STANDARDIZATION OF TREE INJECTION PROCEDURES OF AZADIRACHTIN IN COCONUT (*Cocos nucifera* L.), MANGO (*Mangifera indica* L.) AND NEEM (*Azadirachta indica* A Juss.)

by

SARMISHTA V.

(2017-17-011)

THESIS

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VELLANIKKARA, THRISSUR- 680 656

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DECLARATION

I, hereby declare that the thesis entitled **"STANDARDIZATION OF TREE INJECTION PROCEDURES OF AZADIRACHTIN IN COCONUT (*Cocos nucifera* L.), MANGO (*Mangifera indica* L.) AND NEEM (*Azadirachta indica* A Juss.)"** is a bonafide record of research done by me during the course of research and that this thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara,
Date: 21-10-2019

Sarmishta V.
(2017-17-011)

CERTIFICATE

Certified that the thesis, entitled **"STANDARDIZATION OF TREE INJECTION PROCEDURES OF AZADIRACHTIN IN COCONUT (*Cocos nucifera* L.), MANGO (*Mangifera indica* L.) AND NEEM (*Azadirachta indica* A Juss.)"** is a record of research work done independently by Ms. Sarmishta V. (2017-17-011) under my guidance and supervision and that it is not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Vellanikkara,

Date: 21-10-2019

Dr. E.V.Anoop

(Major Advisor, Advisory Committee)

Professor and head

Department of Forest Products and Utilization

College of Forestry

Kerala Agricultural University

Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Sarmishta. V. (2017-17-011), a candidate for the degree of Master of Science in Forestry with major in Forest Products and Utilization, agree that the thesis entitled **"STANDARDIZATION OF TREE INJECTION PROCEDURES OF AZADIRACHTIN IN COCONUT (*Cocos nucifera* L.), MANGO (*Mangifera indica* L.) AND NEEM (*Azadirachta indica* A Juss.)"** may be submitted by Ms. Sarmishta V., in partial fulfillment of the requirement for the degree.

Dr, E. V. Anoop
(Chairman, Advisory Committee)
Professor and Head
Department of Forest Products
and Utilization
College of forestry, Vellanikkara

Dr. A. V. Santhoshkumar
(Member, Advisory Committee)
Professor and Head
Department of Forest Biology and
Tree Improvement
College of Forestry, Vellanikkara

Dr. S. Gopakumar
(Member, Advisory Committee)
Professor
Department of Natural Resource
Management
College of Forestry, Vellanikkara

Dr. Berin Pathrose
(Member, Advisory Committee)
Assistant Professor
Department of Agricultural Entomology
College of Horticulture, Vellanikkara

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Dedicated to my parents and friends

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Introduction

1. INTRODUCTION

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The forests are known to be present on earth since millions of years, way more earlier than the period when humans existed. They have played a greater decisive role in the survival of many different species including man and have paved the way for the development of modern civilizations. The dependence of man on forest based materials, more importantly wood, is still relatable in this modern era of cement, ceramics, composites, etc. The use of wood can be traced back to our more traditional equipment of old times and its importance still grows in this age backed by the growing economy and more beneficial products of wood being developed through research which makes human life comfortable, in turn boosting demand (Tsoumis, 1991).

The decay of wood is an inevitable process and it is an important phenomenon since it paves way for newer generations otherwise the wood skeleton of the older tree would still be standing in the forest making it more cluttered (Richardson, 2002). The degradation of timbers can be mainly attributed to the insects and fungi. Insects can be dangerous in case of rampant infestation, which can result in extensive deterioration of structural timbers and can lead to frequent replacement of timbers which otherwise would have been serviceable. The wood deterioration in temperate regions can always be pointed to wood boring beetles (Coleoptera) alone or beetles and termites (Blattodea, Termitoidae) when they are present. The attack of beetles is primarily focused on sapwood though with exceptions and it can be recognized by the shape and type of holes and the type of frass (Nunes *et al.*, 2019).

Insect outbreaks are normal in any type of forests which is essential for forest dynamics and such a way the insect population multiplies and sometimes reaches a destructive level leading to extensive damage of trees in large areas of natural and planted forests; destruction of vital ecosystems and considerable hindrance to the national economy. In developing countries like India pest outbreaks can lead to loss of livelihood to forest dependent people and scarcity of food, thus impacting the national economy.

Problems related to the usage of inorganic chemical pesticides was recognized worldwide and was brought to the limelight with the publication of the book 'Silent Spring' by Rachel Carson (1962), leading to the eventual banning of DDT by the US government in 1972. It emphasized the need for more safer natural organic pesticides and other developments across the world paved the way for the importance of organic pesticides such as azadirachtin with better properties than their inorganic counterparts (Morgan, 2009).

Research conducted over the last two decades on various plants to understand the properties of alkaloids and extractives revealed that the neem tree (*Azadirachta indica*) has been at the forefront in catching the attention of entomologists and phytochemists all over the world. Looking at its importance as an effective control for pests, several neem based marketable products have already been produced all over the world (Schmutterer, 1990).

Jones and Gregory (1971) claimed that the solutions required by the trees can be effectively introduced into the trees through the apparatus that has been devised to rejuvenate the health of affected trees and provide protection to trees from possible infection by using systemic fungicides, insecticides, herbicides and even nutrients and hormones. They also reported that the method of injecting chemicals directly into the trees has many advantages over soil and foliar application like proper dosage application, treatment being independent of external agents, smaller dosages are enough and their placement and subsequent movement can be regulated, etc. Thus, this method is not only economically beneficial but also mitigates inherent hazards associated with other methods.

Coconut (*Cocos nucifera* L.) and mango (*Mangifera indica* L.) are commercially the most important fruit crops of India. Whereas neem (*Azadirachta indica* A. Juss.) is a major component of urban and community forests in India since time immemorial. They are important commercial species yielding fruits and other various important commodities like wood, medicines and fibres. The challenge faced by these species is the invasion of various pests. Farmers

currently count on chemical insecticides to control insect pests. The heavy use of inorganic insecticides has led to increased costs, environmental pollution and social problems (Peng and Christian, 2005), and in the reduction of natural predators and parasitoids of the insect pests.

Trunk or soil injection of systemic insecticides is progressively preferred as a method for controlling insect pests in urban and suburban landscapes because of little risks of applicator exposure, drift and impacts on non-target organisms (Mota-Sanchez *et al.*, 2009). Imidacloprid products are commonly used to guard ash trees from *Agrilus planipennis* (Emerald Ash Borer). Neonicotinoid and organophosphate trunk injections are employed to enhance the management of avocado thrips in California avocado groves (Byrne *et al.*, 2014).

Objectives

This study was intended to assess the intake of azadirachtin when applied at basal heights in neem, mango and coconut using tree injection procedures. The study also aimed at standardising tree injection procedures of azadirachtin in these species.

Review of literature

2. REVIEW OF LITERATURE

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2.1. Azadirachtin

Butterworth and Morgan (1968, 1972) claimed that typically the neem tree (*Azadirachta indica* A. Juss.) is a crucial resource of biologically lively compounds and it has been the center of attention of progressively powerful investigations during the past four decades since the extraction of the bio insecticide azadirachtin from the seeds they bear. According to Jarvis *et al.* (1999), azadirachtin is a well oxidized triterpenoid and is one of the most powerful anti-feedant compounds yet revealed. It also influences the regular development and growth of any broad range of insects (Mordue and Blackwell, 1993; Schmutterer, 1995).

Kaushik (2002), stated that the most crucial limonoid contained in the neem seed is azdirachtin. It is also reported to have antifeedant, growth disrupting, and ovicidal activity against a wide range of insect pests. Despite the fact that hundreds of plant-derived nourishing deterrents have been uncovered, none which are alone antifeedants (unlike azadirachtin), have however been exploited commercially (Morgan, 2009).

Schmutterer (1990) stated that special curiosity of entomologists and phytochemists all over the globe is on azadirachtin extracts among the list of various ingredients of vegetation studied since 20 years in addition to other compounds from the neem tree. Several marketable neem-based products have been manufactured in recent years. Thus, the up to date knowledge about the properties of neem derivatives with respect to pest control appears to opportune.

Typically, well known and majority of the useful property of the neem tree is the antifeedant property to insects portrayed even in crude components. Azadirachtin has the greatest biological activity along with antifeedant property. In addition to

this, it also produces developmental abnormalities in just about all insect orders (Subrahmanyam, 1990).

According to Isman *et al.*, 1990, the most potent natural insect antifeedant learned to date is azadirachtin, a ring C-seco tetranortriterpenoid, which is the main active principle. Azadirachtin hinders the reproduction along with molting in numerous varieties of insects (Sieber and Rembold, 1983; Koul, 1984; Koul *et al.*, 1987), considering the neuroendocrine system as a target site.

Herms *et al.* (2009) stated that a number of systemic insecticide products like the formulations of azadirachtin, emamectin benzoate, and imidacloprid can be injected directly into the trunk of ash tree. The ability of mature female beetles of emerald ash borers to produce viable eggs that successfully hatched was reduced by the application of azadirachtin. Several Emerald Ash Borer larvae had commenced feeding on trees treated with TreeAzin (5% azadirachtin) but died whilst still young and tiny. When trees were handled in the first year, abundnce of live larvae had been 75-80% lesser than on control trees. Results of this study hypothesised that in most years, TreeAzin will effectively protect ash trees for a couple of years, and when EAB densities are usually high, yearly applications may possibly be prudent.

2.1.1. Effect of azadirachtin in different species

The neem based formulations have been effective on Banganapalli in which the hopper population was reasonable as compared to Alphonso which recorded higher density of hopper population. These kinds of observations showed that the efficacy of both the azadirachtin (3000 and 10000ppm) seemed to be dependant upon density of hopper. From lower densities, or even as a prophylactic spray, therefore azadirachtin must be desired. As azadirachtin is environmentally friendly, it can be regarded as a follow-up spray (Verghese, 1999).

Azadirachtin does not necessarily kill insects like neurotoxins but influences thier conduct and physiology. Though restrained, the effects of neem such as chemo-sterilization, feeding, growth inhibition, mating interruption, oviposition prevention, and repellency, etc. (Schmutterer, 1995, 2002) are now considered significantly more desirable than a speedy knock-down in integrated infestation management programs since they decrease the risk of subjecting natural enemies to poisoned food or hunger. Despite high selectivity, neem extracts affect four hundred to five hundred species of pests belonging to Blattodea, Caelifera, Coleoptera, Dermaptera, Diptera, Ensifera, Hetroptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Phasmida, Phthiraptera, Siphonoptera, Thysanoptera, etc. Neem formulations furthermore act as nematicides against endoparasitic species of *Globodera* and *Meloidogyne*, ectoparasite varieties of *Hoplolaimus* and *Tylenchorhynchus* and semiendoparasitic types of *Pratylenchus* and *Rotylenchus* nematodes (Musabyimana and Saxena, 1999). In the same way as a fungicide neem products are successful against numerous fungal pathogens. Water snails like *Melinia scabra* (schistosomiasis) and phytophagous land-snails in greenhouses are killed by neem formulations (West and Mordue, 1992). The neem products likewise control many acarines of *Tetranychus* genus, bacterial plant pathogens and animal and plant viruses (Hunter and Ullman, 1992; Schmutterer, 1995; Mordue, 2004).

Special effects of azadirachtin on the prolonged existence, reproduction and transformation of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), melon fly, *Dacus cucurbitae* (Coquillett) and oriental fruit fly, *Dacus dorsalis* (Hendel) exposed as late 3rd instars and pupae to treated sand were determined. Pupal stage was not affected by azadirachtin at the concentrations tested. Adult introduction was completely repressed at concentrations of 14 ppm for *C. capitata* and *D. dorsalis* including ten ppm for *D. cucurbitae*. *D. cucurbitae* was substantially more prone to azadirachtin than *C. capitata*. Even though adult emergence was inhibited, around 95% of treated puparia contained living adults. Adults that emerged from remedies

appeared normal but more of them died compared when with control. Ten days following emergence, 75% *D. dorsalis* and 64% *C. capttata* exposed as larvae and pupae to 4.66 ppm azadirachtin perished. Approximately 24% of *D. cucurbitae* confronted with 2.77 ppm azadirachtin died within just 10 days after emergence. Azadirachtin had no considerable impact on the amount of eggs laid by adult *D, dorsalis* and *C. capitata* that had survived larval-pupal treatments. *D. cucurbitae* controls laid substantially more eggs than grownups that had survived larval-pupal treatments with 1.85 ppm (Stark *et al.*, 1990).

2.1.2. Comparison of azadirachtin with artificial pesticides

2.1.2.1. Range of activity

The synthetic pesticides commonly have a wider range of action, but that is good and bad. Useful insects are killed along with the pests, and no insecticide is much desirable against insects once the pest is beneath tree bark, inside fruit or plant stems.

2.1.2.2. Systemic

Azadirachtin is systemic when compared to artificial insecticides.

2.1.2.3. Resistance

Synthetic insecticides are incredibly open to resistance in the development as they are single compounds. Azadirachtin and most natural insecticides have the advantage here.

2.1.2.4. Mammalian toxicity

Azadirachtin has very low mammalian toxicity (>5000 mg/kg).

2.1.2.5. Permanence and Residues

Their better stableness is both a benefit and drawback to artificial insecticides. Their persistence on food crops is the reason why we certainly have these kinds of strict rules about certification and allowable degrees of elements, and has led to the thoughts of the general public about perils of insecticide use.

2.1.2.6. Availability of supply

Synthetic insecticides are made finally from simple petroleum hydrocarbons. Their supply is therefore relatively limitless and the expense is decided by the cost of petroleum to only a little extent. The manufacturer generates huge added value simply by their production. Their benefit is substantial here.

2.1.2.7. Extraction

No direct comparison could be made between natural product extraction and large-scale synthesis (Mordue, 2004).

2.2. Trunk injection

Norris (1967) stated that people were never more worried regarding the side effects associated with toxic chemicals like pesticides than they could be nowadays. Our ultimate goal by the utilization, consumption and application of therapeutics in preferred organisms is correction of problems associated with the pest or disease situation without deleterious outcomes to the person or associated life.

A new apparatus has been devised to inject solutions under pressure into trees. This equipment should permit rapid launch of certain systemic fungicides, insecticides, herbicides, etc. straight into the xylem and should facilitate their supply throughout the tree (Jones and Gregory, 1971).

Trunk or soil injection associated with systemic insecticides is significantly recommended as a technique for controlling insect infestations in urban and suburban landscapes as a consequence of minimal hazards of applicator exposure, drift and impacts upon non-target organisms. To work with, systemic insecticides must end up being translocated from the root base or injection site to the site of pest feeding. Once in the xylem, chemicals are reliant on the transpiration stream in order to move upward to leaves (Mota-Sanchez *et al.*, 2009).

An exceptional and important feature of the equipment is that the chemicals could be injected into the outermost active xylem tissues. This is accomplished by serving the solution under pressure through an injection head clamped against the surface of the sapwood in order to cover a tiny hole cut to penetrate the exterior two or three annual rings. Rapid uptake in addition to the distribution of solutions will be facilitated because maximum fluid movement occurs in the outer sapwood, and inserted chemicals tend to proceed throughout the current solid wood and into the leaves and roots (Jones and Gregory, 1971).

Costonis (1981), reported that Leonardo da Vinci early acknowledged the value of systemic injection after injecting apple trees with arsenic in order to control apple pests. With the latest advancement of injection procedures for treating the Dutch Elm disease in elm trees an increased fascination with tree injection has developed.

Leonardo da Vinci first systematically looked into a method in which the injection was done straight into the conductive tissues of trees. Yet, until early 20th century, many of the most early trunk injection experiments were not documented (Roach, 1939; Costonis, 1981). In 1970s, a disparaging vascular wilt disease of elm called Dutch elm disease renewed curiosity in tree injection (Jones and Gregory, 1971; Gregory *et al.*, 1973, 1975; Shigo and Campana, 1977), because more ordinary fungicide applications were proven unsuccessful. Quite a few injection methods like

trunk infusion (Schreiber, 1969), and pressurized trunk injections (Filer, 1973; Helburg *et al.*, 1973; Reil and Beutel 1976; Sachs *et al.*, 1977; Kondo, 1978; Darvas *et al.*, 1984; Navarro *et al.*, 1992), were created during this period. Trunk injection was similarly practiced for treating other tree pathogens (Fernández- Escobar *et al.*, 1993), insects and other potent physiological disorders like interveinal chlorosis in the EUROPEAN UNION. Curiosity in trunk injection systems (Doccola *et al.*, 2007; Smitley *et al.*, 2010) in the USA has furthermore elevated, with the introduction of several tree killing pests such as *Adelges tsugae* (hemlock woolly adelgid), *Agrilus planipennis* (emerald ash borer) and *Anoplophora glabripennis* (Asian longhorned beetle),.

According to Harries (1965) several attributes of tree injection or perhaps systemic applications are self-reliant from water requirements and the device used fro application; probability associated with distributing the insecticide to parts of the tree not reached by mechanized methods; prevention of disturbance caused due to weather conditions during the application of sprays and dusts, as well as problems associated with sun and rain on the insecticide deposits; much less threat to other individuals who can be involved and also to other creatures; chance of better effectiveness towards sucking pests; and elimination of exterior residues along with less antithesis between chemical and biological control.

Numerous insects, diseases, and nutrient deficiencies in trees are taken care of by various macro and microinjection strategies. Nevertheless the chemicals are not efficiently delivered into pines using injection techniques. As a reaction to wounding, oleoresin is released and filled quickly into the holes drilled into pines (Grosman *et al.*, 2002). A simple injection apparatus named the systemic tree injection tube (STIT) was introduced by Helson *et al.* (2001), and it was capable of successfully injecting several conifer species with neem oil (James *et al.*, 2006).

The wounds brought on by injection are generally associated with columns of occluded (compartmentalized) xylem and killed bark. These columns extend both up and down the trunk and is extended into the roots. Forty percent or even more of the transport system can be blocked. Presently, there are other techniques for the uptake of nutrients, biocides, and growth regulators by trees. Brand new technologies need to end up being developed by advertising and improving these options (Perry *et al.*, 1991).

These days, trunk injection is a great optional method for applying chemical with certain positive aspects: (1) effective utilization of chemical compounds, (2) limited environmental exposure, and (3) when soil and foliar applications are either inadequate or challenging to apply, it is beneficial (Stipes, 1988; Sanchez-Zamora and Fernandez-Escobar, 2004). Trunk injection directly into branches, stems or roots requires wounding of the tree, which has ramifications to the well being of tree. The problem frequently raised usually is, whether the advantage gained simply by trunk injection overshadow the risk of the injury caused by treatment. This specific query of cost-benefit is unquestionably legitimate. Meanwhile, this problem should also be considered in opposition to environmental exposures when trees and shrubs are sprayed or pesicides are applied to the soil. A fundamental presumption is usually that the importance of the tree and its treatment is higher than keeping the trees alive (Doccola and Wild, 2012).

Doccola and Wild (2012) stated that crucial factors weigh in to wound responses in trees which require care and attention. Such as (1) the tree species, (2) well being of tree, (3) the attributes associated with the chemical applied and (4) the frequency at which the chemicals are applied. These concerns present a larger and more complicated model and bring over into trunk injection practices. In order to apply trunk injections successfully, we need a fundamental idea of the (1) way of applying the chemicals, (2) the chemical applied, and (3) form of tree. The purpose of this document is to suggest trunk injection as an optional application way for

systemic pesticides to (1) safeguard trees against damaging insects, (2) to reduce possible environmental exposures, and (3) in order to handle tree injury reactions.

