 1L/day or 2L/day in sand media.

GENERAL DISCUSSION AND CONCLUSIONS

A study was carried out to determine the propagation methods of fever tea by stem cuttings and seeds as well as to determine the growth, yield and quality response of fever tea as affected by fertigation frequency and growing medium. This study was conducted at the Hatfield Experimental Farm in a mist bed and in a tunnel as well as at the Department of Botany in growth chambers. The investigated variables were: cutting position (apical vs. basal), media (pine bark vs. sand) and hormone (Seradix No.2 vs. no-hormone). For seed germination: continuous light, continuous dark, alternate light: dark, constant and alternate temperatures were studied. The following was investigated: growth, yield and quality of fever tea as affected by fertigation frequency (0.4L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and growth medium (pine bark vs. sand).

Vegetative propagation of fever tea by stem cutting is possible. The optimum rooting of this species can be achieved if factors that affect rooting are considered. These factors are cutting position, rooting media and rooting hormone. The root development of fever tea depended on the number of days after planting. Apical cuttings rooted earlier than basal cuttings regardless of the growing media. It took 10 days to root most of the apical cuttings whereas basal cuttings showed maximum rooting from 15 days after planting. Pine bark growing medium gave good results as compared to sand medium for both apical and basal cuttings in terms of the root length of the cuttings. Basal cuttings resulted in thicker stems and more number of leaves as compared to apical cuttings. Seradix No.2 hormone increased fresh mass, stem circumference, number of roots and number of leaves for both apical and basal cuttings. However, for commercial production of fever tea growers are advised to propagate stem cuttings using both apical and basal cuttings in pine bark medium. The cuttings can be ready for transplanting at 15 to 20 days after establishment and Seradix No.2 hormone (0.3% IBA) is recommended to promote rooting of the cuttings.

The germination percentage and the germination rate of fever tea was affected by photoperiod (light and dark) and temperature (both under constant and alternate conditions). Germination of fever tea seeds started after 8 days and germinated up to 30 days from sowing. Seeds germinated in constant and alternating temperatures ranging from 15 to 30 ^{o}C. There were highly significant differences in germination percentage due to temperature treatments and the highest was 86% at continuous light. The mean germination time was higher in continuous

light than in alternating light and dark treatments. Fever tea seeds were positively photoblastic and they failed to germinate in the dark. According to these investigations, germinating seeds under continuous light at 20 to 30 ^{o}C constant temperatures can obtain better germination of this species. It is recommended that when planting fever tea seeds directly to the soil, it should be planted shallowly covered with a small layer of soil to encourage light to penetrate into the soil. It is also advisable to plant on sandy soils and avoid clay and silty loam because sandy soil has high transmission of light and therefore this will be an advantage for fever tea seeds.

Fever tea (*Lippia javanica*) could be propagated successfully in a tunnel. There was a significant effect of fertigation frequency on plant height of fever tea at 8, 16 and 32 weeks after planting. Plants grew taller when fertigated with 0.4L/day, 1L/day and 2L/day. The maximum height was reached at 32 weeks after planting with a fertigation frequency of 2L/day. Growing medium also affected plant height of fever tea. When plants were younger, they grew taller in pine bark media than when they were older. Sand media increased plant height of fever tea at 16 weeks and 32 weeks after planting.

Stem circumference and number of branches of fever tea were significantly affected by fertigation frequency and growth medium. In terms of the growth medium, at 16 and 32 weeks after planting, plants showed thicker stems when grown in sand than in pine bark. Contrastingly, when plants were younger they showed thicker stems in pine bark than in sand. At 16 weeks after planting, plants fertigated with 1L/ day had thicker stems.

The essential oil yield of fever tea was not affected by fertigation frequency. The significant effect was on growth medium. Plants grown in pine bark produced more essential oil than plants that were grown in sand. The oil glands that developed in plants grown in pine bark appeared larger than the ones developed in plants grown in sand.

The chemical compounds of fever tea were not affected by fertigation frequency and growth medium. In this study the chemical compounds detected from essential oils of fever tea were monoterpenes (i.e. α-pinene, sebinen, 1.8 cinede, myrcene, ipsenone and ipsedienone) and the sesquiterpenes (i.e. β-caryophyllene and germacrene-D). Compounds that gave the smallest chemical percentages and the shortest time to be detected were α-pinene, sebinen, 1.8 cinede, myrcene, ipsenone and ipsedienone. Compounds found with the highest chemical percentages

with highest peaks were β-caryophyllene and germacrene-D. For the production of fever tea essential oil, plants should be grown in pine bark, but for the production of tea, more vegetative growth was produced in sand. The recommended fertigation frequency that improved plant height, stem circumference, number of branches and fresh mass of fever tea was either 1L/day or 2L/day in sand.

