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Title: Effects of Chitin-Chitosan Nanoparticles on the physical and Decay Resistance Properties *Antheocephalus chinensis* (Lamk.) Wood

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Programme: Bachelor of Science in Forestry

EFFECTS OF CHITIN-CHITOSAN NANOPARTICLES ON THE PHYSICAL AND DECAY RESISTANCE PROPERTIES OF *Anthocephalus chinensis* (Lamk.) WOOD

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Course Title: Project Thesis

Course No: FWT- 4114

(This thesis paper has been prepared and submitted in partial fulfillment of the requirement for the degree of Bachelor of Science in Forestry)

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Declaration

I, Hira Khanom student ID: 110528, declare that the thesis is based on my original and own work and that it has not been submitted or accepted for a degree in any other university.

I, hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans, and for the title summary to be made available to outside organizations.

HIRA KHANOM

Dedicated to-

My beloved parents

ACKNOWLEDGEMENT

First of all, I am very grateful to almighty Allah who enabled me to complete this thesis work.

I would like to express my sincere gratitude and profound appreciation to Dr, Ashaduzzaman, Associate Professor, Forestry and wood Technology Discipline, Khulna University, Khulna for his continuous supervision, scientific guidance, helpfulness, inspiration and thoughtful suggestion in completing the work.

I am also thankful to my friends GM. Ahsanuzzaman, Nasrin Sultana Hena and Jamila Khanom. I also provide thanks to my all other classmate for their technical assistance and all other effort to complete my thesis work.

Finally, I provide my gratefulness to my parents for their sacrificed for education and all other happiness,

Hira Khanom

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ABSTRACT

This study was undertaken to find out the effect of chitin-chitosan nano-fibre on the physical and decay resistance properties of wood. *Neolamarckia cadamba* (Roxb.) sapwood was treated with different concentration of aquous solution of chitin-chitosan by vacuum impregnation method. The chitin-chitosan nano fibres were extracted from tiger shrimp (*Peneus monodon*) waste by applying nano- technology. Chitin-chitosan treated wood gained 1.32%, 1.14% and 1.24% when treated with 20%, 30% and 40% solution respectively. The specific gravity increments were 0.37, 0.39, 0.39 and 0.39. Without this the void volume were73.5%, 73.9% and 74.1%, water absorption were 81.4%, 84.8% and 84.5%, water repellent efficiency 7.7%, 6.0% and 6.3 %, anti-swelling efficiency were 33.4%, 30.1% and 32.9% and leachability were 29.2%, 30.6% and 34%. The decay resistance test was done by *Trametes versicolor* and the result of mass loss 0%, 20%, 30% and 40% of chitin-chitosan showed7.4%, 4.3%, 3.2% and 3.4% respectively. And the treated wood blocks showed higher resistance (lower mass loss). Based on the weight loss chitin-chitosan has antifungal properties.

Chapter 1

Introduction

1.1 Background and justification

Wood products can be protected from the attack of decay fungi, harmful insects, or marine borers by applying chemical preservatives. Preservative treatments greatly increase the life of wood structures, thus reducing replacement costs and allowing more efficient use of forest resources (Hill, 2006). The degree of protection achieved depends on the preservative used and the proper penetration and retention of the chemicals. Some preservatives are more effective than others, and some are more adaptable to certain use requirements. To obtain long-term effectiveness, adequate penetration and retention are needed for each wood species, preservative, and treatment method (Lebow, 2010).

Wood preservatives must meet two broad criteria: (1) They must provide the desired wood protection in the intended end use, and (2) they must do so without presenting unreasonable risks to people or the environment. Because wood preservatives are considered to be a type of pesticide. And all types of treated wood evaluated release small amounts of preservative components into the environment (Lebow, 2010). So it is very important do make bio preservatives or preservatives which are not harm to the environment.

Nanotechnology is defined as the development and application of materials, devices and systems using particles in the size range of into 100 nanometers with fundamentally new properties and functions because of their structure. Chitin is a white, hard inelastic, nitrogenous polysaccharide found in the exoskeleton as well as in the internal structure of invertebrates. The waste of these natural polymers is a major source surface pollution in coastal areas (Dutta *et al.*, 2004).Chitin content in fish industrial waste is 8-33% which is thrown if not used. . The production of chitin chitosan may recover this problem. Yield of dry chitin from prawn shells was 16.7% (Ifuku *et al.*, 2013) 1300 million tons of chitin produced per year in marine ecosystems Traditional

isolation of chitin from crustacean shell waste consists of three basic steps: demineralization (calcium carbonate and calcium phosphate separation), deproteinization (protein separation), and decolorization (removal of pigments) (Nessaa *et al.*, 2010). Chitin and chitosan, together with their antifungal and bactericidal character, used as in medical treatment and wastewater treatment (Jayakumara *et al.*, 2011; Maram *et al.*, 2013; Ifuku, 2014).