The launch and movement of liquid pesticides by injection is reliant on the vasculature of the tree. Eventually, trees are usually extremely linked systems (Shigo, 1989, 1991). Non-woody fibrous roots take up water in addition to solutes (i.e., nutrients and mineral deposits) in dissolved form through the root-soil environment (rhizosphere). Hydraulic movement through the xylem is reliant from stomates as transpiration, because the moisture is lost from leaf to the ambient environment. Upward movement of systemic pesticides likewise rely on the surge of sap in trees.

The distribution, paradigm and size of vessels differ in trees. Hardwoods may be arranged as diffuse porous or ring porous; softwoods are regarded as non-porous species. Angiosperms have large, broad vessels together with relatively higher flow rates, whereas gymnosperms depend only on relatively smaller diameter tracheids to transport water. The water flow rate varies along with different tree species (Doccola and Wild, 2012).

2.2.1. Tree injection compared to soil and trunk spray applications

Normally water soluble pesticides are absorbed differentially by tree roots when compared to insoluble chemicals (Wislocki, 1989). Imidacloprid in addition to acephate are labeled in the United States of America for soil application, yet limited in localities associated with ground water concern (e.g., Long Island, New York, U. S.). Doccola and Wild (2012) claimed that in coarse textured, sandy soils as well as localities together with high rainfall, we will find the possibility for pesticide leaching. The pesticidal treatment of eastern hemlock (*Tsuga canadensis*) for hemlock woolly adelgid (*Adelges tsugae*) is an instance. Eastern hemlock is the new riparian species, that develops in moist soils, and beside waterways and streams. In these environments, the practice of application of trunk sprays increases typically the

prospect of exposure to non target organisms. Trunk injection of pesticides is usually an optional technique for apllying chemicals where these conditions are present. Imidacloprid is applied straight to the vascular tissues through tree injection which in turn is conducted upward within those tissues; the process limits the possible for accidental exposures.

2.2.2. Advantages of trunk injection

According to Doccola and Wild (2012), canopy sprays are employed to manage insects that is involved in defoliating, but limited reach is usually an issues in very tall (more than 15 mts) trees, where treatment from hydraulic sprayers is not enough. Using trunk injections resolves such problems; the chemicals proceed within the vascular system into the canopy for systemic activity. Systemic injections are employed to efficiently manage borers that nourish underneath the bark, where active elements sprayed onto the tree surfaces might not exactly sink into biologically vibrant levels. Soil applications are likewise used, but have several restrictions. For instance, they might end up being slower in action, require larger quantities of product or even frequent applications, may drift off track, and be susceptible to microbial degradation. In conclusion, trunk injections may be a lot more cost-effective to make use of. Even if hydrolysis occur inside the plant, systemically injected chemicals might offer better residual activity in contrast to other methods like spray and drench which often are liable to leaching, microbial degradation or photolysis. Continual spraying practices every season are essential for satisfactory pest handling. Soil applications of systemic pesticides are tend to be made at substantially larger amounts (5 to 10 times) compared to trunk injection in order to recompense binding to soil, leaching, microbial degradation, soil moisture and the vagaries of pH.

2.2.3. Trunk injection as a substitute

These days, trunk injection is a good substitute method for applying chemical with specific positive aspects: (1) effective utilization of chemical compounds, (2) limited potential ecological havocs, and (3) when soil and foliar applications are either inadequate or challenging to apply, it is beneficial (Stipes, 1988; Sanchez-Zamora and Fernandez-Escobar, 2004). Trunk injection is employed when trees are susceptible to be attacked by destructive or persistent infestations. It may be used efficiently for tall trees. They are implemented in trees growing in ecologically sensitive areas (near water and in sandy soils). Trunk injection will generate injuries, nevertheless the advantage of the introduced chemistry for protecting trees frequently surpass the drilling wound. The brand new model weighs the prospective of off track outcomes of application to the consequences of the drilled wound made by trunk injection. Unintentional non target exposures take account of toxicity to the applicator, aquatic arthropods, earthworms, fish and pollinators. Pesticides are by design, harmful, albeit useful substances. Trunk injection is an approach to convey specific toxicants to the harmful insect infestations and also to limit non-intended exposures. Tree injection will be an alternative methodology for apllying systemic insecticides with regard to tree protection (Doccola and Wild, 2012).

2.3. Species

2.3.1. Coconut

Coconut (*Cocos nucifera*) belongs to the family of the Arecaceae (Palmae), the subfamily Cocoideae. It is a tree that is grown because of its multi uses, primarily for its medicinal and nutritional values. Different products of coconut are coconut cake, coconut leaves, coconut oil, coconut shell, coconut toddy, coir pith, copra, raw kernel, tender coconut water, wood based products, etc. All parts are used in the day to day life of the folks in the conventional coconut growing areas (DebMandal and Mandal, 2011).

He also claimed that the coconut palm is a significant fruit tree on earth that provides nourishment for lots of people, particularly in the subtropical and tropical regions and with its various uses it is normally known as the "tree of life". Since, for hundreds of years, coconut products have attained appreciated and important place in Indian folk medicine. It is considered to be antiblenorrhagic, antibronchitis, antigingivitic and febrifugal.

According to Bedford (1976), in several tropical countries over-aged coconut palm plantations will end up being cut during the subsequent decades to pave way to new plantations with high yielding varieties. One of the most practical idea is to transform the stems into timber. However, the coconut palm stem has some unique properties which make specific attention in processing essential.

He also reported that *Oryctes rhinoceros* L. is a destructive pest of the palm. It occurs in India and Ceylon and distributed eastwards throughout south eastern parts of Asia, and has already been unintentionally introduced into several South Pacific islands (Catley 1969). Rhinoceros beetles attack the palm by boring in to the stem to nourish. In New Britain and New Ireland it has been found to attack primarily palms that have been old and was seldom found on very young, post-seedling palms or individuals around 2 or 3 years of age. Gressitt (1953) made similar findings in Palau Islands. The number of palms infested mottled broadly from a single area to another.

2.3.1.1. Coconut wood

According to Wilson (1985), coconut palm has no vascular cambium (lateral developing tissue) they do not increase in diameter together with age. It is unusual to discover a stem above 30 cm in diameter. Minor variations in diameter from one stem to another or among different locations, certainly are a representation of the growing problems for the individual stem throughout early stages of its life. Taper is usually very slight (about five mm) and old stem heights of tall varieties are of the order of 20m thus providing maximum wood volume for each stem of approximately

1 m^3. The wood volume in a mature or fully developed plantation is about one hundred m^3/ha.

Wilson (1985) also reported that many hardwoods and softwoods display density gradients from the centre of the stem towards the outside and from the bottom of the trunk towards the top. This is due to the later formed wood in a cross-section is generally slower developing and is composed of cells with thicker walls. With coconut stems the gradients are more noticable, but for different factors. Palm wood includes a number of dispersed vascular bundles set within a matrix of a lot more or less spherical parenchyma cells. The vascular bundles are much more ample towards periphery of the stem. A typical stem at one metre height would have about 10 bundles/cm^2 in the core portion and approximately 50 bundles/cm^2 in dermal wood.

Durability and density are incredibly strongly related so the density distribution decides the sawing and cutting pattern that must end up being chosen if high strength wood is to be developed. High-density zone is pretty narrow as the logs have small diameter. Thus only a few small-dimension pieces of best strength may be produced from a stem.

Up to the six metre mark the high-density zone (outer $1/3^{rd}$ radius) accounts for 20% of the total Stem volume yet by the time allowances are made for saw kerf and other wastage, the net recoverable extremely high density wood drops to below 10% of the total. The maximum size of member of which can be cut out of this high density zone is usually 100× 50mm. Although the quantity of such material for each stem is low, the quality is uniformly large. As the palm provides no branches there are no knots in the wood. Therefore no piece is destabilized by the occurrence of natural defects (Wilson, 1985).

2.3.1. 2. Uses of wood

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Wilson (1985) claimed that the denser grades of coconut wood works extremely well as construction material while the lower grades are well suited for joinery and interior use. It has proved financial to construct dwelling houses completely of coconut wood. The denser material makes appealing furniture and is likewise traditionally used for utility products. The roundwood has excellent strength qualities and is also sutable for transmitting poles and fence posts provided the problems associated with regard to preservation treatment can end up being overcome.

2.3.1.3. Anatomy

In a young coconut stem the cell walls are relatively skinny and the basic density of wood in two zones could be about 90 kg/m^3 and 300 kg/m^3 respectively. These wood cells are not dead as in natrual trees, however, and the walls carry on and increase in thickness and when the palm is mature the density in these two regions end up being as high as 250 kg/m^3 and 900 kg/m^3.

Just about all tissues in the basal parts of mature palms (including the ground parenchyma cells) possess thicker cell walls. On top of the trunk the vascular bundles are more abundant and up to I75 bundles/cm^2 have been found outside an overmature stem with a height of 19.5 mts. The cells in these zones in no way develop thick walls, on the other hand, the basic density in this zone had been only 250kg/m^2. In the core region of the coconut stem (at 19.5 mts) the bundle frequency was 68/cm^2. The basic density varies from stem to stem (Wilson, 1985).

2.3.2. Mango

Mango (*Mangifera indica* L.) belongs to family Anacardiaceae and is called as king of fruits with regard to its delightful flavor, exceptional flavor, nutritive value and sweetness. Mango is cultivated in almost 100 countries. It is also important

ornate and shade tree, which protects the soil against erosion and possess various therapeutic qualities. Various pests attack mango trees, which were studied specifically (Sen, 1955; Herren, 1981; Tandon and Verghese, 1985). The nymphs and female bugs draw sap from flowers, fresh fruit peduncles, shoots and soft leaves. As an outcome, the afflicted inflorescences get dried and are shriveled. Severe pest attacks influence the fruit set and results in immature fruit drop. Sooty mould develops over the secreted honey dew (Tandon and Lal, 1978). Photosynthetic activity will be impacted as a result of sooty mould developed on leaves (Pruthi and Batra, 1960). Karar *et al.* (2009) stated that mealybug (*Drosicha mangiferae* Green.) is another severe insect pest infestation of mango crop in Pakistan and is a developing risk to the mango orchards of India.

The mango fruit is an essential source of nourishment for bats, birds, insects, and mammals. Although cultivated extensively, mangoes favor comparatively hot, frost-free weather with a dry winter season. High humidity and rain during flowering stage and fruiting stage decreases yields. The tree normally flowers in winter, with fruits maturing in mid-summer months. Mango trees are generally between 10 to 33 ft tall yet can reach up to 100 feet in some wild conditions. It has an evergreen canopy with a generally spreading habit. The mango canopy is a good refuge and shade for animals and humans. Mangoes are very well adapted to farming in a variety of soils and is being grown commercially for ages. These days, mangoes are renowned and consumed world wide. Mangoes one of the most popular tropical fruit (Bally, 2006; Chavan and Rasal, 2012).

A mango orchard might have a broad range of diseases. Diseases, disorders and pest problems in the plant produce some noticeable effects identified as symptoms. Thus, illnesses in a plant may be portrayed in a definite series of indicators and symptoms or indicator complexes occurring in various morphological parts like flowers, fruit, leaves, trunk etc of the tree (Prasad *et al.*, 2006).

2.3.2.1. Mango wood

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Mango wood is brown and lustrous without distinct sapwood. Mature trees often contain a little dark brown wood which is most likely the true heartwood. Mango wood has irregular, strongly interlocked grain and is moderately coarse in texture. Wood which is marketed is generally discolored. This staining is normally favored by customersand is easily avoided during processing. In typical discolored wood, the pores are black and the encircling wood is streaked with blue-gray, green, pink, and sometimes yellow discoloration. Generally, mango wood is featured by irregular grain. Figuring will take the kind of dimples, mottling, ripples, pronounced fiddleback, or even a swirl. Mango grown in the Hawaii islands weigh about 40 pounds/cft. This is similar to the wood grown in Puerto Rico (Longwood 1961) and in India. It truly is a little lighter than white ash and little heavier compared to black walnut. Where relative humidity changes are large, mango wood has a little lateral shrinkage drying and then remains stable when in service. If the wood is highly figured, the longitudinal shrinkage occasionally exceeds 0.5%, which is fairly huge in contrast to most woods, nevertheless this has not triggered any seasoning problems in Hawaii. Wood of specific gravity similar to Hawaii-grown wood have been studied in our country and was found to end up being similar to black ash and black cherry in bending strength, crushing strength and stiffness, but more on the order of butternut or silver maple in shock resistance. Its hardness may be just scarcely dented with a fingernail just like that of black walnut.

When working with mango wood, the machining properties vary along with grain irregularities and is sawn easily. In wavy-grained wood, grain tearouts are normal when planed, shaped or turned (Longwood, 1961). Highly figured wood has been tried as a sliced face veneer in plywood manufacturing. Such wood is usually highly delicate as veneer because of its extremely wavy grain. The wood breakes without difficulty while handling and chipped. Mango wood is seasoned easily. Thick timber can be kiln-dried from the green condition by the way of a quite harsh

schedule devoid of defects. In the wet climate of Hilo, mango wood will get habitually discolored if set out for normal airdrying. The wood staining can be eliminated kiln drying of freshly sawn timber immediately. Mango is furthermore greatly prone to discoloration and insect attack when in log form and demands quick processing. The wood is not tolerant to degradation and is highly vulnerable to destruction from insects in Hawaii even when used indoors as furnishings. Pressure-treatement of preservatives is requied for any indoor or outdoor uses. Excellent transmission and retention of preservatives can be accomplished. Although the mango wood is not greatly long-lasting, it is easily treated with preservatives. Mango wood has specific gravitiy around 0.5 and modulus of rupture around 56.9-27.6 MPa (Rajput *et al.*, 1996; Jain *et al.*, 2000).

2.3.2.2. Uses of wood

Wood is used in the Hawaiian island on a small scale for craftwood products, furniture, gunstocks, and paneling. As a craftwood, it is employed generally to make plates and bowls. For this specific purpose, varicolored, stained wood is usually preferred along with extremely figured wood. Furniture comprised of cabinets, chairs and occasional tables which is very appealing and also have given good service. Paneling used in houses and chapels has equally been extremely successful. Mango provides a strange color for gunstocks. Besides having these kinds of unusual decorative uses, mango is a good common utility hardwood ideal for crates, pallets and upholstery framing (Skolmen, 2000). Wood of mango finds place in handicraft industries of India including export markets (Kumar *et al.*, 2015).

2.3.3. Neem

Neem (*Azadirachta indica*) usually known as 'Indian Lilac' or 'Margosa'. It belongs to the family Meliaceae, subfamily Meloideae and tribe Melieae. Neem is usually the most flexible tree of tropics, along with enormous prospectives. It owns highest number of useful non-timber products like bark, flowers, fruits, gum, leaves,

neem cake, oil and seeds unlike other tree species (Girish and Shankar, 2008). They also reported that neem is a huge tree that grows to about 25 m high and has a semi-straight to straight trunk, 3 m in girth and spreading twigs to form a broad crown. A neem tree usually starts fruiting after 3-5 years. Within a decade, the tree becomes fully productive. Coming from the tenth year, it produces around 50 Kg of fruits every year.

Srivastava *et al.,* 1997 reported that neem is an attractive broad-leaved medium to large tree, which attains an elevation around 20m and a girth of 2.5 meters in favorable localities. Neem usually possesses a stout and rather short stem together with wide spreading branches in addition to glabrous twigs forming a round to oval crown. A neem tree usually bears fruits approximately after five years and becomes completely productive in ten years.

Neem tree is a significant resource of biologically vibrant compounds and offers the center of progressively intensive experiments in past decades after the extraction of azadirachtin from the neem seeds (Butterworth and Morgan, 1968, 1972). Derevatives from different components from the neem tree have already been declared to own biological activity, like anti-inflammatory, anti-tumour, bactericidal, immune-stimulating, but mainly, pesticidal and pest anti-feedant effects (Jarvis *et al.*, 1999). The minor non-timber products of neem are identified to have antidermatic, antifungal, antiallergenic, anti-inflammatory, antiscabic, antifeedent, antipyorrhoeic, cardiac, diuretic, larvicidal, nematicidal, pesticidal, spermicidal and some other biological activities. Due to these properties, neem has found huge uses rendering it a green, eco-friendly value (Girish and Shankara, 2008).

Kaushik (2002) reported that the neem tree yields a wide array of triterpenoids like azadirachtin, azadirachtol, nimbin, salanin, etc., and 30–50% oil. The triterpenoids are generally referred as limonoids.

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Neem is found all over India apart the Himalayan foothills, which is a moderately fast-growing tree. Many commercially utilizable by-products are obtained from the neem tree. Neem has several household uses and has lately been declared to be an excellent source of azadirachtin, which is an antifeedant present in its seeds.

According to Morgan (2009) neem tree, is indigenous to the Indian sub-continent, but it is currently spread extensively in the drier tropics, including Africa, Central and South America, Indochina and Indonesia. It has furthermore taken to China, Fiji, Mauritius and Northern Australia. Koul *et al.*, 1990 stated that neem tree is a source of natural insecticides which is an Indian native, is fast-growing shade tree and continues to be widely grown in Africa, Australia, the Carribean, and Central and South America. Neem is a tropical evergreen tree and is deciduous is nature in drier areas, which is native to Indian sub-continent (Girish and Shankara, 2008).

Neem is a valuable tree, besides its potential to produce azadirachtin. It is a tree which is small-to-medium sized. It grows fast in degraded soil or perhaps, semi-arid conditions with an average of a minimum of 400 mm rainfall per annum and poor soil. It can endure high temperatures and remains to be green after the leaves of other trees have fallen in drought. It is a tree species that yields fuel in the drier tropics. It is a potential windbreak, to stop desertification and is a shade tree. The seeds are rich in bitter-tasting non-edible oil (40%). The seed cake is likewise bitter and unpalatable to livestock, even though they are loaded with proteins. It also has pesticidal properties and is a soil improver (Morgan, 2009).

Even though the neem seeds and foliage have been utilized conventionally since ages to manage unwanted pests (Koul *et al.*, 1990), current interest in neem as a crop protectant schedules to the effort of Pradhan *et al.* (1962), who confirmed that dilute derevatives of seed completely averted desert locust (*Schistocerca gregaria*) feeding. The main active principle present in neem seeds azadirachtin (AZA), which

is a ring C-seco tetranortriterpenoid, is considered the most important natural pest antifeedant uncovered. More recently, azadirachtin offers a demonstration to irmly intervene with reproduction and molting and in many varieties of pests (Sieber and Rembold, 1983; Koul, 1984; Koul *et al*., 1987; Shalom *et al*., 1988), which makes the neuroendocrine system as the target site. Thus, azadirachtin is dually beneficial as a bio pest control agent since it offers antifeedant as well as poisonous (which regulates insect growth) qualities against pests. Over two hundred species of insect pests in seven orders usually are identified to be vulnerable to the bioactivity of azadirachtin (Saxena, 1989), addressing over 90% of the insect species which is so far analyzed (Isman *et al*., 1990).