GENERAL SUMMARY

Fever tea is an important medicinal plant used to treat various diseases such as malaria, colds, fever and stomach pains. A study was carried out to determine the propagation methods of fever tea by stem cuttings and seeds as well as to determine the growth, yield and quality response of fever tea as affected by fertigation frequency and growth medium. The study was conducted at Hatfield Experimental Farm in a mist bed and in a tunnel, as well as at the Department of Botany in the growth chambers. The effect of cutting position (apical vs. basal), media (pine bark vs. sand) and hormone (Seradix No.2 vs. no-hormone) were studied. For seed germination, continuous light, continuous dark, alternate light: dark, constant and alternate temperatures were studied. For growth, yield and quality, fertigation frequency (0.4L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) effect and growth media (pine bark vs. sand) were studied.

In vegetative propagation of fever tea by stem cuttings, apical cuttings rooted earlier than basal cuttings regardless of the growth medium. It took 10 days to root most of the apical cuttings whereas basal cuttings showed maximum rooting from 15 days after planting. Pine bark improved the root length of both apical and basal cuttings than sand. Basal cuttings had thicker stems and more leaves as compared to apical cuttings. Seradix No.2 hormone increased fresh mass, stem circumference, number of roots and number of leaves for both apical and basal cuttings.

Fever tea was tested at constant temperature regimes (15, 20, 25 and 30 ^{o}C with continuous light or dark periods and alternating temperatures of 20:30 and 16L: 8D (light: dark) combinations respectively. Germination of fever tea seeds started after 8 days and germinated up to 30 days from sowing. Seeds germinated in constant and alternating temperatures ranging from 15 to 30 ^{o}C. There were highly significant differences in germination percentage due to temperature treatments and the highest was 86% at continuous light. The mean germination time was higher in continuous light than in alternating light and dark. Fever tea seeds were positively photoblastic and they failed to germinate in the dark. According to these investigations, germinating seeds under continuous light at 20 to 30 ^{o}C constant temperatures can obtain better germination of this species.

Maintenance of nutrients and water at optimum levels within the rhizosphere of plants is a primary factor to achieve high yield, to improve quality and increase fertilizer and water use efficiencies. However, the response of fertigation frequency and growing medium to fever tea growth, development, oil quality and yield was not known. Studies were conducted in a tunnel to determine the influence of fertigation frequency and growing medium on the growth, oil yield and oil quality of fever tea. Treatments used were five fertigation frequencies (0.5L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and two growing media (pine bark and sand). Measurements of plant growth included plant height, stem circumference and number of branches at 8, 16 and 32 weeks after planting.

At 8 weeks after planting, plants grown in pine bark had better growth and increased plant height and stem circumference as compared to plants grown in sand. At 16 and 32 weeks after planting, plants grown in sand increased plant height, stem circumference and number of branches as compared to pine bark. In other words, fever tea plants grew better in pine bark at early growth stages and at later stages plants grew well in sand. Plants grew taller when fertigated with 0.4L/day, 1L/day and 2L/day. The maximum height was reached at 32 weeks after planting with a fertigation frequency of 2L/day. Fertigation frequency of 2L/day was more adequate to sustain plant growth and development of fever tea. The recommended fertigation frequency and growth medium that improved plant height, stem circumference, number of branches and fresh mass of fever tea was either 1L/day or 2L/day in sand.

Extraction of fever tea oil was determined by hydro-distillation. The essential oil yield of fever tea was not affected by fertigation frequency. The significant effect was due to growth medium. Plants grown in pine bark gave more essential oil than plants in sand. The oil glands that developed in plants that grew in pine bark appeared larger than the ones developed in plants that grew in sand.

Composition of *L. javanica* oil prepared from fresh leaves was determined by gas chromatography (GC) and mass spectrometric (MS) techniques. The chemical compounds of fever tea were not affected by fertigation frequency and growth medium. In this study the chemical compounds detected from essential oils of fever tea were monoterpenes (i.e. α-pinene, sebinen, 1.8 cinede, myrcene, ipsenone and ipsedienone) and the sesquiterpenes (i.e. β-caryophyllene and germacrene-D). Compounds that gave the smallest chemical percentages and the shortest time to be detected were α-pinene, sebinen, 1.8 cinede, myrcene, ipsenone

and ipsedienone. Compounds found with the highest chemical percentages with highest peaks were β-caryophyllene and germacrene-D. For the production of fever tea essential oil, plants should be grown in pine bark medium but for the production of tea, more vegetative growth was produced in sand.