Anthocephalus chinensis (Lamk.) also known as kadam, is a tropical tree species that is native to South Asia. Because of its very fast growth, its ability to grow on a variety of soils, its favorable silvicultural characteristics and the absence of serious pests and diseases. This species is also expected to become increasingly important for wood industries, particularly when supplies for plywood from natural forests decrease. However, the wood is rated as non-durable, graveyard tests in Indonesia show an average life in contact with the ground of less than 1.5 years. The timber air dries rapidly with little or no degrade. Kadam wood is very easy to preserve using either open tank or pressure-vacuum systems (Krisnawati *et al.*, 2011).

During the nineteenth century the demand for durable construction particularly for rail road, trucks and bridges were increases. So, necessary for the industrial revolution, the scarcity of naturally durable timbers and inability to control regulate the immediate environment led to the development of timber preservation industry (Freeman et al., 2003). Spurred on by initial success it was surmised that provided the timber, the preservative and the treatment process were all appropriate, it should be possible to ensure that treated timber retains its integrity to as long as is desired (Hill, 2006). Though for protection we use preservatives (chemical preservatives) these has some draw backs-

- Creates health hazard to the human and animal.
- High toxicity and metal contamination.
- Disposed of the end of the product release toxic material into environment and make environmental hazard.
- During combustion or where loose wood dust particles or other fine toxic residues are generated or where treated wood comes into direct contact with food and agriculture.

For the above reason most of the countries banned or restricted toxic metal containing preservatives. Today, increasing emphasis is placed on using preservatives that are targeted more specifically to particular application. Such preservatives are safer to use and potentially less damaging to the environment. The chitin and chitosan are completely bioorganic material and it has some amount of antimicrobial properties. Antimicrobial properties of chitosan is more than chitin, which doesn't causes harm to the environment.

1.2 Objectives of the study

The objective of the research are-

- To impregnate the chitin-chitosan nanoparticles into wood to improve the properties of wood.
- Evaluate the physical and decay resistance properties of chitin-chitosan treated wood.

Chapter 2

Literature Review

2.1. Concept of nanoparticles

A nanofibre/ nanoparticle is generally defined as a fiber of less than 100 nm diameter and an aspect ratio of more than 100 (Xia *et al.*, 2003). Properties of NF are quite different from those of microfibers, because nanofibres have a characteristic morphology, an extremely high surface-to-volume ratio, and unique optical and mechanical properties thus, it is important to develop a novel method of preparing nanofibres. The electro-spinning process is well known for producing artificial nanofibres from a wide range of polymers.

2.1.1. Chitin nanoparticle

Chitin nanoparticle is a natural, renewable and biodegradable polymer, the second most abundant natural polymer after cellulose Chitin is non-toxic, odorless, biocompatible with living tissues, biodegradable (Dutta *et al.*, 2004; Mincea *et al.*, 2012). Nanoparticle may get from crab shell (100nm length and 6.1nm), mushroom, prawn shell, squid pen e-chitin, and commercial chitin: powder, the width of nanoparticles are 10-20nm. It is prepared by the deacetylation of chitin. Since large quantities of crab and shrimp shells are produced annually as food waste, further utilization of chitins as functionalized materials is desired (Ifuku, 2014). The chitin nanowhiskers are currently obtained as aqueous suspensions which are being studied and used as reinforcing additives for high performance environment-friendly biodegradable nanocomposite materials, as biomedical composites for drug/gene delivery.

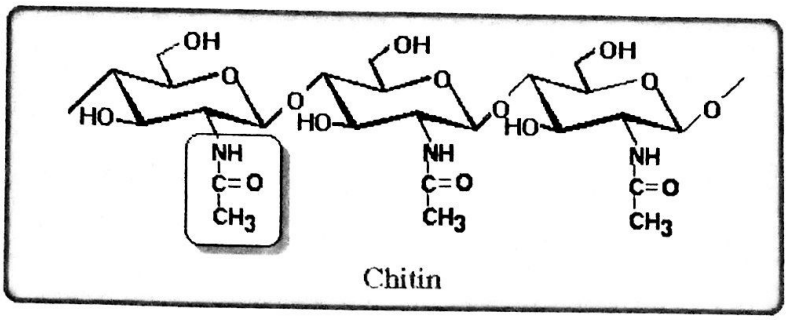

Figure 1: Chemical structure of chitin. (Bordenave, 2009)

Nano scaffolds in tissue engineering and cosmetic orthodontics. The possible applications of the chitin nanowhiskers with synthetic polymers, such as (polyvinyl alcohol) (PVA) and polycaprolactone (PCL) to give corresponding composite materials depend on the procedures that assure the compatibility between chitin and the polymeric matrix (Mincea *et al.*, 2012). The main sources of raw material for the production of chitin are cuticles of various crustaceans, principally crabs and shrimps. In crustaceans or more specifically shellfish, chitin is found as a constituent of a complex network with proteins onto which calcium carbonate deposits to form the rigid shell.

2.1.2. Chitosan nanoparticle

Chitosan nanoparticle is a fiber-like substance derived from chitin, a homopolymer of ß-(1→4)-linked *N*-acetyl-D-glucosamine. Chitosan is widely distributed in marine invertebrates, insects, fungi, and yeast. The shell of selected crustacean consists of 30-40% protein, 30-50% calcium carbonate and calcium phosphate, and 20-30% chitin. Above 280°C thermal degradation occurs and polymer chains rapidly break down (Nessaa *et al.*, 2010). Different types of chitosan derivatives are use into food industry because of its biocompatibility, biodegradability, safety and also another interesting biological activities. Much attention has been paid to their applications especially in biomedical, food, biotechnology and pharmaceutical fields (Rinaudo *et al.*, 2015).