New shoots arise after the leaf fall since neem tree is deciduous. The bark of the tree is dark grey and thick with many longitudinal furrows on the surface. The heartwood is usually red to reddish-brown which darkens on contact with air and the sapwood is greyish-white. The wood is durable, easily workable, hard to very hard, interlocked narrowly, medium to coarse textured, moderately heavy and not attacked by insects (Nair, 1988). He also reported that neem wood, besides construction, is employed in the broad array of artefacts starting from boats, home furniture, to toys and is a source of fuelwood.

2.3.3.1. Neem wood

According to Dhillon *et al*., (2008) nineteen Meliaceous genera are addressed in Indian sub continent by one or more species. Neem is precious among the fourteen native Indian timber trees of Meliaceous genera. Meliaceous timbers possess variations within their anatomy. Neem wood is just like mahogany and the workability is similar to that of teak, yet takes poor polish.

Neem tree has many financial benefits over other multi-functional tree species grown within India. Even though the key utilization of the tree is for seed production in order to extract oil, the tree could yield wood after 35 to 45 years of planting. The

wood is not easily attacked by insects and is durable. Timber is moderately refractory and when sawn moist, it seasons well. The timber is easily workable, but do not take good polish (Girish and Shankara, 2008).

The wood is moderately heavy, medium to coarse textured and narrowly interlocked-grained. The heartwood is red whenever exposed and later on turns reddish brown whereas the sapwood is grayish-white. The wood is not very lustrous and is slightly aromatic (Dhillon *et al.*, 2008).

Following substantial study the wood was recommended for basic construction, and is very good for outdoor use, as well as for timber house construction (Punhani, 1995). The timber is usually durable even in outdoor conditions. Insects do not attack the wood. It is easily worked by hand or machine. It lends itself for broad carving. It has a very good calorific value but is not recommended as fuel wood as it gives fumes in conventional chulhas (Dhillon *et al.*, 2008).

2.3.3.2. Uses of wood

Girish and Shankara (2008) stated that the neem wood is used as farming and gardening implements, carts, boats, construction articles, furniture, idols, working tools.

Neem wood can be used for beams, boat construction, building houses, cart axle assemblies, cigar boxes, designed images, door/window frames, furnitures, farming implements, naves, posts, ship, toys and yorks. It has a long life of more than a century. Neem tree has a number of monetary advantages (Dhillon *et al.*, 2008).

Neem wood is hard and heavy, and is employed in making pious icons within few regions of India. Apart from end splitting, the wood seasons well without defects. As neem wood is durable and termite tolerant, it can be used in making

fence posts, furniture and poles for house construction. There is a flourishing market in several European countries for pale neem wood for producing furniture (Koul *et al.*, 1990). The tree is capable of resprouting after trimming, and regrowth after pollarding can make it suited to production of poles. Neem grows in no time and is also an excellent source of firewood and fuel with a high calorific value for the charcoal (Koul, 2004).

2.3.3.3. Anatomy

Neem wood is diffuse porous, with paratracheal banded or vasicentric axial parenchyma. It possess uniseriate to multiseriate rays. Vessel length and diameter shows a strong negative correlation. The inner wall surface of vessel is spiral thickened. Sometimes extractives are present in axial, ray parenchyma, vessels and fibres in the heartwood. Presently there is an increase in succinate dehydrogenase in addition to acid phosphatase activities in sapwood-heartwood interface that may possibly be connected to heartwood development. Peroxidase activity is higher close to the cambial zone, showing its credible function in lignifications (Gibson, 1973; Young, 1974).

Structural characterization of the wood features basic value in order to understand its nature. Several researches have been conducted about the anatomy (Datta and Samanta, 1983) and mechanical and physical properties of neem wood (Pearson and Brown, 1981). These researches did not consider the climatic conditions of the localities in which often the trees were being grown. Many reviews recommend the likely correlation of environment and wood anatomy (Baas, 1973; Carlquist, 1977, 1982; Van Vliet, 1979), while other studies show no such correlation (Gibson, 1973; Young, 1974).

The bark is bulky, furrowed longitudinally and obliquely, dark grey outside and reddish brown inside. The heartwood is usually red, hard and durable. The wood is smelly (Dhillon *et al.*, 2008).

Wood anatomy of neem have been described by Pearson and Brown (1932); Pennigton and Styles (1975); Datta and Samantha (1983) and Nair (1988). Neeem wood possesses difused porous and the pores are extremely little to large. The vessel outline is generally spherical and infrequently angular in cross section view. Vessels are in solitary or even radial multiples or in clusters (Nair, 1987).

2.3.3.4. Insecticidal potential of neem

The neem tree is a source of natural compounds with properties such as feeding deterrent, insect growth regulator action and powerful pesticide. However, little information is available associated with these natural products on termites. Bark feeding of neem caused considerable termite mortality (56.4%) when compared with mortality induced by wood feeding (27.1%) or even *Pseudotsuga menziesii* wood (12.5%). Although, neem is not completely termite resistant, the antifeedant properties of neem bark and wood as well as the toxicity of neem bark support neem trees for arboriculture in areas liable to termite attack (Delate and Grace, 1995).

The tea mosquito bug, *Helopeltis theivora*, is among the most disparaging sucking insect pest of tea in North East India. Integration of biopesticide and synthetic insecticides together with successful spraying methods, were experimented in order to trim down the load of the man-made chemicals in tea along with their bad consequence on the final product. The insecticidal property of neem have been applied to fix many infestation problems. In this research, various azadirachtin concentrations have been evaluated at various dosages contrary to *Helopeltis theivora* to learn their controlling efficiency. Azadirachtin concentration of 3,000 ppm and 10,000 ppm, reduced 30–43% of tea mosquito bug population. Therefore, azadirachtin concentration as well as its dilutions were reported to obtain desired bioactivity (Roy *et al.*, 2010).

The neem tree has been known for its unique properties against insect infestations. It is usually grown in sub-tropical and tropical areas foe providing

shade, reforestation and for the production of raw materials for medicines in addition to natural pesticides. A tranortriterpenoid limonoid named azadirachtin obtained from neem seeds is the principle element liable for the toxic outcomes in pests. The antifeedant and physiological effects of neem are recorded in six global conferences and also a huge scientific literature. Nisbet (2000), reviewed the behavioral and physiological attributes of azadirachtin, including outcomes on pest reproduction and the physiological effects assessed as abnormal and delayed moults, growth reduction and improved mortality. These outcomes were categorized in two ways: direct outcomes on cells and indirect effects applied via the endocrine system. The differential effects among animal phyla and non-target organisms are mentioned and indicate its prospective success being a safe pesticide.

2.4. Sapwood

Sapwood is the conductive tissue of plants, made up of cellulose, lignin and other substances. Cellulose ($C_6H_{10}O_5$) is a natural polymer made up of glucose molecules linked collectively in long chains (Raven, 1981). Lignin is an intricate organic polymer that features to boost wood. Cell wall of plants is made up of cellulose, which composes of 44.4% carbon (Heukelekian and Waksman, 1925). The xylem protoplast cease to work when fully developed, leaving cell wall intact. Water conduction occurs through the remaining lumen. The lumen concurrently functions as a continuous and intensive conductive and adsorptive system (Doccola and Wild, 2012).

Materials and methods

3. MATERIALS AND METHODS

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3.1. Materials

The study was conducted to standardize tree injection procedures for coconut, mango and neem using azadirachtin under the prevailing agro – climatic conditions. The study was conducted in the Department of Forest Products and Utilization, College of Forestry, Kerala Agricultural University, Vellanikkara during 2017-2019.

3.1.1. Species Studied

Stem wood of three species like coconut (*Cocos nucifera* L.), mango (*Mangifera indica* L.) and neem (*Azadirachta indica* A. Juss.) were used in the study along with their leaves after the application of azadirachtin through injection.

3.1.2. Location

This study was conducted in Thrissur district (between N 10° 11' 8.16" and N 10° 41' 2.76" latitude; E 75° 58' 2.64" and E 76° 53' 29.04" longitude) of central Kerala, India. Stem wood of three different species viz., coconut, mango and neem of three different girth classes were collected from saw mills located in Palakkad and Thrissur district.

3.2. Methods

The methods adopted to carry out the study are discussed below.

3.2.1. Selection of Samples

Wood samples belonging to coconut, mango and neem were collected from Thrissur and Palakkad districts. Wood billlets of 1m length were collected from the butt end of logs obtained from sawmills. Round wood belonging to three girth classes like 50 – 60 cm, 60 – 70 cm, 70 – 80 cm were selected, and one wood disc from four

trees belonging to each size class were obtained. The billets were converted into discs of 1 inch (2.54 cm) thickness for further analysis. Thus two discs were made out of one tree. One set of disc was used to study the heart wood/ sapwood and dermal wood / core wood whereas the other set of discs were used to study basic density and moisture content. Altogether, four discs of one inch thickness were obtained from the size class 50-60 cm, four discs from the size class of 60-70 cm, four discs from the size class 70-80 cm were collected from coconut, mango and neem. Thus thirty six discs of one inch (2.54 cm) thickness were obtained.

3.2.2. Heartwood sapwood ratio

In order to determine heartwood sapwood ratio, the tree trunks were sliced into discs of 1 inch (2.54 cm) thickness. Though there is no pronounced colour differentiation between heartwood and sapwood of mango and neem, it was differentiated using a marker. Then the area of heartwood and sapwood was calculated along with their thickness and ratio by using the software digimizer.

3.2.3. Core wood dermal wood ratio

Coconut palmwood with density over 400 kg m^{-3} were regarded as dermal wood and under 400 kg m^{-3} as core wood. The variation between dermal wood and core wood was likewise marked with the aid of a marker and then further studies were carried out.

3.3. Physical properties

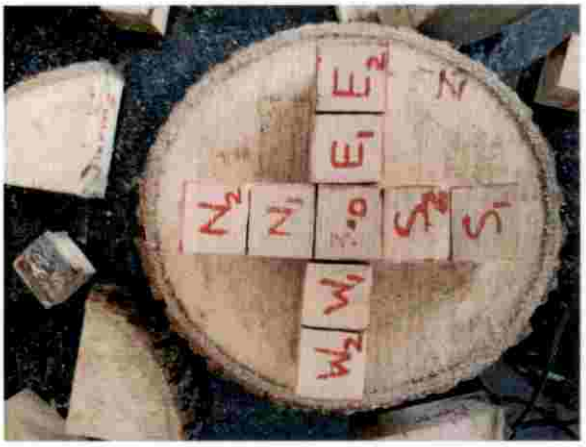
Small clear wood specimens of 2 cm×2 cm× 2.5 cm dimension were made out according to IS: 1708 (ISI, 1986) for estimating the basic density and moisture content of wood samples of coconut, mango and neem.

Plate 1. Collection and preparation of samples

Collected samples

Sawing of samples

Converted discs



Small clear wood samples

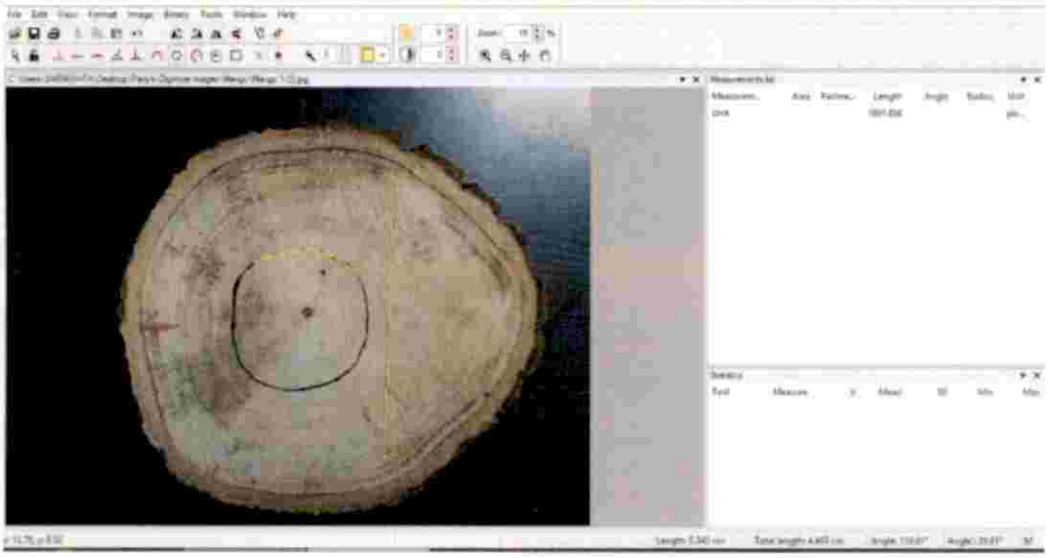
Sanding

Marking high density wood area of coconut palm

Plate 2. Analysis of physical parameters

Marked wood disc

Using digimizer



Measured sample using digimizer

Precision electronic balance
(Shimadzu AUY 220)

Oven drying of samples

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3.3.1. Basic density

Green volume of the samples was estimated using the immersion method. The samples were oven dried at103°C ± 2°C until the weight became constant with regard to the determination of oven dry weight by using a precision electronic balance (Shimadzu AUY 220) and were weighed correct to 0. 001g.

Basic density (standard specific gravity) for wood specimens from four different directions of each disc was determined using the following formula,

$$\text{Basic density (kg m}^{-3}) = \frac{\text{Oven dry weight}}{\text{Green volume}}$$

3.3.2. Moisture content

In order to determine moisture content, the samples were weighed to an accuracy of 0.001 in a weighing balance (Shimadzu AUY 220) and then dried in a hot air oven at a temperature of 103°C ±2°C till constant weight. The final weight has been taken as oven dry weight. The moisture content of each specimen was calculated using the formula.

$$\text{Moisture content (\%)} = \frac{\text{Green weight} - \text{Oven dry weight}}{\text{Oven dry weight}} \times 100$$

3.4. IRGA

Infrared Gas Analyzer (LI-6400, Portable Synthesis System) was used to measure the photosynthesis and transpiration rate. Fifteen readings were taken for each parameters from coconut, mango and neem prior to tree injection.

3.5. Tree injection

One tree each from coconut, mango and neem were selected. The trees selected were watered one day prior to tree injection. The coconut palm selected was of Pushkala dwarf variety to ensure the easy collection of palm leaves. Each tree was

marked at a basal height of 20 cm from ground. Holes were made at an angle of 45° to make sure that there was no oozing out of chemicals. A drilling bit of 0.6 cm diameter was used for drilling.

3.5.1. Instrument

The instrument used was "EcoJect" system which is a safe and simple micro injection tool for injecting systemic pesticides into trees. The EcoJect system mainly consists of three components like the nozzle, the canister and the pump. The narrow end of the nozzle was inserted into a pre- drilled injection site in a tree. The wide end of nozzle is used to securely push the nozzle into the pre- drilled injection site and is mated with a loaded canister. A clean, sharp 15/64 inch helix drill bit is required for drilling injection sites. The canisters come in 8 ml and 20ml capacities. These canisters are re-usable. The tree injection toolkit is given in Plate 3.

The EcoJect pump consists of a manifold, loading gun and a cylinder. The cylinder comes in 3l, 6l and 12l capacities. The EcoJect pump must be pressurized with compressed air between 100 and 150 PSI

3.5.2. Chemical used

Systemic insecticide, Azajet (50,000ppm) was used to inject the trees.

3.6. Leaf sample collection

The leaflets from coconut frond were collected. The leaflets were collected devoid of midrib from middle of the frond. A single frond was collected from the coconut palm using the formula.

$\frac{n}{2} + 1$, where 'n' is the number of fronds.

In case of mango and neem, the whole canopy was divided into three strata and the third mature leaf was collected from each twig. The leaves were collected

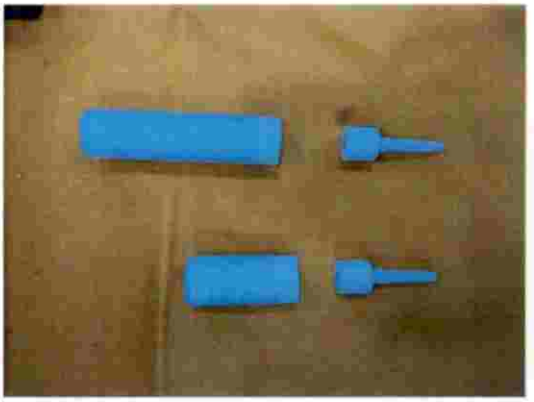
after 1hr, 2hr, 6hr, 2nd day, 7th day, 14th day, 20th day, 28th day, 40th day and 55th day of tree injection.

3.7. Packaging and forwarding samples

The samples collected were cleaned of dust and other particles. Coconut leaflets were cut into small pieces. The samples belonging to coconut, mango and neem were packed separately in zip lock covers and were packed in air tight thermocol boxes along with dry ice. It was made sure that the samples reached the laboratory (International Institute of Biotechnology and Toxicology, Kancheepuram) within two days.

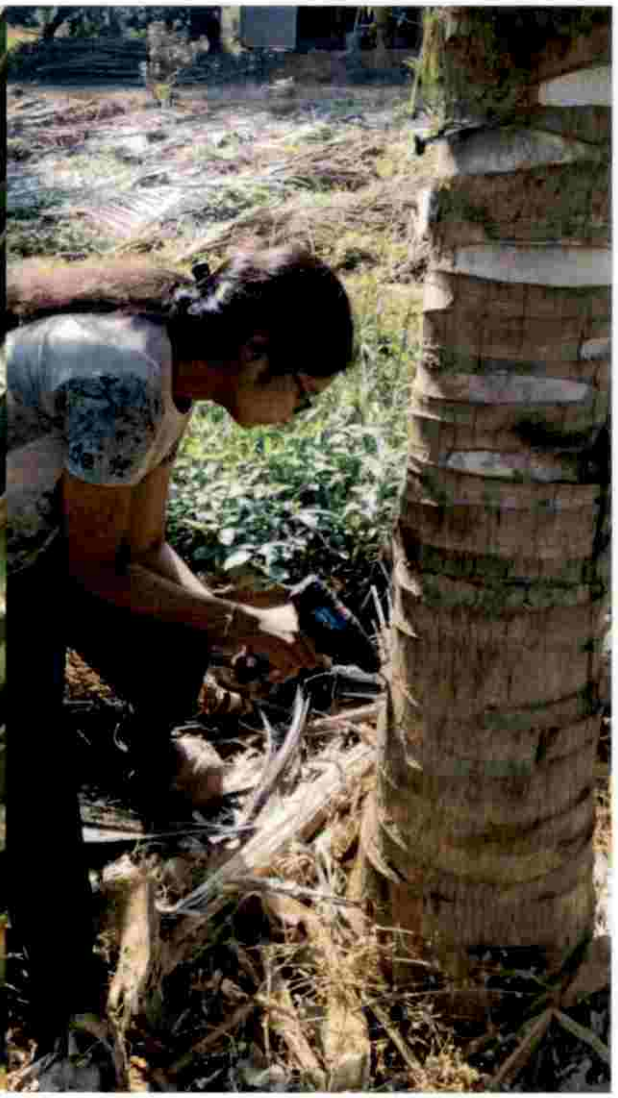
Plate 3. Tree injection kit and its usage

Tree injection kit



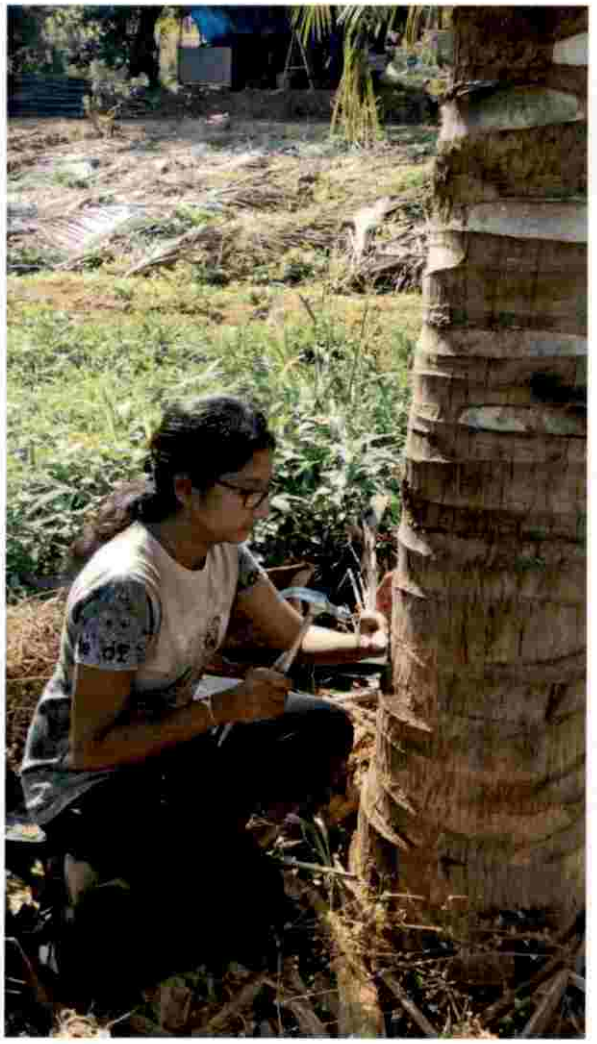
Nozzles

Using IRGA to analyse physiological parameters



Drilling hole to incorporate nozzle

Plate 4. Injecting tree trunk



Fixing needle

Injected tree

3.8. High Performance Liquid Chromatography (HPLC)

HPLC was used for analysis of azadirachtin residue in different samples.