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APPENDIX

SUMMARY OF ANALYSIS OF VARIANCE (ANOVA)

Table A1 Analysis of variance for fresh mass, root number and root length of fever tea cuttings at 5 days after planting

Sources of variation	DF	Mean Squares[z]		
		Fresh mass (10^{-4}mg)	Root number (10^{-2})	Root length (10^{-2} cm)
Cutting (C)	1	6	289	2289
Media (M)	1	689	449	7323
C x M	1	725	11	2621
Hormone (H)	1	1737*	2527**	20131**
C x H	1	149	559	7664
M x H	1	47	211	2104
C x M x H	1	16	102	8262
Error	71	401	266	2430

[z]F – values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

Table A2 Analysis of variance for fresh mass, root number, root length and leaf number of fever tea cuttings at 10 days after planting

Sources of variation	DF	Mean Squares[z]			
		Fresh mass 10^{-4}mg)	Root number (10^{-2})	Root length (10^{-2} cm)	Leaf number (10^{-2})
Cutting (C)	1	1025	24955	19287	1758
Media (M)	1	1975	23852	1166126**	187
C x M	1	785	9915	848	111
Hormone (H)	1	3054	63462*	155484	162
C x H	1	419	3086	63496	92
M x H	1	38	30	4795	69
C x M x H	1	42	1334	92744	131
Error	59	1303	9745	99089	575

[z]F – values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

Table A3 Analysis of variance for fresh mass, root number, root length and leaf number of fever tea cuttings at 15 days after planting

Sources of variation	DF	Mean Squares[z]			
		Fresh mass (10^{-4}mg)	Root number (10^{-2})	Root length (10^{-2} cm)	Leaf number (10^{-2})
Cutting (C)	1	1	4138	40452	946526*
Media (M)	1	46183	77653	1610376**	834533*
C x M	1	87	490	258244	74099
Hormone (H)	1	16518	75544*	459	1
C x H	1	2571	140	1414	192665
M x H	1	443	6184	250576	209697
C x M x H	1	29	39743	34338	61425
Error	59	2580	12761	133370	96545

[z]F – values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

Table A4 Analysis of variance for fresh mass, root number, root length and leaf number of fever tea cuttings at 20 days after planting

Sources of variation	DF	Mean Squares[z]			
		Fresh mass (10^{-4}mg)	Root number (10^{-2})	Root length (10^{-2} cm)	Leaf number (10^{-2})
Cutting (C)	1	1443	8156	224401	1280
Media (M)	1	7	90	29970	6865
C x M	1	9359	159654**	546809	6303
Hormone (H)	1	6825	53810	475856	61
C x H	1	16065*	82759*	285809	18251
M x H	1	8587	1148	48760	19386
C x M x H	1	5663	7106	30421	25584
Error	65	3530	16197	175724	8390

[z]F – values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

Table A5 Analysis of variance (ANOVA) of germination percentage and mean germination rate of fever tea with temperature treatments

Source of variation	DF	Mean squares	
		Germination % (10^{-2})	MGT (10^{-2})
Temperature	4	168333**	3005
Error	15	25556	1077

[z]F – values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

Table A6 Analysis of variance (ANOVA) of germination percentage and mean germination rate of fever tea with photoperiod treatments

Source of variation	DF	Mean squares	
		Germination % (10^{-2})	MGT (10^{-2})
Temperature	1	267593*	11136**
Error	18	43838	947

[z]F – values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

Table A7 Analysis of variance for plant height, number of branches and stem circumference of fever tea at 8,16 and 32 weeks after planting as affected by fertigation frequency and growing media

Sources of variation	DF	Mean Squares[z]							
		Plant height (10^{-2}cm)			Stem circumference (10^{-2}mm)			Number of branches (10^{-2})	Fresh mass (10^{-2}g)
		(8wks)	(16wks)	(32 wks)	(8wks)	(16wks)	(32 wks)	(32 wks)	(32 wks)
Fertigation frequency	4	196836	252827*	3287013 *	859044	4670664*	5501341*	129517*	4307782
Media	1	216600**	14236253*	14756233*	1675682	2992479*	4258299*	3041720*	16506417
F x M	4	546850	632734	433900	885899	498109	339590	213903	715575**
Error	163	292231	202257	195252	301324	215649	185256	307956	156013

[z]F – values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

Table A8 Analysis of variance (ANOVA) of fever tea chemical compounds as affected by fertigation frequency, media and retention time

Sources of variation	DF	Mean squares[z]
		% Compounds (10^{-2} min)
Fertigation frequency	4	2490
Media	1	4607
F x M	4	2335
Compounds	7	92979*
F x C	53	3435
M x C	7	5170
F x M x C	47	2506
Error	102	8659

[z]F – values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

Table A9 Analysis of variance (ANOVA) of fever tea essential oil yield as affected by growing media

Source of variation	DF	Mean Squares[z]
		Oil yield (%) (10^{-2})
Fertigation frequency	4	500
Media	1	597*
F x M	4	110
Error	30	800

[z]F – values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)