3.8.1. Materials

3.8.1.1. Reagents

1. Milli-Q- water– Supplied by Millipore India Limited.
2. Acetonitrile (Purity – 99.9%) - Supplied by Merck India Limited.
3. Methanol (Purity – 99.7%) - Supplied by Merck India Limited.
4. Sodium chloride – Supplied by Sigma Aldrich.
5. Magnesium Sulfate - Supplied by Merck India Limited.
6. Celite 545 - Supplied by Merck India Limited.

3.8.1.2. Equipment and Glassware

1. Pipettes, measuring cylinder, glass columns, Borosil glass works Ltd, Mumbai.
2. Mettler Toledo analytical balance, Mettler AG, Switzerland.
3. Sartorius analytical balance, Sartorius Mechatronics India Private Limited, Bangalore.
4. Spinix - Vortex shaker, Tarsons.
5. Buchi Rotavapor temperature controlled vacuum rotary evaporator, M/s. Buchi Labortecnik, AG, Switzerland.
6. End over end mechanical shaker, Toshniwal Instruments Pvt. Ltd., Mumbai.
7. Sonicator, Ultra Sonic, Chennai.
8. Oasis HLB 20cc, SPE, part# WAT 094226, Water.
9. Robot coupe, ETL LISIEA, USA.
10. Cooling Centrifuge, Remi.

3.8.2. Experimental Procedure

3.8.2.1. Preparation of Reference Standard Solution

Accurately 25.47 mg working standard of azadirachtin (40%) was weighed into 100 ml volumetric flask, dissolved in methanol: water (90:10) and brought up to the mark with same solvent to obtain stock solution. From this stock solution, a working standard of 1 µg/ml was used for the analysis.

3.8.2.2. Preparation of Sample Solution

The leaf samples were homogenized in frozen state (using dry ice in Robot coupe). The homogenized sample (25 g) was weighed in an Erlenmeyer flask and 25 ml of water, 1 g NaCl, 3 g $MgSO_4$ and 100 ml of acetonitrile were added to it. The sample was extracted in an end-over-end mechanical shaker for about 1 hour and filtered through 1 g of Celite. The residual material was rinsed with 50 ml acetonitrile and the filtrate was collected. The combined filtrate was centrifuged (5000 rpm, 5 min). To the accurately measured 5 ml of supernatant of extract, 250 mg of primary secondary amine (PSA), graphitized carbon bloch (GCB), C_{18} and florisil and 400 mg of $MgSO_4$ were added to a 15 ml centrifuge tube. The content was vortexed (1 min) and centrifuged (5000 rpm, 5 min). The filtrate was concentrated to dryness (N_2 evaporator, 40°C) and re-dissolved the residues in 5 ml mobile phase and analyzed into the HPLC.

3.8.2.3. HPLC parameters

Instrument	:	Agilent HPLC 1290 series system equipped with quaternary pump, degasser column and diode- array detector (DAD), with chem-station software– Supplied by Agilent, USA.
Column	:	Phenomenex C-18, 25 cm x 4.6 mm Stainless Steel 5 µ Particle size

Mobile Phase	:	Acetonitrile : Water (35:65) (v/v)
Flow Rate	:	1.0 ml/mm approx.
Column Temperature	:	40°C
Wave Length	:	215nm Band width: 4 nm
Injection Volume	:	20 µL
Run time	:	20 min
Retention time (approximate)		
Azadirachtin - A	:	10-12 minutes (approx.)
Azadirachtin - B	:	11-13 minutes (approx.)

3.8.3. Formula for calculation

$$\textit{A}\text{zadirachtin content } \left(\frac{g}{g}\right) = \frac{A \times B \times C}{D \times E} \times F$$

Where,

A - Peak area of Azadirachtin in sample solution (Aza A+B) (mAU)

B - Final Volume of the sample (ml)

C - Concentration of the standard (µg/ml)

D - Peak area of Azadirachtin in working standard (Aza A +B (mAU)

E - Weight of the sample (g)

F - Dilution Factor

3.9. Anatomical Properties

The anatomical features assessed differed for the trees and coconut palm. Fibre morphology, vessel morphology in addition to ray morphology were evaluated for mango and neem whereas, the features assessed for coconut palm include fibre morphology, vascular bundle sheath thickness, vascular bundle diameter, vascular bundle frequency, and vessel diameter.

3.9.1. Microtomy

Wood specimens collected with the help of wood increment borer of 1cm in diameter were used were used for anatomical studies. The specimens were softened by keeping them in water as they were fresh samples. Thin microscopic sections (tangential and cross sections) of 10µm – 15µm thickness were prepared using a sliding microtome (Motic BA 210).

3.9.2. Staining procedure

Permanent slides of sections were prepared using the practice outlined by Johansen (1940). For this, sections were stained using safranin and later washed by means of a series of alcohol solutions at different concentrations (70%, 90% and 95%) to make sure complete dehydration. They were later dipped in acetone followed by xylene and finally mounted using DPX to prepare permanent slides (size 75mm × 25 mm ×1 mm) and covered using cover slips.

3.9.3. Maceration

Maceration of the wood samples was done by Jeffrey's method (Sass, 1971). For maceration, freshly prepared Jeffrey's solution (10%) was used that was prepared by mixing 10 g Potassium dichromate and 14 ml of Nitric acid and volume was made upto 100 ml. Radial shavings of wood were taken from the wood specimens collected using an increment borer. These chips were boiled in the maceration chemical for

fifteen to twenty minutes so that the individual fibres get separated. Subsequently, these test tubes were kept undisturbed for 5 to 10 minutes so that the fibres settle down. The solution was discarded and the resultant material was washed in distilled water until traces of acid were removed. The samples were stained using safranine and mounted temporarily using glycerol.

3.9.4. Image analysis

Microscopic examination and quantification of sections were undertaken using an Image Analyzer (CatCam 500E series). The image analyzer offers quick and accurate information replacing the more repetitious traditional methods. The digital camera provides digitized pictures which are analysed by the software (CatCam 500E series). The software provides several classes of measurements like length, diameter, area and count.

3.9.5. Observations

From the macerated fibres, observations such as fibre length, fibre diameter, fibre wall thickness, in addition to fibre lumen diameter were collected. From microscopic sections, vascular bundle diameter, bundle sheath thickness, vascular bundle percentage in addition to vessel diameter were assessed using an Image Analyzer. Each measurement was repeated fifteen times for all the above characters and expressed in micrometers (μm).

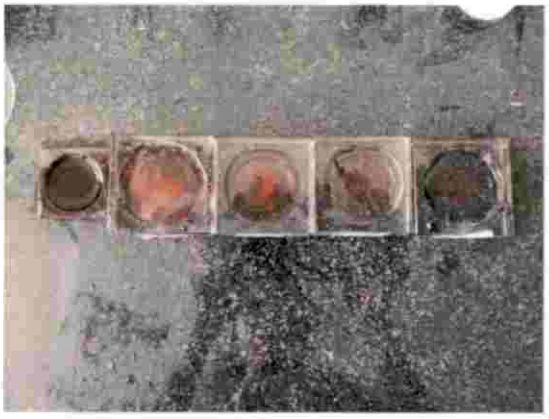
3.10. Statistics

The present study was an attempt to standardize the tree injection procedures using azadirachtin in coconut, mango and neem. Fifteen replications were used in the study and the data was analyzed by one-way ANOVA. The significance of each mean property value was determined with the Duncan's multiple range test using the SPSS (Version 21). Correlation between various parameters were also evaluated.

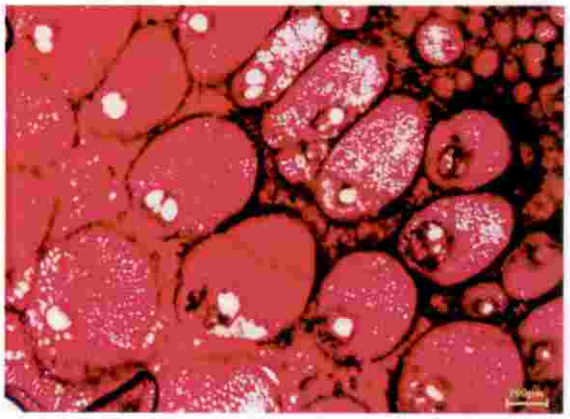
Plate 5. Analysis of anatomical parameters

Collection of sample with an increment borer

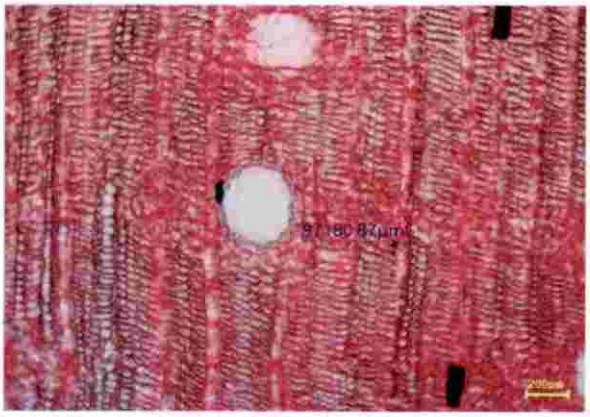
Sectioning of wood samples



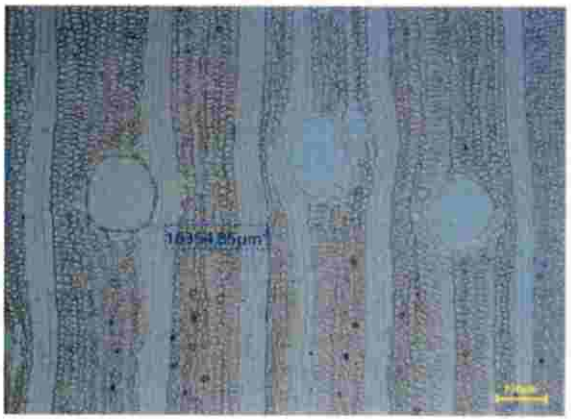
Staining of micro sections



Vessels of coconut



Vessels of mango



Vessels of neem

Results

4. RESULTS

The results obtained in the present study entitled "Standardization of tree injection procedures of azadirachtin in coconut (*Cocos nucifera* L.), mango (*Mangifera indica* L.) and neem (*Azadirachta indica* A Juss.)" are presented in this chapter.

4.1. Physical parameters

The results obtained from the analysis of physical properties like basic density, moisture content and high density wood of coconut and sapwood thickness of mango and neem in order to decide the depth of injection is been discussed below.

4.1.1. Basic Density

Basic density of different species belonging to three size classes is given in Table 1. and Figure 1. In coconut, the mean basic density was 355.27 kg/m^3 in the size class of 50-60 cm, 378.56 kg/m^3 in the size class 60-70 cm, 329.15 kg/m^3 in the size class 70-80 cm. In general, the average basic density was 354.33 kg/m^3 irrespective of the size class. Analysis of variance indicated no significant difference in basic density between the three size classes of coconut.

In mango, analysis of variance indicated no significant difference in basic density between three size classes in mango. The basic density of mango was 522.19 kg/m^3 in the size class 50-60 cm, 526.48 kg/m^3 in the size class of 60-70 cm, 531.92 kg/m^3 in the size class 70-80 cm. In general, the average basic density was 497.98 kg/m^3 irrespective of size class.

Analysis of variance indicated no significant difference in basic density between the three size classes in neem. The basic density of neem wood was 73.73 kg/m^3 in the size class 50-60 cm, 724.28 kg/m^3 in the size class 60-70 cm and 682.88 kg/m^3 in the size class 70-80 cm. In general, the average basic density was

704.85 kg/m^3 irrespective of size class, indicating that neem is denser than coconut palm wood and mango.

Table 1. Basic density of coconut palm, mango and neem wood of three size classes

Size class	50-60 (cm)	60-70 (cm)	70-80 (cm)	Mean
Coconut	355.27 ± 30.0	378.56 ± 23.19	329.15 ± 27.92	354.33^{c}
Mango	522.19 ± 2.42	526.48 ± 1.9	531.92 ± 4.63	497.98^{b}
Neem	730.73 ± 4.03	724.28 ± 5.07	682.88 ± 1.79	704.85^{a}
Mean	531.62^{ab}	543.11^{a}	482.43^{b}	

Analysis of variance for moisture content of coconut palm wood belonging to three size classes did not show significant difference in three species.

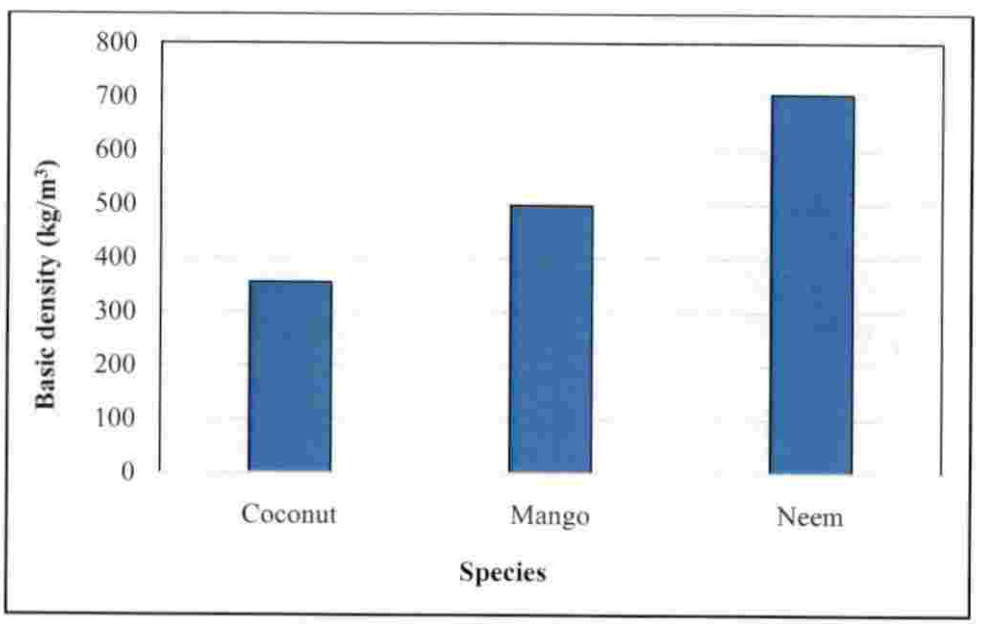


Figure 1. Basic density (kg/m^3) of coconut palm, mango and neem wood

4.1.2. Moisture Content

Moisture content of different species belonging to three size classes is given in Table 2. and Figure 2. Analysis of variance of moisture content of coconut palm

wood belonging to three size classes (50-60 cm, 60-70 cm, 70-80 cm) did not show significant difference. The value was 116.14 in the size class 50-60 cm, 88.22 in size class 60-70 cm, 130.26 % in the size class of 70-80 cm. In general, the average moisture content of coconut palm wood was 107.87% irrespective of size class.

The average moisture content of mango wood was 73.27% irrespective of size class, whereas it was 74.13% in the size class 50-60 cm, 71.71% in the size class 60-70 cm and 73.96% in the size class 70-80 cm. Analysis of variance for moisture content of mango wood belonging to three different size classes showed no significant difference.

The moisture content of neem wood was 39.44% in the size class 50-60 cm, 35.12% in size class 60-70 cm and 35.18% in the size class 70-80 cm. In general, the average moisture content of neem wood was 36.58% irrespective of size class. The analysis of variance did not show significant difference between the three different size classes of neem.

In general, the average wood moisture of coconut palm, mango and neem were 107.87%, 73.27% and 36.58% respectively. These results indicated that coconut palm wood has very high moisture content.

Table 2. Moisture content (%) of coconut palm, mango and neem wood of three size classes

Size class	**50-60 (cm)**	**60-70 (cm)**	**70-80 (cm)**	**Mean**
Coconut	116.14 ± 2.55	88.22 ± 7.12	130.26 ± 2.32	107.87^{a}
Mango	74.13 ± 4.55	71.71 ± 1.19	73.96 ± 1.31	73.27^{b}
Neem	39.44 ± 1.65	35.12 ± 0.96	35.18 ± 0.77	36.58^{c}
Mean	72.9^{ab}	65.02^{b}	79.8^{a}	

Analysis of variance for moisture content of coconut palm wood belonging to three size classes did not show significant difference

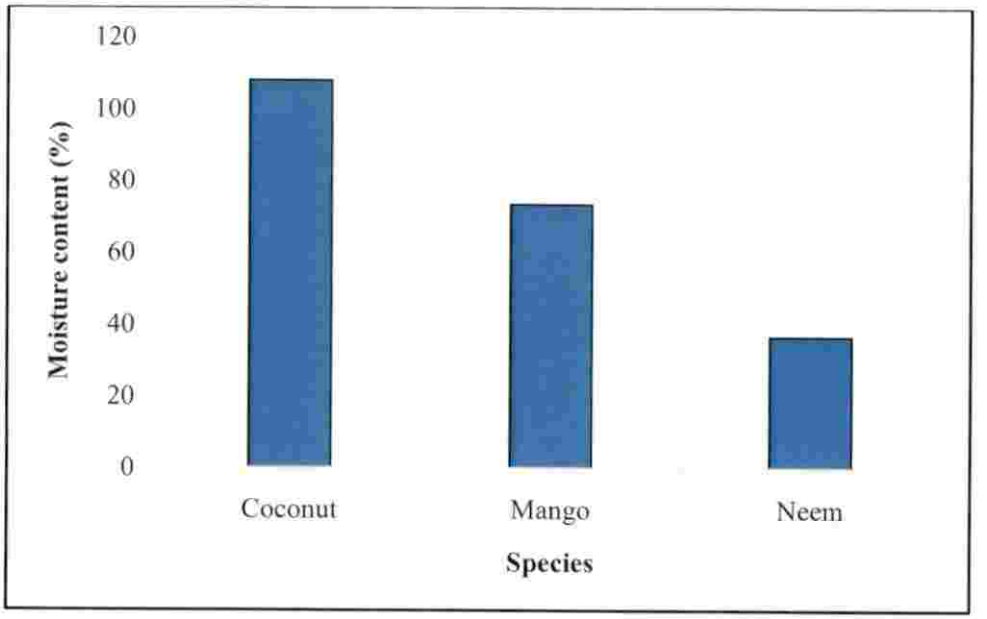


Figure 2. Mean moisture content (%) of coconut palm, mango and neem wood

4.1.3. High density wood thickness of coconut palm wood

Information regarding the high density wood thickness of coconut is given in Table 3. The variation in high density wood thickness (HDT) was in the range of 1.854 cm to 5.045 cm in the size class 50-60 cm, 2.118 cm to 4.443 cm in the size class 60-70 cm and 2.138 cm to 5.242 cm in the size class 70-80 cm. Average thickness of high density wood was estimated to range from 1.854 cm to 5.242 cm irrespective of size class, and highest average high density wood thickness was 4.89 cm.

Analysis of variance did not show significant difference for the high density wood thickness of coconut palm wood between three size classes. It means that the thickness of physiologically dead wood (high density wood) does not vary with the change in size class. This was helpful to draw a conclusion and fix the depth of injection to 6 cm deep so as to ensure the delivery of chemicals injected to the live part of wood.

Table 3. High density wood thickness of coconut palm wood

Sample no.	Size class (cm)	HDT1	HDT2	HDT3	HDT4	HDT AVG (cm)
1	50-60	5.05	4.58	4.35	4.27	4.56
	60-70	3.66	3.08	2.73	3.27	3.19
	70-80	2.32	2.58	2.52	2.47	2.47
2	50-60	2.14	1.89	1.95	1.85	1.96
	60-70	2.96	2.55	2.12	3.47	2.77
	70-80	2.84	2.67	2.68	2.14	2.58
3	50-60	2.69	3.19	3.77	4.32	3.49
	60-70	3.50	3.72	3.36	4.44	3.75
	70-80	4.65	4.77	4.89	5.24	4.89
4	50-60	3.57	3.71	3.04	2.60	3.23
	60-70	4.10	3.60	4.04	3.83	3.89
	70-80	4.33	4.83	4.48	4.71	4.59

Analysis of variance was not significant for the high density wood thickness of coconut palm wood between three size classes

4.1.4. Sapwood thickness of mango

Information regarding the sapwood thickness of mango is given in Table 4. The variation in sapwood thickness was in the range of 3.21 cm to 9.45 cm in the size class 50-60 cm, 2.46cm to 9.97cm in 60-70cm size class, 2.98cm to 16.90cm in the size class of 70-80cm. Average thickness of sapwood was estimated to range from 2.46cm to 16.90cm irrespective of size class, and highest average sapwood thickness was 8.70 cm and was never smaller than 3.27cm.

Analysis of variance was not significant for the sapwood thickness of mango wood between three size classes. It means that the thickness of physiologically live wood (sapwood) does not vary with the change in size class. This was helpful to draw a conclusion and fix the depth of injection to 3 cm deep so as to ensure the delivery of chemicals injected to the live part of wood.

Table 4. Sapwood thickness of mango

Sample no.	Size class (cm)	SWT 1 (cm)	SWT 2 (cm)	SWT 3 (cm)	SWT 4 (cm)	AVG SWT (cm)
1	50-60	3.54	3.21	3.79	5.50	4.01
	60-70	3.84	4.10	3.40	2.75	3.52
	70-80	2.98	3.05	3.29	3.75	3.27
2	50-60	5.94	4.92	6.24	9.45	6.64
	60-70	5.46	4.61	4.99	2.46	4.38
	70-80	3.45	6.17	16.90	4.61	7.78
3	50-60	4.40	9.21	6.20	7.81	6.90
	60-70	8.73	8.44	4.68	4.23	6.52
	70-80	10.53	7.97	8.80	7.51	8.70
4	50-60	8.91	4.57	8.56	5.31	6.84
	60-70	5.45	9.97	6.93	9.09	7.86
	70-80	7.92	8.53	5.79	8.73	7.74

Analysis of variance was not significant for the sapwood thickness of mango wood between three size classes

4.1.5. Sapwood thickness of neem

Information regarding the sapwood thickness of neem is given in Table 5. The variation in sapwood thickness was in the range of 2.95cm to 4.95cm in the size class 50-60cm, 2.79cm to 3.75cm in 60-70cm size class, 3.27cm to 8.51cm in the size class of 70-80cm. Average thickness of sapwood was estimated to range from 2.79cm to 8.51cm irrespective of size class. And highest average sapwood thickness was 6.90 cm and was never smaller than 3.02cm.

Analysis of variance was not significant for the sapwood thickness of neem wood between three size classes, which means that the thickness of physiologically live wood (sapwood) does not vary with the change in size class. This was helpful to draw a conclusion and fix the depth of injection to 3 cm deep so as to ensure the delivery of chemicals injected to the live part of wood.

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Table 5. Sapwood thickness of neem

Sample no.	Size class (cm)	SWT 1 (cm)	SWT 2 (cm)	SWT 3 (cm)	SWT 4 (cm)	AVG SWT (cm)
1	50-60	4.95	4.46	4.50	4.43	4.58
	60-70	3.08	3.01	2.96	3.04	3.02
	70-80	6.12	5.97	7.00	8.51	6.90
2	50-60	3.12	3.20	4.22	3.49	3.50
	60-70	3.90	3.46	3.75	3.13	3.56
	70-80	3.97	3.53	3.27	3.88	3.66
3	50-60	3.18	3.32	2.95	3.21	5.17
	60-70	3.24	3.27	3.65	3.38	5.39
	70-80	4.99	5.18	5.79	4.80	5.19
4	50-60	3.35	3.05	3.07	3.34	3.20
	60-70	3.13	2.79	3.72	3.22	3.22
	70-80	4.93	5.36	5.44	5.20	5.23

Analysis of variance was not significant for the sapwood thickness of neem wood between three size classes

Table 6. Mean table of high density wood of coconut palm and Sapwood thickness of mango and neem

Species	50-60 (cm)	60-70 (cm)	70-80 (cm)
Coconut	3.31±0.26[a]	3.34±0.16[a]	3.72±0.30[a]
Mango	6.10±0.52[a]	5.42±0.57[a]	7.13±0.93[a]
Neem	3.62±0.16[b]	3.46±0.18[b]	5.19±0.35[a]

4.2. Anatomical properties

The results of anatomical studies like vessel diameter, vascular bundle frequency, vascular bundle sheath thickness, fibre length, fibre diameter, fibre lumen diameter of coconut, vessel diameter, ray height, ray width, vessel frequency, fibre length, fibre diameter and fibre lumen diameter of mango and neem conducted are presented below in Table 7.

4.2.1. Vessel diameter

Analysis of variance for vessel diameter of coconut palm wood, mango and neem showed a significant difference, and the values ranged from 101.47 µm to 118.93 µm in coconut, 225.60 µm to 275.16 µm in mango and 133.42 µm to 164.47 µm in neem. Mango showed a high mean value of 245.41 µm and coconut palm wood showed a mean value of 110.20 µm.

4.2.2 Fibre length

Fibre length showed a significant difference between three species. The values ranged from 1578.59 µm to 1654.83 µm in coconut palm wood, 222.83 µm to 285.25 µm in mango and 522.69 µm to 614.33 µm in neem. Coconut palm wood showed the highest mean value of 1611.17 µm and mango showed the lowest mean value of 257.12 µm.

4.2.3. Fibre diameter

Fibre diameter showed a significant difference between the three species of coconut, mango and neem. The values of fibre diameter ranged from 16.67 µm to 20.08 µm in coconut with a mean value of 18.28 µm, 11.52 µm to 16.63 µm in mango with a mean value of 13.36 µm and 16.2 µm to 17.93 µm in neem with a mean value of 17.07 µm. Coconut had the widest diameter whereas mango had the smallest diameter of 13.36 µm.

4.2.4. Lumen width

Analysis of variance for fibre lumen width of coconut palm wood, mango and neem showed a significant difference even though the values ranged from 14.09 µm

to 16.22 µm in coconut palm wood, 7.26 µm to 11.08 µm in mango and 10.69 µm to 14.07 µm in neem. The mean value was highest for coconut palm wood (14.99 µm) and lowest for mango (8.98 µm).

4.2.5. Vessel frequency

Analysis of variance for vessel frequency indicated no significant variation between the species. The values ranged from 3/mm^2 to 5/mm^2 in coconut, 3/mm^2 to 7/mm^2 in mango, and 5/mm^2 to 6/mm^2 in neem. The mean values for vessel frequency were 4/mm^2, 4.8/mm^2 and 5.4/mm^2 for coconut, mango and neem respectively. Neem showed highest vessel frequency, followed by mango and coconut.

4.2.6. Ray height

The values of ray height ranged from 207.78 µm to 247.68 µm in mango with a mean value of 228.41 µm and 846.03 µm to 875.44 µm in neem with a mean value of 861.53 µm. Analysis of variance showed no significant difference between mango and neem.

4.2.7. Ray width

Analysis of variance did not show any significant difference between mango and neem though the values widely ranged from 21.94 µm to 32.65 µm in mango with a mean value of 29.20 µm and 175.72 µm to 187.82 µm in neem with a mean of 181.15 µm. Neem had the highest ray width when compared with mango.

4.2.8. Bundle sheath thickness

The bundle sheath thickness of coconut palm wood indicated no significant difference between the size classes. The values ranged from 306.89 µm to 347.16 µm with a mean value of 318.22 µm.

4.2.9. Vascular bundle diameter

Analysis of variance showed no significant difference in vascular bundle diameter of the coconut palm though the values ranged from 311.39 µm to 354.91 µm with a mean value of 334.16 µm.

Table 7. Anatomical properties of cconut palm, mango and neem wood

	Coconut	**Mango**	**Neem**
Vessel diameter (µm)	110.20±2.94^{c}	245.41±9.80^{a}	151.87±5.35^{b}
Fibre length (µm)	1611.172±15.56^{a}	257.12±11.41^{c}	563.73±15.88^{b}
Fibre diameter (µm)	18.28±0.55^{a}	13.36±0.90^{b}	17.07±0.38^{a}
Lumen width (µm)	14.99±0.44^{a}	8.98±0.65^{c}	12.14±0.56^{b}
Vessel frequency (per mm^{2})	4±0.45^{a}	4.8±0.66^{a}	5.4±0.24^{a}
Ray height (µm)	-	228.41±6.57	861.53±5.33
Ray width (µm)	-	29.20±1.89	181.15±2.05
Bundle sheath thickness (µm)	318.22±7.31	-	-
Vascular bundle diameter (µm)	334.16±7.92	-	-

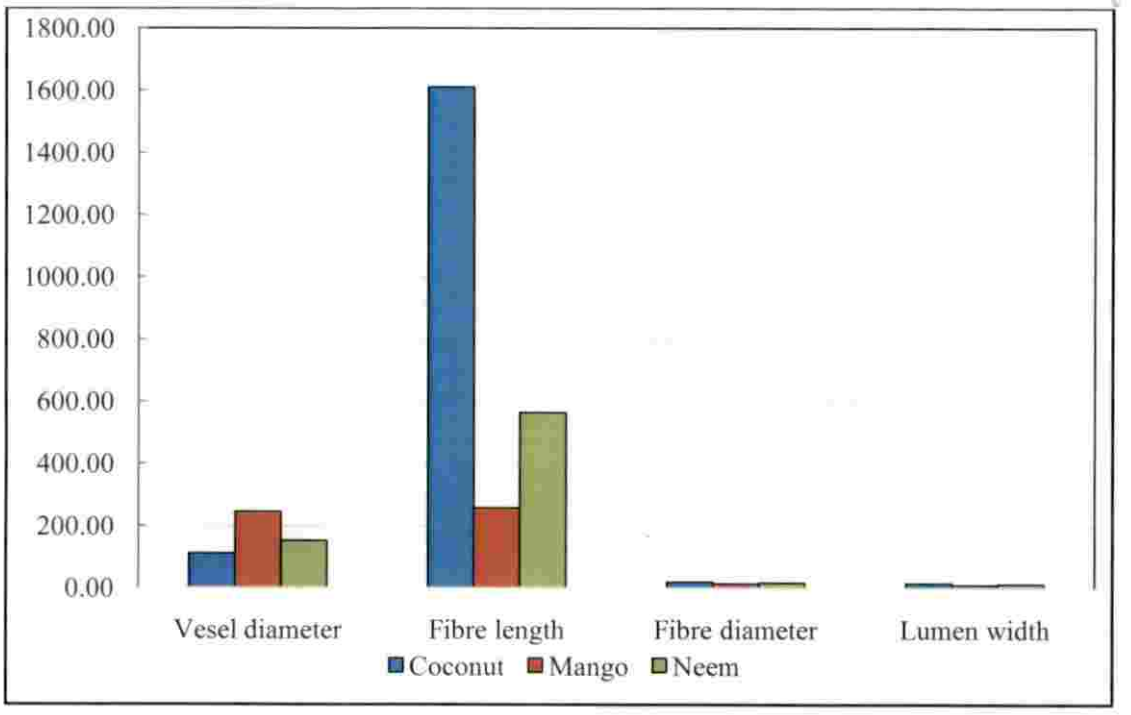

Figure 3. Vessel diameter, fibre length, fibre diameter and lumen width of coconut palm, mango and neem wood

4.3. Physiological parameters

Results of physiological parameters conducted during the time of tree injection in coconut palm, mango and neem are discussed and represented graphically in Figure 4,5,9,7 and 8.

4.3.1. Photosynthesis

Photosynthesis was significant between the three species of coconut, mango and neem. The values ranged from 1.9µmol of $CO_2/m^2/s$ to 2.53 µmol of $CO_2/m^2/s$ in coconut with a mean of 2.22 µmol of $CO_2/m^2/s$ in coconut palm, 1.05 µmol of $CO_2/m^2/s$ to 1.22 µmol of $CO_2/m^2/s$ with a mean value of 1.14 µmol of $CO_2/m^2/s$ in mango and from 1.1 µmol of $CO_2/m^2/s$ to 1.72 µmol of $CO_2/m^2/s$ with a mean of 1.40 µmol of $CO_2/m^2/s$ in neem. Photosynthesis was observed to be the highest in coconut palm followed by neem and mango.

4.3.2. Transpiration

The transpiration values of transpiration ranged from 0.33 mmol of $H_2O/m^2/s$ to 0.89 mmol of $H_2O/m^2/s$ in coconut palm, 2.46 mmol of $H_2O/m^2/s$ to 3.06 mmol of $H_2O/m^2/s$ in mango and 1.27 mmol of $H_2O/m^2/s$ to 1.79 mmol of $H_2O/m^2/s$ in neem. The mean values of transpiration were 0.58 mmol of $H_2O/m^2/s$, 2.61 mmol of $H_2O/m^2/s$ and 1.47 mmol of $H_2O/m^2/s$ in coconut palm, mango and neem respectively. The analysis of variance indicated a significant variation between the species.

4.3.3. Stomatal frequency

The values of stomata per mm^2 area of leaves varied widely within the range of $142/mm^2$ to $173/mm^2$ in coconut, $530/mm^2$ to $545/mm^2$ in mango and $258/mm^2$ to $272/mm^2$ in neem. The mean values ranged from $161/mm^2$, $536/mm^2$ and $265/mm^2$ in coconut, mango and neem respectively. The analysis of variance showed a significant difference between the species. Stomatal rate was highest in mango and lowest in coconut.

4.3.4. Leaf temperature

Analysis of variance showed a significant difference between coconut palm, mango and neem. The values of leaf temperature ranged from 33.7°C to 34.21°C in coconut with a mean value of 34.06°C, 31.41°C to 31.69°C with a mean value of 31.52°C and 31.18°C to 31.44°C in neem with a mean value of 31.29°C.

4.3.5. Leaf moisture

Leaf moisture indicated a significant difference between the three species. The values ranged from 81.25% to 86.51% with a mean value of 84.03% in coconut, 85.45% to 101.65% in mango with a mean value of 93.81% and 75.74% to 82.11% with a mean value of 79.36% in neem.

Table 8. Physiological parameters of coconut palm, mango and neem tree

	Coconut	**Mango**	**Neem**
Photosynthesis (µmol of $CO_2/m^2/s$)	2.22 ± 0.11^a	1.14 ± 0.03^b	1.40 ± 0.11^b
Transpiration (mmol of $H_2O/m^2/s$)	0.58 ± 0.09^c	2.61 ± 0.11^a	1.47 ± 0.10^b
Stomatal frequency (per mm^2)	161 ± 5.72^c	536.2 ± 3.04^a	265 ± 2.49^b
Leaf temperature (°C)	34.06 ± 0.09^a	31.52 ± 0.06^b	31.29 ± 0.05^c
Leaf moisture (%)	84.03 ± 0.87^b	93.81 ± 2.78^a	79.36 ± 1.24^b

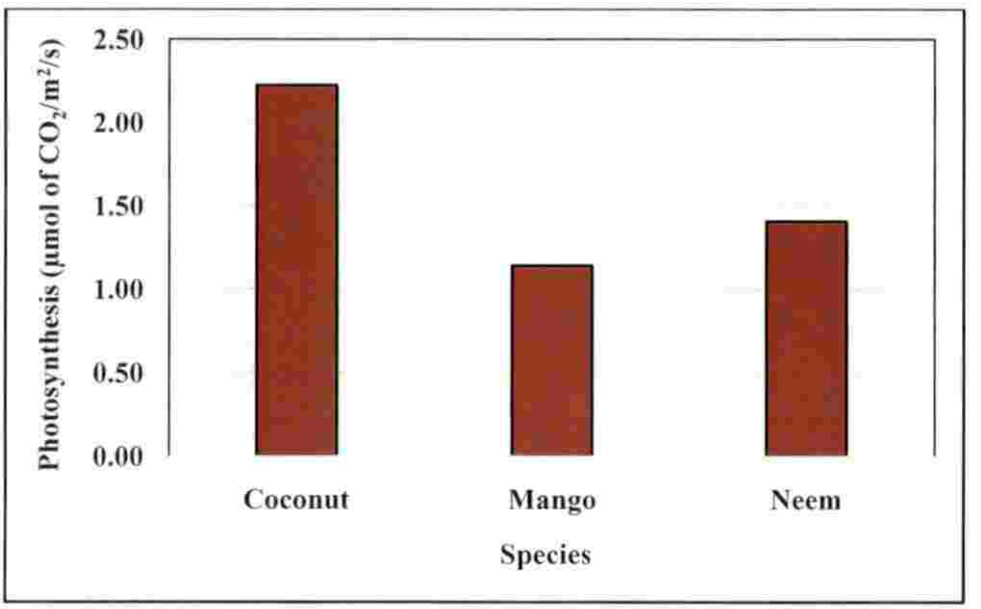

Figure 4. Photosynthesis of coconut palm, mango and neem tree

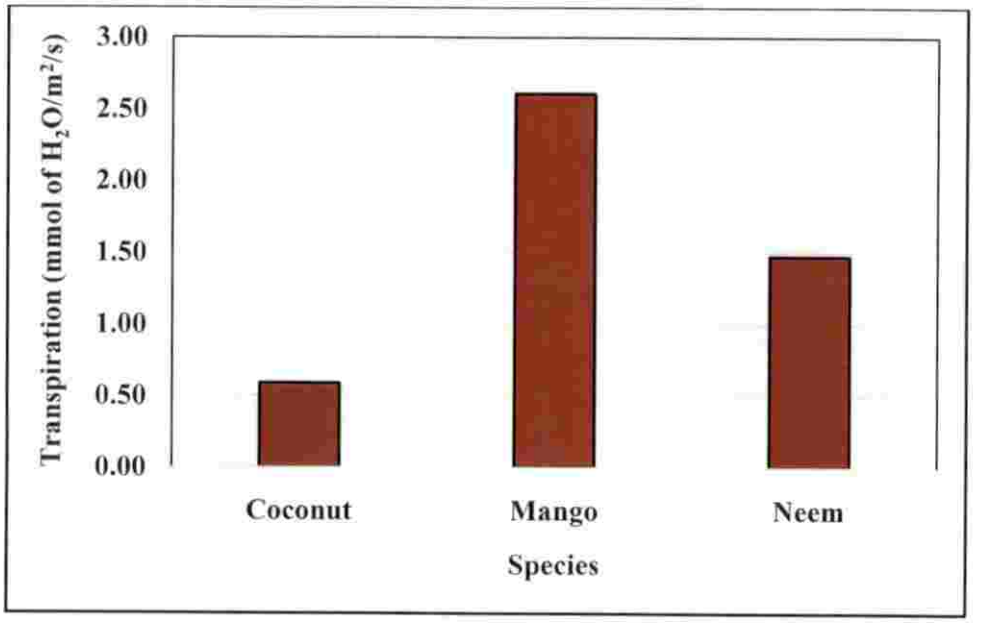

Figure 5. Transpiration of coconut palm, mango and neem tree

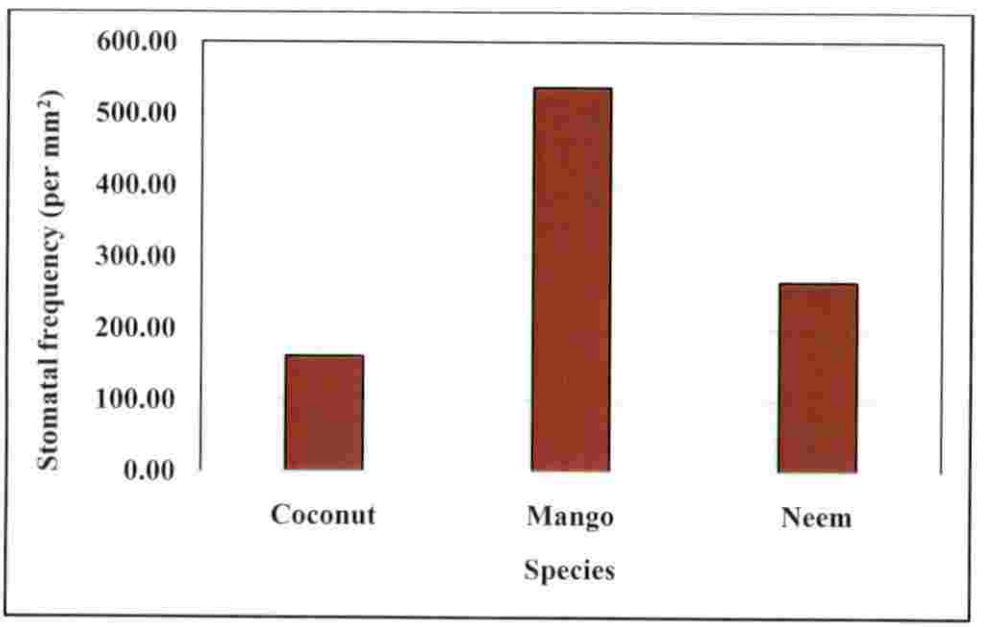

Figure 6. Stomatal frequency of coconut palm, mango and neem tree

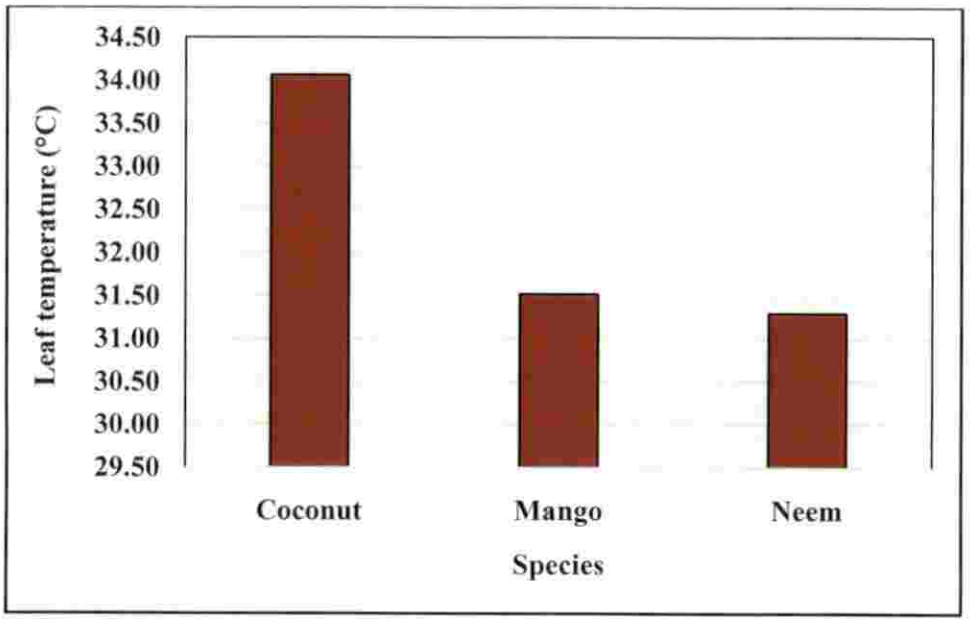

Figure 7. Leaf temperature of coconut palm, mango and neem tree

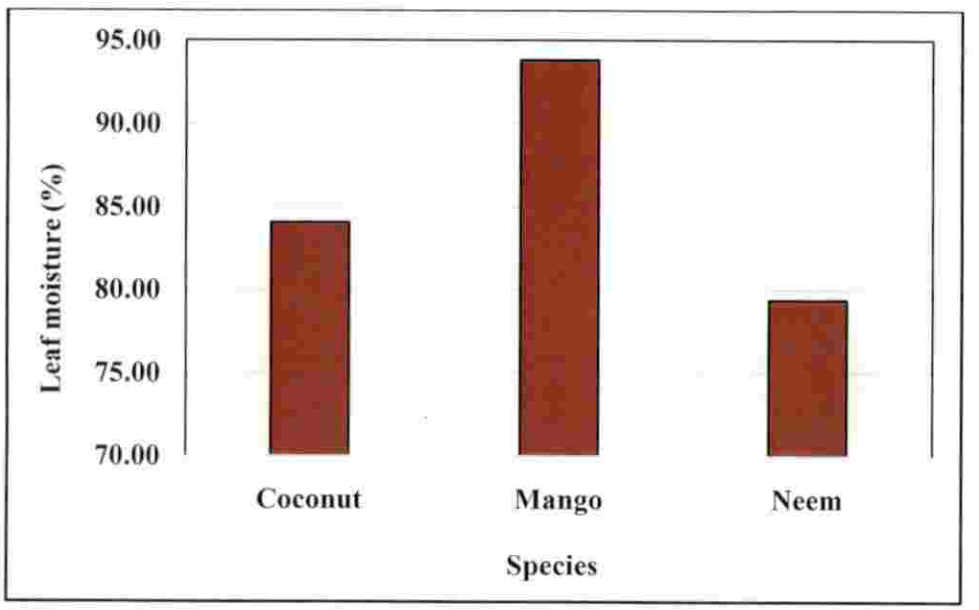

Figure 8. Leaf moisture of coconut palm, mango and neem tree

4.4. Interrelationship between anatomical and physiological properties

4.4.1. Coconut

Table 9 gives the correlation between different anatomical and physiological properties of coconut palm. Transpiration was positively correlated to photosynthesis at 0.01 of significance and vessel diameter was positively correlated to fibre length. There were no negative correlations between the anatomical and physical properties of coconut.

4.4.2. Mango

Photosynthesis was positively correlated to leaf temperature at 0.05 level of significance, and negatively correlated to leaf moisture at 0.01 level of significance. At the same time, leaf temperature was also negatively correlated to leaf moisture at 0.05 level of significance. Correlation between anatomical and physiological properties is given in Table 10.

4.4.3. Neem

Correlation between anatomical and physiological properties of neem is given in Table 11. In case of neem, transpiration is positively correlated to leaf temperature at 0.01 level of significance and leaf moisture was positively correlated to vessel frequency. Whereas, stomatal frequency is negatively correlated to leaf moisture at 0.05 level of significance.

Table 9. Correlation between anatomical and physiological properties of coconut palm

	Photosynthesis	Transpiration	Stomatal frequency	Leaf temperature	Leaf moisture	Vessel diameter	Fibre length	Fibre diameter	Lumen width	Vessel frequency
Photosynthesis	1									
Transpiration	.989**	1								
Stomatal frequency	.010	-.088	1							
Leaf temperature	.693	.610	-.019	1						
Leaf moisture	-.498	-.400	-.325	-.839	1					
Vessel diameter	.601	.593	.287	.086	.166	1				
Fibre length	.632	.606	.100	.314	.077	.932*	1			
Fibre diameter	.078	-.025	.690	.092	-.023	.635	.638	1		
Lumen width	.054	-.093	.796	.442	-.640	.069	.106	.691	1	
Vessel frequency	-.366	-.424	.860	-.344	-.163	-.134	-.384	.322	.568	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 10. Correlation between anatomical and physiological properties of mango

	Photosynthesis	Transpiration	Stomatal frequency	Leaf temperature	Leaf moisture	Vessel diameter	Fibre length	Fibre diameter	Lumen width	Vessel frequency
Photosynthesis	1									
Transpiration	.679	1								
Stomatal frequency	.384	.731	1							
Leaf temperature	.940*	.805	.360	1						
Leaf moisture	-.971**	-.817	-.571	-.933*	1					
Vessel diameter	.157	-.427	.010	-.125	-.039	1				
Fibre length	-.160	.316	.466	.027	.054	-.116	1			
Fibre diameter	-.019	-.337	.278	-.342	.020	.787	-.259	1		
Lumen width	.125	.207	.219	.009	-.223	-.271	-.645	.273	1	
Vessel frequency	-.344	-.671	-.541	-.543	.423	.184	-.853	.434	.559	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 11. Correlation between anatomical and physiological properties of neem

	Photosynthesis	Transpiration	Stomatal frequency	Leaf temperature	Leaf moisture	Vessel diameter	Fibre length	Fibre diameter	Lumen width	Vessel frequency
Photosynthesis	1									
Transpiration	.848	1								
Stomatal frequency	.054	.468	1							
Leaf temperature	.698	.964**	.521	1						
Leaf moisture	.161	-.365	-.880*	-.508	1					
Vessel diameter	.247	.550	.215	.647	-.540	1				
Fibre length	.047	.046	-.069	.135	.202	-.417	1			
Fibre diameter	-.425	-.059	.854	.016	-.762	-.111	-.091	1		
Lumen width	.089	.011	.143	.000	.200	-.720	.850	.183	1	
Vessel frequency	-.309	-.758	-.820	-.849	.880*	-.699	.123	-.466	.173	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4.5. Azadirachtin uptake

The HPLC results of leaves of coconut palm, mango and neem are discussed below.

4.5.1. Coconut

Azadirachtin was found to be accumulated in leaves of coconut palm. Leaves of mango and neem did not show traces of azadirachtin in detectable levels. Azadirachtin traces were found on coconut leaves collected on the second day after injection was above detectable levels (0.14µg/g). Peak area of Azadirachtin - A in sample solution was 5.84 mAU and 5.55 mAU when compared to the peak area of Azadirachtin - A in standard solution (17.57 mAU). Peak area of Azadirachtin - B in sample solution was 2.72 mAU and 2.98 mAU when compared to the peak area of Azadirachtin - B in standard solution, which was 6.18 mAU. Altogether, peak area of Azadirachtin - (A + B) in sample solution was 8.56 mAU and 8.53 mAU when compared to the peak area of Azadirachtin - (A + B) in standard solution, which was 23.75 mAU. The chromatogram for sample solution is given in Figure 10 and Table 13, and the chromatogram for standard solution of azadirachtin is given in Figure 9 and Table 12.

Table 12. Chromatogram for standard solution (1ppm) of azadirachtin in coconut palm leaves

Peak No.	Compound name	RT (min)	Peak area
1	Azadirachtin A	10.64	17.57
2	Azadirachtin B	11.93	6.18

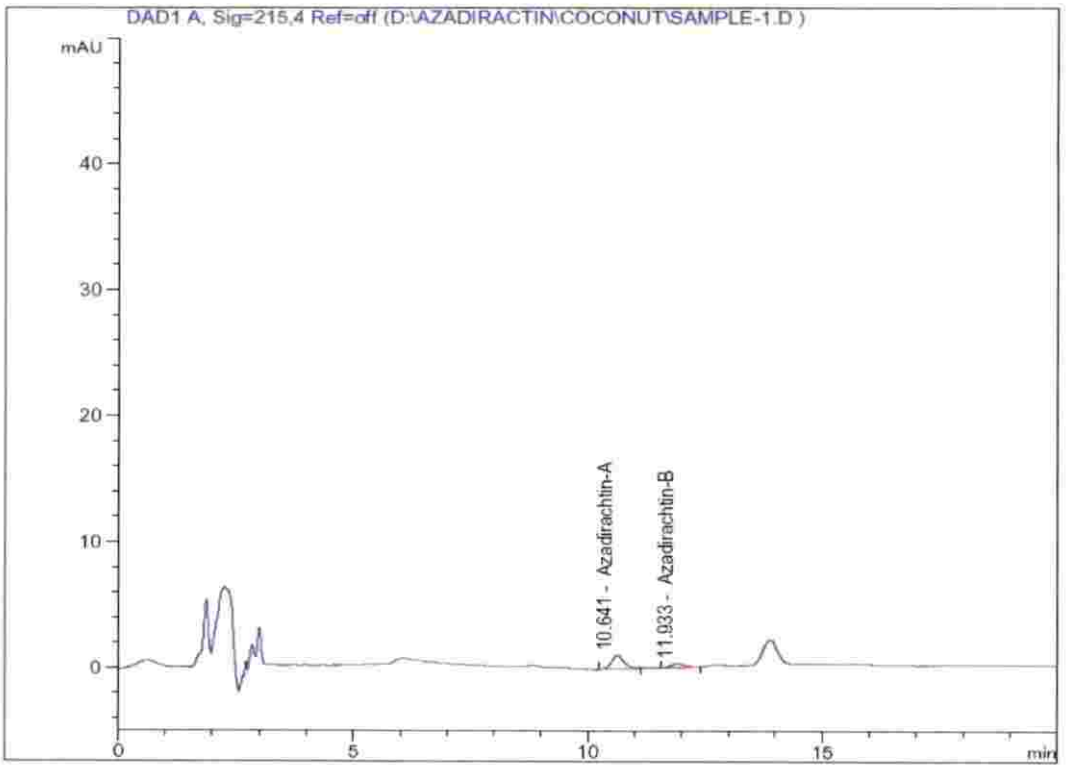

Figure 9. Chromatogram for standard solution of azadirachtin in coconut palm leaves

Table 13. Chromatogram for sample solution of azadirachtin in coconut palm leaves

Peak No.	Compound name	RT (min)	Peak area
1	Azadirachtin A	10.66	5.84
2	Azadirachtin B	11.96	2.72

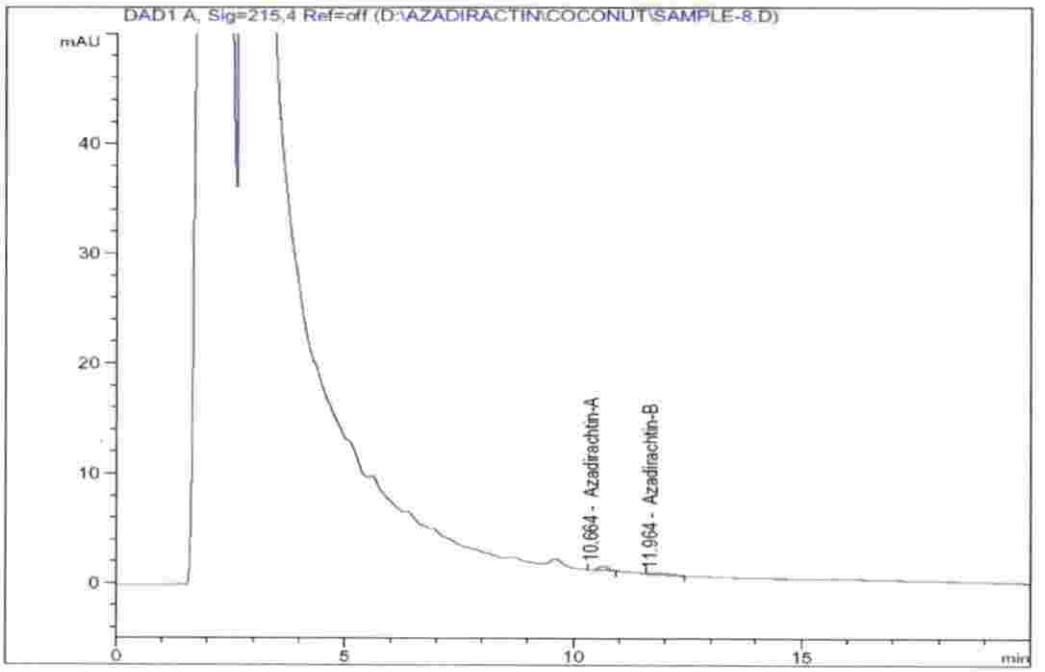

Figure 10. Chromatogram for sample solution of azadirachtin in coconut palm leaves

4.5.2. Mango

In mango, the peak area of Azadirachtin - A in standard solution was 18.07 mAU, peak area of Azadirachtin - B in standard solution was 5.42 mAU and peak area of Azadirachtin - (A + B) in standard solution was 23.49 mAU. The average residue content of Azadirachtin was below detectable level for all the samples. The chromatogram for sample solution is given in Figure 12 and Table 15. And the chromatogram for standard solution of azadirachtin is given in Figure 11 and Table 14.

Table 14. Chromatogram for standard solution of azadirachtin in mango leaves

Peak No.	Compound name	RT (min)	Peak area
1	Azadirachtin A	10.53	18.07
2	Azadirachtin B	11.78	5.42

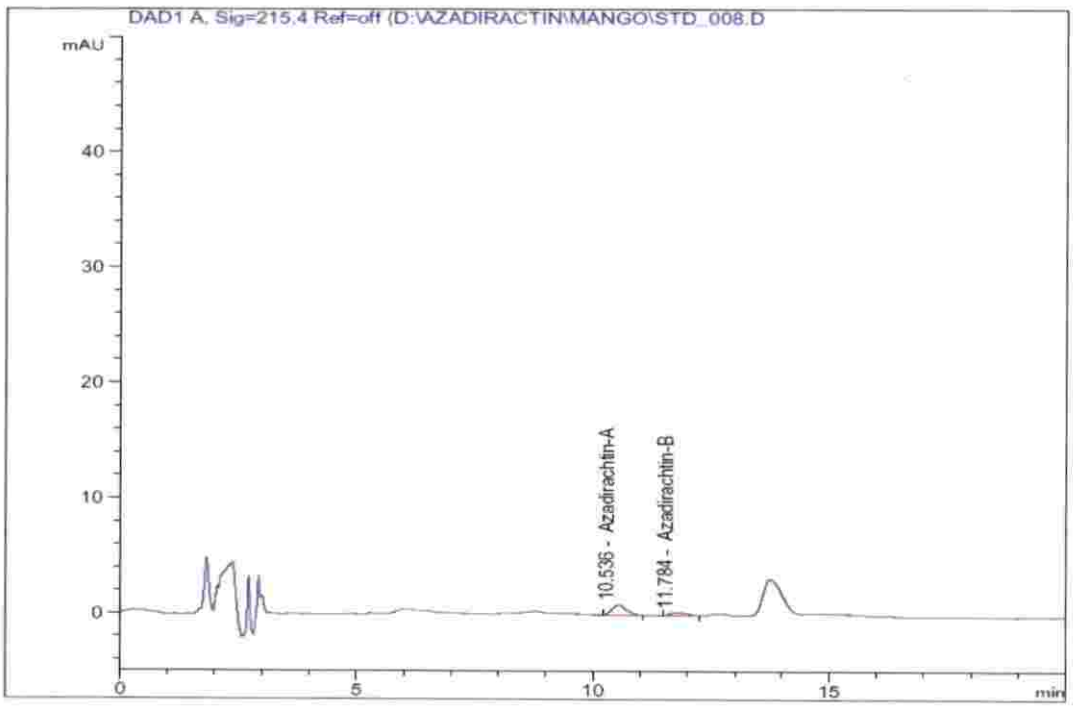

Figure 11. Chromatogram for standard solution of azadirachtin in mango leaves

Table 15. Chromatogram for sample solution of azadirachtin in mango leaves

Peak No.	Compound name	RT (min)	Peak area
1	Azadirachtin A	0	0
2	Azadirachtin B	0	0

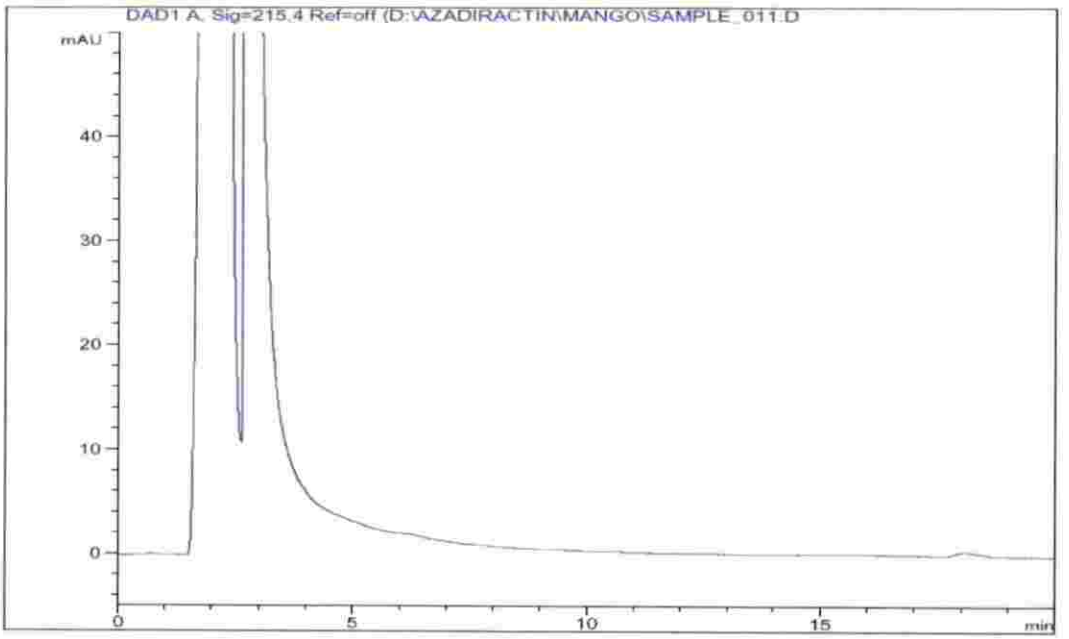

Figure 12. Chromatogram for sample solution of azadirachtin in mango leaves

4.5.3. Neem

In neem, peak area of Azadirachtin - A in standard solution was 18.10 mAU, Peak area of Azadirachtin - B in standard solution was 5.25 mAU and the peak area of Azadirachtin - (A + B) in standard solution was 23.35 mAU. The average Azadirachtin residue content was below detectable level in all the samples of neem leaves. The chromatogram for sample solution is given in Figure 14 and Table 17. And the chromatogram for standard solution of azadirachtin is given in Figure 13 and Table 16.

Table 16. Chromatogram for standard solution of azadirachtin in neem leaves

Peak No.	Compound name	RT (min)	Peak area
1	Azadirachtin A	10.57	18.1
2	Azadirachtin B	11.83	5.25

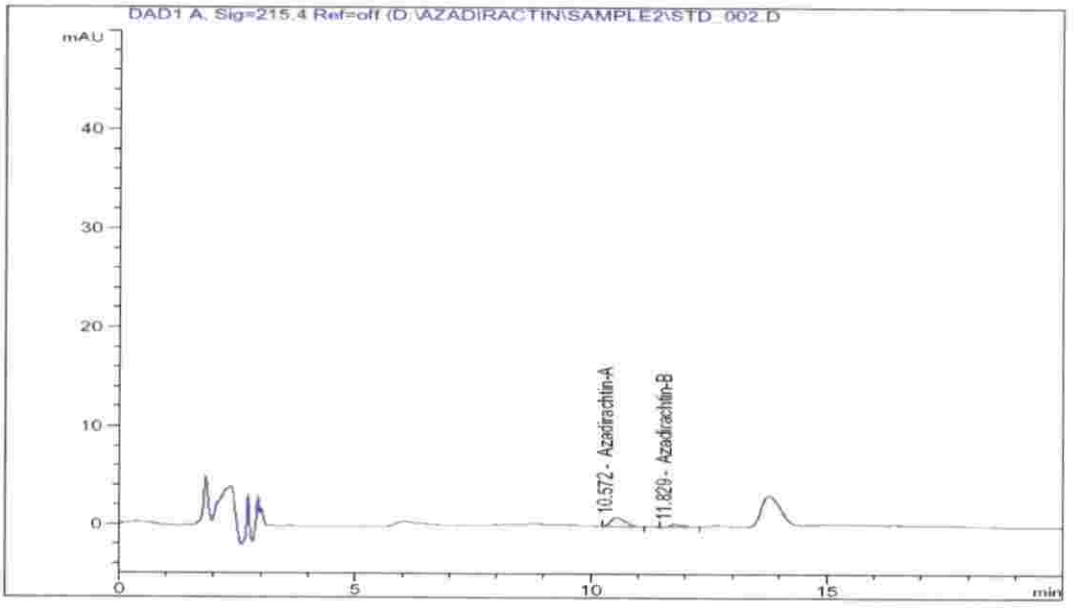

Figure 13. Chromatogram for standard solution of azadirachtin in neem leaves

Table 17. Chromatogram for sample solution of azadirachtin in neem leaves

Peak No.	Compound name	RT (min)	Peak area
1	Azadirachtin A	0	0
2	Azadirachtin B	0	0

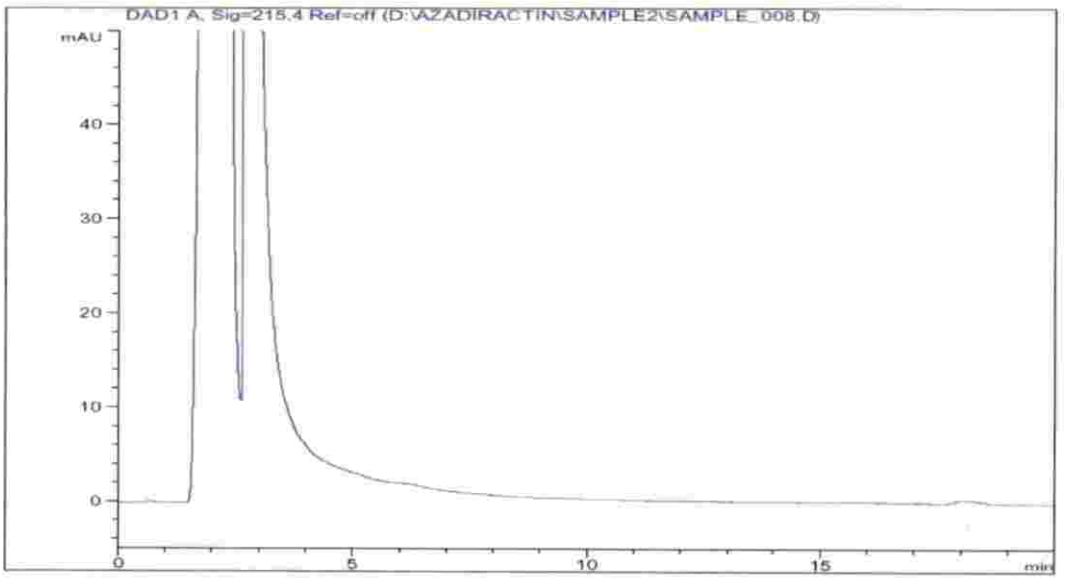

Figure 14. Chromatogram for sample solution of azadirachtin in neem leaves

Discussion

5. DISCUSSION

The results of the study entitled "Standardization of tree injection procedures of azadirachtin in coconut (*Cocos nucifera* L.), mango (*Mangifera indica* L.) and neem (*Azadirachta indica* A Juss.)" are discussed hereunder.

The study investigated the suitable depth to which the tree injection can be applied in coconut, mango and neem. The intake of azadirachtin was also correlated with the anatomy and physiology of trees. Residual nature of azadirachtin after injection was assessed by conducting High Performance Liquid Chromatography.

5.1. Physical properties

As one of the main objectives of the study was to understand and estimate variation in physical properties of coconut, mango and neem wood, the present work focused on some important physical properties such as basic density, moisture content, sapwood thickness, sapwood area, sapwood – heartwood ratio and bark thickness prior to tree injection procedure.

5.1.1. Basic density

Basic density is one of the most important wood property, which highly correlated with other wood properties and morphological, physiological and ecological characters (Jerome *et al.*, 2006). Wood density is a basic biophysical property that can often serve as a vigorous proxy for an array of simple and complex hydraulic traits over a range of scale from tissue to whole plants (Chave *et al.*, 2009; Meinzer *et al.*, 2008; Pratt *et al.*, 2007; Zanne *et al.*, 2010; Anderegg and Meinzer, 2015)

Analysis of variance conducted revealed that there is no significant difference in basic density between the three size classes of coconut, mango and neem stem wood.

The finding that the basic densities of four replications from each size classes of coconut palm wood can be supported by the work of Fathi (2014), which suggested that the variation in basic density might be due to palm age, palm varieties and/ or soil and climate conditions. Investigation of Hopewell *et al.* (2010), based on the variation of density pattern with age was not so clear cut. Such a situation may perhaps occur in palms of different varieties or from different climatic sites (Meylan, 1978).

In contrast, the density of mango and neem wood decreases from core towards outside. This is due to the deposition of lignin and other extraneous materials in heartwood. Number of cells per unit area also increases in the heartwood area. It is also an important factor in determining the mechanical properties of wood. Anderegg and Meinzer, 2015 stated that in *Eucalyptus camaldulensis*, growth at higher temperatures led to higher wood density, lower hydraulic conductance, and a shift toward smaller vessel diameters.

5.1.2. Moisture content

A large number of studies have quantified anatomical and physiological differences in wood in species in divergent environments, especially along moisture gradients (Alder *et al.*, 1996; Maherali and DeLucia 2000; Anderegg and Meinzer, 2015).

Moisture content of coconut palm wood, mango and neem was not found to differ between the three size classes (50-60 cm, 60-70 cm, 70-80 cm). The moisture content profile of coconut shows an opposite pattern compared to green dicot wood of mango and neem, which showed high moisture content in the core, decreasing towards the periphery, contrary to the pattern generally observed in typical green wood (Hopewell *et al.*, 2010). This variation in moisture helps to determine the part or depth of wood which will actively participate in conducting the injected chemical to the canopy.

The density of wood depends on specific gravity and moisture content. In hardwoods, the difference in moisture content between heartwood and sapwood differs with species. Moisture makes up part of the weight of each product in use; therefore, the density must reflect this fact (Simpson and TenWolde, 1999). Thus the basic density of core zone of coconut palm wood was very less and the moisture was highest as compared to the high density dermal zone. Similarly, the moisture content of heartwood was less and basic density was more in mango and neem wood when compared with sapwood of the same. This value should always be considered an approximation because of the natural variation in anatomy, moisture content, and ratio of heartwood to sapwood that occurs (Simpson and TenWolde, 1999).

5.1.3. High density wood thickness of coconut palm

High density wood thickness of coconut palm wood was not significant between three size classes. The average high density wood thickness was 3.5 cm and was not more than 4.89 cm. Thus the depth of 6 cm was fixed for injecting azadirachtin into coconut palm wood irrespective of size class. The result of ANOVA meant the thickness of high density wood does not follow a trend to increase or decrease with an increase in size classes. Nevertheless, there are several studies showing a significant increase in dermal wood percentage with increasing age (Alex, 2017), which indirectly agrees with the statement that over mature coconut palm wood have higher sawn wood outturn (Gnanaharan and Dhamodaran, 1989). Yet, interpretation of the finding that the thickness of dermal wood does not follow a pattern of either increase or decrease with size class will not be complete as there are no clear scientific facts.

5.1.4. Sapwood thickness of mango

Sapwood thickness of mango wood did not differ between three size classes. Thus it was decided to inject the trees at 3 cm deep since the study did not show the presence of physiologically dead heartwood shallower than 3 cm. This is because of

the fact that the mango trees have a very large sapwood width (Lu and Malik, 2005). In a large mango tree, the boundary between sapwood and heartwood is usually indistinct and heartwood formation occurs only after 20-30 years growth (Lu and Chacko, 1996). There are no scientific studies showing an increasing or decreasing pattern of sapwood thickness in mango stem wood with varying size class.

5.1.5. Sapwood thickness of neem

Estimating sapwood area is significant for ecohydrological studies such as transpiration and water balance experiments (Clearwater *et al.*, 1999; Benson *et al.*, 2018), which are essential for conducting tree injections. Sapwood thickness of neem wood did not differ between three size classes. Examinations of tree-stem cross sections often disclose a central region of darkened heartwood, surrounded by a ring of lighter colored, conductive sapwood (Githiomi and Dougal 2012; Lin *et al.*, 2012). Thickness of sapwood varied with several size classes without showing a trend of increase or decrease. Thus the depth of 3 cm was fixed for neem stem at basal height for injection irrespective of size class.

5.2. Anatomical properties

Though there is little information on the wood behavior of evergreen species (Sass *et al.*, 1995; Alves and Angyalossy, 2000), wood anatomy interacts with other components in the hydraulic continuum, together with the root–rhizosphere interface and the water–air interface in stomatal pores on leaves (Barnard *et al.*, 2011). Numerous reports put forward the possible correlation of wood anatomy and environment (Baas, 1973; Carlquist, 1977, 1982, 1985; Van Vliet, 1979), whereas other studies indicate no such relationship (Young, 1974; Nair, 1988).

5.2.1. Vessel diameter

Vessel diameter of coconut palm wood, mango and neem differed between the species. The mean vessel diameter of coconut was 110.20 µm, mango was 245.41 µm and for neem was 151.87 µm. The average vessel diameter differs with various species. And reduced xylem conductivity could be explained by the decrease of vessel diameter, according to the Poiseuille equation (Lovisolo and Schubert, 1998). Moreover, Xylem conductivity is determined by the structure and size of the vessels (Schultz and Matthews, 1993; Tyree and Ewers, 1991).

5.2.2. Vessel frequency

Vessel frequency showed no significant difference between the species though the vessel frequency changes with different species. The mean values for vessel frequency were $4/mm^2$, $4.8/mm^2$ and $5.4/mm^2$ for coconut, mango and neem respectively. Neem showed highest vessel frequency, followed by mango and coconut. This variation can be accounted to the statement given by Wagner *et al.*, 1998 that within two congeneric pairs of chaparral shrubs growing together in the same habitat, there may be tradeoffs between mechanical strength and conductive efficiency of the stem xylem which correspond to differences in transport physiology and life history traits of sprouter versus non-sprouter species.

5.3. Physiological parameters

5.3.1. Photosynthesis

Photosynthesis differed between the three species of coconut, mango and neem. Gamon *et al.* (1997), reported that evergreens exhibited significantly reduced midday photosynthetic rates relative to annual and deciduous species. Photosynthesis was observed to be the highest in coconut palm followed by neem and mango. This can be related to the study of Pallas *et al.* (1967), in one way that when soil water was

adequate, photosynthesis was proportional to light intensity because the injection was done in coconut at 0930 hrs, when the light intensity was high and the soil moisture was high. The result did not match the study of Pallas *et al.* (1967), the other way which showed that the lowering of leaf temperature at the higher vapor pressure deficits may thus increase photosynthesis because the leaf temperature was highest for coconut (34.06°C), when compared to mango and neem at the time of injection.

5.3.2. Transpiration

Transpiration differed between the species. The mean values of transpiration was the highest for mango (2.61mmol of H_2O /m^2/s), followed by neem (1.47 mmol of H_2O /m^2/s) and coconut (0.58 mmol of H_2O /m^2/s). This trend has a relation with the soil moisture content and stomatal rate of each species. Mango leaves had the highest stomatal density (536.2/mm^2) followed by neem (265/mm^2) and coconut (161/mm^2). The study conducted by Urban and Jannoyer (2002), shows that mature mango leaves are efficiently protected against excessive loss of water by transpiration. This feature is connected with high stomatal frequency. This contrast might account to the availability of moisture in the soil as the tree was well irrigated. As transpiration is positively correlated with radiation level, air vapor pressure deficits and soil water potential, the transpiration rate of trees change for different species and environmental conditions (Pallas *et al.*, 1967). In a study conducted by Lovisolo and Schubert (1998), it was clear that in both irrigated and stressed plants, the total plant transpiration was linearly correlated with sap flow throughout the shoot. There are reports that diurnal and seasonal time courses of transpirational rate can be quite variable, especially during the hottest hours of the day in summer, with transpiration rate peaking at 10.00–12.00 h (Rodríguez-Gamir *et al.*, 2010), which was against the findings of this study as transpiration was assessed in the early morning hours for mango. Transpiration rate is different for dwarf and tall varieties of coconut palm. High transpiration in dwarf genotypes resulted in elevated intake of water in comparison to other varieties and hybrids. Tall varieties, on the other hand,

show a more conservative water use (Gomes and Prado, 2007). The present study helped to arrive to the conclusion that dwarf coconut responds positively for tree injection as compared to tall varieties of coconut palm.

5.3.3. Stomatal frequency

Stomatal frequency varied between the species. Stomatal frequency was the highest in mango (536.2/mm^2) and the lowest in coconut (161/mm^2). Comprehending stomatal functioning and their role is important since stomata control both photosynthesis, which is nothing but the production potential, and transpiration, which in turn influences irrigation management (Urban and Jannoyer, 2002). Stomata play a major role in water uptake and in determining transpiration. This relation had been published in the work of Pallas *et al.* (1967), which indicated that the percentage of open stomata at high radiation levels were always substantially greater than under low radiation, which was a photoactive effect. The greater the number of open stomata, the less is leaf resistance. This partially explains any kind of increase in transpiration with increase in radiant energy. Even in this study, the transpiration was highest in mango (2.61 mmol of H_2O /m^2/s) with high stomatal frequency 536.2/mm^2.

5.3.4. Leaf temperature

Leaf temperature varied significantly between coconut palm, mango and neem. The mean values of leaf temperature were 34.06°C, 31.52°C and 31.29°C in coconut, mango and neem respectively. This is evident from the statement that leaf temperature increases with an increase of leaf size (Gates, 1968) as the leaf size of coconut palm was highest. It has been stated by Anderegg and Meinzer (2015), that rising temperature will affect physiology and xylem anatomy. The relevance of leaf temperature was seen in the work of Pallas *et al.* (1967), where leaf temperature was highly correlated with transpiration under moist conditions. With decreasing soil water, transpiration was decreasing; in the mean time, leaf temperatures rose. At high

soil water, leaf temperature ranged from a fraction of 1°C to a few degrees over ambient, probably as a result of elevated transpirational cooling (Pallas *et al.*, 1967), which was same in this study, because the leaf temperature of coconut was highest (34.06°C) whereas the transpiration was least (0.58 mmol of H_2O /m^2/s) in coconut palm.

5.3.5. Leaf moisture

Leaf moisture differed between the three species. The mean values were 84.03%, 93.81% and 79.36% in coconut, mango and neem respectively. The variation between the species can be attributed to plant's water relations in terms of the environmental evaporative demand as well as the supply or loss of water from leaves via measures of stomatal conductance and transpiration (Flanagan and Ehleringer 1991; Roden and Ehleringer 1999; Cernusak *et al.*, 2003; Keitel *et al.*, 2003). In turn, leaf water can prove pivotal for ecophysiological interpretations and for plant breeding programs (Farquhar *et al.*, 2007; Kahmen *et al.*, 2008).

5.4. Interrelationship between anatomical and physiological properties

Anderegg, and Meinzer (2015) stated that temperature can affect hydraulic vulnerability to cavitation, both through effects on conduit size and xylem anatomy and through effects on xylem fluid viscosity, although most of the evidence for this comes from studies of contrasting existing environments. They also concluded that wood anatomy and plant hydraulics are known to vary in time and in space within the same species. Wood anatomy and plant hydraulics are known to vary in time and in space within the same species (Anderegg and Meinzer, 2015). However, correlation between anatomical properties and physiological aspects was not observed in any of the species was studied.

5.4.1. Coconut

In coconut palm, the vessel diameter was positively correlated to fibre length. Even though the vessel diameter and fibre length were positively correlated to each other, literatures were not found in order to support this statement. Transpiration was positively correlated to photosynthesis at 0.01 of significance. The mean value of transpiration was 0.58 mmol of H_2O /m^2/s and photosynthesis was 2.22 µmol of CO_2/m^2/s. It showed a trend of increased photosynthesis with increasing transpiration. In a study conducted by Repellin *et al.* (1997) and Gomes *et al.* (2008), the main factor limiting photosynthesis in young coconut seemed to be stomatal closure.

5.4.2. Mango

In this study, photosynthesis was positively correlated to leaf temperature at 0.05 level of significance, in which photosynthesis was 1.14 µmol of CO_2/m^2/s and leaf temperature was 31.52°C. Chaves *et al.* (1992) stated that the effects of water deficits on photosynthetic capacity in lupins were shown to be based mostly of leaf temperature. As temperature increases, net photosynthetic rate increases, reaches a maximum rate at an optimum temperature, and then declines at supraoptimal temperatures (Kobza and Edwards, 1987). The relationship between net photosynthetic rate and leaf temperature is influenced by illuminance (Chmora and Oya 1967), vapour pressure of the air (Kriedemann 1968), temperature (Mooney and Shropshire 1967), and light intensity history (Kriedemann 1968; Ludlow and Wilson, 1971).

It was also found that photosynthesis was negatively correlated to leaf moisture at 0.01 level of significance. Photosynthesis was 1.14 µmol of CO_2/m^2/s and leaf moisture was 93.81%. Stomatal rate was found to be the highest in mango with a mean value of 536/mm^2. Poor control of stomata might have accounted for the loss of moisture. Short-term responses to water stress such as low stomatal

conductance to water vapor and leaf water potential with negative consequences for the net photosynthetic rate and transpiration rate have been demonstrated (Repellin *et al.*, 1997; Gomes, *et al*, 2008). An early physiological response to water deficit is often stomatal closure, which results in decreased photosynthesis, through limited CO_2 availability in the mesophyll (Cornic, 2000; Gomes, *et al*, 2008). They also concluded that the decrease in photosynthesis is the result of stomatal closure.

At the same time, leaf temperature of 31.52°C was negatively correlated to leaf moisture of 93.81% at 0.05 level of significance. Transpiration rate and leaf temperature are the result of the interaction of many simultaneous environmental factors interacting with a leaf to a degree determined by several plant properties (Gates, 1968). Loss of moisture with increase in temperature is common. The fact that stomatal rate being highest and it's poor control in mango can also attribute to this negative correlation. Other than this, the leaf temperature might decrease because of the high rate of transpiration and stomatal conductance, because leaves lose temperature through evaporative cooling which is an indirect measure of transpiration. In turn, changes in transpiration rate can cause changes in the temperature and water potential of the leaf (Farquhar and Sharkey, 1982). In *Lupins albus*, stomata were more open at higher temperatures (25°C) than lower temperatures (15°C), in either well-watered or water-stressed conditions (Correia *et al.*, 1999), which have accounted for this negative correlation.

5.4.3. Neem

Transpiration of 1.47 mmol of H_2O /m^2/s was positively correlated to leaf temperature of 31.29°C at 0.01 level of significance. It is well known that temperature affects the stomatal aperture of leaves (Jones, 1992). This was contrasting to the study results drawn by Correia *et al.* (1999), who stated that leaves lose temperature through evaporative cooling and therefore is an indirect measure of transpiration. Changes in transpiration rate can cause changes in the temperature and

water potential of the leaf (Farquhar and Sharkey, 1982). Gates (1968) stated that energy absorbed by a leaf goes into the evaporation of moisture which is eliminated from the leaf by the combination of diffusion and air movement. An increase of air temperature raises transpiration rate and leaf temperature for all values of internal diffusion resistance. The lower the internal resistance the more rapidly transpiration increases with air temperature, and the leaf temperature increases somewhat less rapidly.

Stomatal frequency (265/mm^2) is negatively correlated to leaf moisture (79.36%) at 0.05 level of significance in neem wood. Chaves *et al* (2009) stated that stomatal closure is known to have a more inhibitory effect on transpiration of water from leaves. In a study conducted by Kundu and Tigerstedt (1999), the positive relationships between stomatal density on one hand and stomatal conductance on the other, appear to offer a physiological basis for the variation in growth of the provenances.

Leaf moisture (79.36%) was positively correlated to vessel frequency (5.4/mm^2). Neem had the least moisture content and highest vessel frequency among the three species. This is contrary to the work of February and Manders (1999), who suggested that forest species are probably genetically adapted to the environments in which they live, as a result of which vessel morphology of these woody species lack the plasticity to adapt to changes in soil type and soil moisture. Woody plants increase transport efficiency by either producing more cross sectional xylem or changing anatomical features that affect conductivity such as vessel size and frequency (Ewers 1985). However, there was no detection of azadirachtin in neem leaves despite having the highest vessel frequency.

5.5. Azadirachtin uptake

Azadirachtin was found to be accumulated in the leaves of coconut palm. Leaves of mango or neem did not show any traces of azadirachtin in detectable

levels. Azadirachtin traces were found on coconut leaves above detectable levels (0.1439 µg/g) collected on the second day after injection. The difference in between species in detecting azadirachtin might be because of the statement given by Rodríguez-Gamir *et al.* (2010), in which they revealed that morphological and anatomical dissimilarities between rootstocks may be liable for certain physiological differences. The coconut palm selected was a short variety high yielding palm named 'Pushkala'. Tomlinson (2006) stated that the stem cross-sectional area is occupied by interconnected vascular bundles retaining conductive ability throughout the life span of the palm. The transport capacity of this massive hydrosystem increases by the cube of the stem diameter, whose cells are capable to retain their viability over a century. Higher transpiration in dwarf genotypes resulted in elevated intake of water as in comparison to other varieties and hybrids. Tall varieties, on the other hand show a more conservative water use, which might have accounted for the uptake of azadirachtin by the dwarf variety of coconut palm rather than tall mango and neem tree (Gomes and Prado, 2007). Dosage of azadirachtin was only 40 ml which was a contrast against the study of Doccola *et al.* (2003), in which the dosage was based on diameter at breast height and the mean dosage applied per 2.5 cm dbh was 2 ml. If this was followed, the dosage to be applied to the trees would have been 68.8 ml, 54.4 ml and 58.4 ml for coconut, mango and neem respectively. This might have made the detection of azadirachtin residue in mango and neem trees difficult. However, the effect of azadirachtin injection cannot be expected to last for more than two days in contrast to study where a lethal concentration of the insecticide appears to have persisted in the leaves of treated palms for about six months after the application of 5 ml of undiluted 60 per cent water soluble concentrate of monocrotophos (Kanagaratnam and Pinto, 1985).

Summary

VI. SUMMARY

Trunk or tree injection is a modern method of treating medium and large trees against pests by applying systemic pesticides to its trunk. This method is helpful in controlling insect pests in urban landscapes because it minimizes the risk of the applicator to the chemical and off target exposure. Moreover, the chemical used in this study is azadirachtin, which is a neem based bio pesticide. This is eco friendly and does not affect the large living organisms.

Coconut (*Cocos nucifera* L.) and mango (*Mangifera indica* L.) are commercially the most important fruit crops of India. Whereas neem (*Azadirachta indica* A. Juss.) is a major component of urban and community forests in India since time immemorial. They are important commercial species yielding fruits and other various important commodities like wood, medicines and fibres. The challenge faced by these species is the invasion of various pests. Farmers currently count on chemical insecticides to control insect pests. The heavy use of synthetic pesticides has caused an increased cost in managing pests, environmental pollution and other social problems. Heavy use of inorganic insecticides since ages has led to the destruction of natural predators and parasitoids of the pests. The study was conducted to standardize tree injection procedures for coconut, mango and neem using azadirachtin under the prevailing agro – climatic conditions. The study was conducted in the Department of Forest Products and Utilization, College of Forestry, Kerala Agricultural University, Vellanikkara during 2017-2019. The summary of the findings is provided below.

- Basic density was not significant between the three size classes of coconut. In general, the basic density of coconut palm was 354.33 kg/m^3.
- In mango, basic density was not significant between three size classes. In general, the basic density ranged from 519.63 kg/m^3 to 539.52 kg/m^3 irrespective of size class.

- The basic density was not significant between the three size classes of neem. In general, the basic density ranged from 679.59 kg/m^3 to 738.42 kg/m^3 irrespective of size class.
- In general, the average basic density of coconut palm wood, mango wood and neem wood were 354.33 kg/m^3, 526.87 kg/m^3 and 712.6276 kg/m^3, indicating that neem is denser than coconut palm wood and mango.
- Moisture content was not significant for coconut palm wood belonging to three size classes (50-60cm, 60-70cm, 70-80cm) did not show significant difference. In general, the moisture content of coconut palm wood ranged from 74.33% to 133.09% irrespective of size class.
- Moisture content was not significant between the three different size classes of mango. The moisture content of mango wood ranged from 65.12% to 79.76% irrespective of size class.
- The moisture content of wood was not significant between the three different size classes of neem. In general, the moisture content of neem wood ranged from 33.23% to 41.18% irrespective of size class.
- In general, the average wood moisture of coconut palm, mango and neem were 111.54%, 73.27% and 36.58% respectively. This result indicated that coconut palm wood possess high moisture content.
- High density wood thickness of coconut palm wood did not show any significant difference between three size classes. And average high density wood thickness was 3.5cm and was not more than 4.89cm. It means that the thickness of high density wood does not vary with the change in size class. Thus a depth of 6 cm was fixed to inject chemicals into coconut palm.
- Sapwood thickness of mango wood was not significant between three size classes. And average sapwood thickness was 6.18cm and was never smaller than 3.27cm. Indicating the thickness of sapwood does not vary with the

change in size class. Thus a depth of 3 cm was fixed to deliver chemicals into the tree trunk.

- Sapwood thickness of neem wood was not significant between three size classes. And average sapwood thickness was 4.38cm and was never smaller than 3.02cm. It means that the thickness of physiologically live wood (sapwood) does not vary with the change in size class, which made to fix the depth as 3 cm.
- Vessel diameter was significant for coconut palm wood, mango and neem. Mango showed a high mean value of 245.41 µm and coconut palm wood showed a mean value of 110.20 µm.
- Fibre length showed a significant difference between the three species. Coconut palm wood showed the highest mean value of 1611.17 µm and mango showed the lowest mean value of 257.12 µm.
- Fibre diameter showed a significant difference between coconut, mango and neem. Coconut had the widest diameter of 18.28 µm, whereas mango had the smallest diameter of 13.36 µm.
- Fibre lumen width of coconut palm wood, mango and neem showed a significant difference. The mean value was highest for coconut palm wood (14.99 µm) and lowest for mango (8.98 µm).
- Vessel frequency indicated no significant variation between the species. Neem showed highest vessel frequency of 5.4/mm^2, followed by mango (4.8/mm^2) and coconut (4/mm^2).
- Ray height was not significant between mango and neem.
- Ray width was not significant between mango and neem. Neem had the highest ray width of 181.15 µm when compared with mango with a mean value of 29.20 µm.

- The bundle sheath thickness of coconut palm wood indicated no significant difference between the size classes. The values ranged from 306.89 µm to 347.16 µm with a mean value of 318.22 µm.
- Vascular bundle diameter of the coconut palm was not significant though the values ranged from 311.39 µm to 354.91 µm with a mean value of 334.16 µm.
- Photosynthesis between the three species of coconut, mango and neem showed no significant difference. Photosynthesis was observed to be the highest in coconut palm (2.22 $\mu mol^2/m^2/s$) followed by neem (1.40 $\mu mol^2/m^2/s$) and mango (1.14 $\mu mol^2/m^2/s$).
- The mean values of transpiration were 0.58 $mmol/m^2/s$, 2.61 $mmol/m^2/s$ and 1.47 $mmol/m^2/s$ in coconut palm, mango and neem respectively. The analysis of variance indicated a significant variation between the species.
- The mean values ranged from $161/mm^2$, $536/mm^2$ and $265/mm^2$ in coconut, mango and neem respectively. The analysis of variance showed a significant difference between the species. Stomatal frequency was highest in mango ($536/mm^2$) and lowest in coconut ($161/mm^2$).
- Leaf temperature was significant between coconut palm, mango and neem. The values for leaf temperature were 34.06°C, 31.52°C and31.29°C for coconut, mango and neem respectively.
- Leaf moisture indicated a significant difference between the three species. Mango had the highest leaf moisture followed by coconut and neem.
- In coconut, transpiration was positively correlated to photosynthesis and vessel diameter was positively correlated to fibre length. There were no negative correlations between the anatomical and physical properties of coconut.
- Mango showed a positive correlation between photosynthesis and leaf temperature, and a negative correlation between leaf moisture and

photosynthesis. At the same time, leaf temperature was also negatively correlated to leaf moisture.

- In case of neem, transpiration is positively correlated to leaf temperature and stomatal rate is negatively correlated to leaf moisture.
- Azadirachtin was found to be accumulated in leaves of coconut palm and leaves of mango and neem did not show traces of azadirachtin in detectable levels.
- Azadirachtin traces were found on coconut leaves collected on the second day after injection was conducted above detectable levels (0.14µg/g).

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STANDARDIZATION OF TREE INJECTION PROCEDURES OF AZADIRACHTIN IN COCONUT (*Cocos nucifera* L.), MANGO (*Mangifera indica* L.) AND NEEM (*Azadirachta indica* A Juss.)

by

SARMISHTA V.

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ABSTRACT

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ABSTRACT

Tree injection is a new technology that is employed to apply fungicides, nutrients and pesticides in large trees in order to avoid drifting of these chemicals and affecting non target organisms. It eliminates the wastage of chemicals to be applied in trees as the quantity used is little compared to other conventional methods of application. This study aimed at standardizing the tree injection procedures in Indian conditions. Stem wood of three species like coconut (*Cocos nucifera* L.), mango (*Mangifera indica* L.) and neem (*Azadirachta indica* A. Juss.) were used in the study along with their leaves after the application of azadirachtin through injection. The depth to which the tree injection can be applied was determined by studying the thickness of the conducting tissues in these species using the software Digimizer. Three size classes like 50-60cm, 60-70cm, 70-80cm were studied. Thickness of high density wood in coconut palm and sapwood in mango and neem does not vary with the change in size class. Average high density wood thickness in coconut palm was 3.5cm and was not more than 4.89cm. Thus a depth of 6 cm was fixed so as to ensure the delivery of chemicals into the most active part of the stem. In case of mango tree, the average sapwood thickness was 6.18cm and was never smaller than 3.27cm. Similarly, average sapwood thickness in neem was 4.38cm and was never smaller than 3.02cm. Thus 3 cm was the depth fixed to inject chemicals in mango and neem. Systemic insecticide, Azajet (50,000ppm) was used to inject the trees. Each tree was marked at a basal height of 20 cm from ground. Holes were made at an angle of 45° to make sure that there was no oozing out of chemicals. The EcoJect pump consisting of a canister, nozzle and a cylinder with compressed air between 100 and 150 PSI was used to inject the chemicals. Two canisters of 20 ml each (40ml/tree) were used to deliver the chemical into the tree trunk. Physiological parameters like photosynthesis, transpiration, leaf temperature and leaf moisture were analyzed using Infrared Gas Analyzer (LI-6400, Portable Synthesis System). Stomatal rate was

studied by the replica method. A correlation analysis was conducted between the anatomical and physiological properties of the three tree species. The traces of azadirachtin in the leaves were determined by collecting the leaf samples during specific time intervals like 1hr, 2hr, 6hr, 2 days, 7 days, 14 days, 20 days, 28 days, 40 days and 55 days of tree injection by using High Performance Liquid Chromatography (HPLC). There were no correlations between the anatomical and physiological parameters. Azadirachtin traces were found only in coconut palm on the second day of injection with a peak area of 0.14µg/g. Other trees showed no sign of azadirachtin in their leaves. The traces of the bio pesticide did not last for a week as there was no further detection of azadirachtin in the samples collected after 7 days of injection.