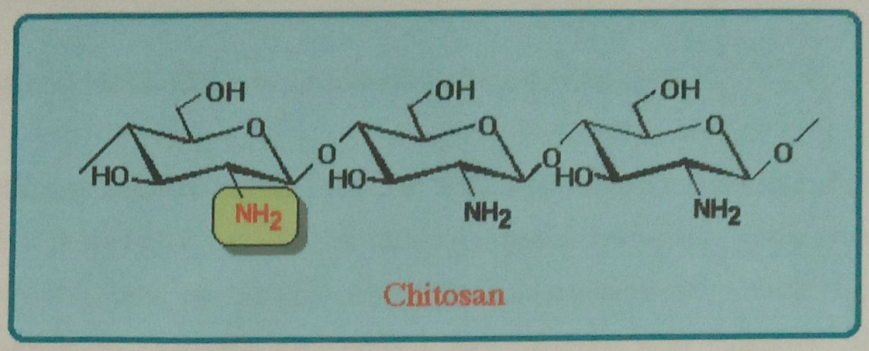

Figure 2: Chemical structure chitosan. (Bordenave, 2009)

Cellulose: **R** = OH

Chitin: **R** = NHCOCH3

Chitosan: **R** = NH-H

2.1.3. Anti-fungal properties of chitin-chitosan nanoparticle

Chitin-chitosan nanoparticles has biocompatibility, biodegradability, cellular binding, anti-bacterial and anti-fungal properties (Sashiwa *et al.*, 2004). Chitosan nanoparticle also has antimicrobial properties. Cationic nature of chitosan restraining the movement of microbiological substances and oligomeric chitosan prevents the growth of cells by preventing the transformation of DNA into RNA (Sashiwa *et al.*, 2004). Recent studies have been focused on the developmentof antibacterial surfaces to attain high functionality and high-value products. Poly (ethylene terephthalate) (PET) is a basic material in the textile and plastics industries. Accordingly, the improvement of the antibacterial properties of PET is important for a wide range of industrial applications. Huh et al. in (Sashiwa *et al.*, 2004). Prepared chitosan-grafted PET (C-PET) and quaternized chitosan-grafted PET (QC-PET). Against S. aureus, C-PET and QC-PET showed high growth inhibition in the range of 75–86% and still retained 48–58% bacterial growth inhibition after laundering Chitosan and its derivatives are useful as carriers in drug delivery systems, as antibacterial agents, and in other medical applications. (Sashiwa *et al.*, 2004).

2.1.4. Production of chitin-chitosan nanoparticle from prawn shell

Preparation method of chitin nanofibre from crab shell is applicable to a variety of prawn shells, since prawn shell is also made up of a hierarchical organized structure. Three types of prawn shells were mainly used as starting materials: *Penaeusmonodon* (black tiger prawn), *Marsupenaeus japonicas* (Japanese tiger prawn), and *Pandalus eous Makarov* (Alaskan pink shrimp). These species are widely cultivated around the world as important food sources. The shells are often thrown away as food industrial waste. Chemical methods were the first approach used in deproteinization. A wide range of chemicals have been tested as deproteinization reagents including NaOH, Na_2CO_3, $NaHCO_3$, KOH, K_2CO_3, $Ca(OH)_2$, Na_2SO_3, $NaHSO_3$, $CaHSO_3$, Na_3PO_4 and Na_2S and NaOH are the preferential reagents. The second steps is demineralization consists in the removal of minerals, primarily calcium carbonate. Demineralization is generally performed by acid treatment using HCl, HNO_3, H_2SO_4, CH_3COOH and HCOOH. Here, HCl are mostly used, HCl to convert one molecule of calcium carbonate into calcium chloride (Rinaudo *et al.*, 2015). After removing proteins and minerals, purified wet chitins extracted from the shells and disintegrated using a grinder. A uniform structure of the chitin nanofibres may be observed. The fiber structure was very similar to that of nanofibres obtained from crab shells. Since the prawn exoskeleton is made up of a finer structure than crab shell, the mechanical disintegration of prawn shell was easier than that of crab shell (Ifuku, 2014).

2.1.5. Use of chitin and chitosan nanoparticle

Chitin is widely used to immobilize enzymes and whole cells; enzyme immobilization has applications in the food industry, such as clarification of fruit juices and processing of milk when a- and b-amylases or invertase are grafted on chitin (Rinaudo *et al.*, 2015). On account of its biodegradability, nontoxicity, physiological inertness, antibacterial properties, hydrophilicity, gel-forming properties and affinity for proteins, chitin has found applications in many areas other than food such as in biosensors. Chitin-based materials are also used for the treatment of

industrial pollutants and adsorbs silver thiosulfate complexes and actinides. Chitin can be processed in the form of films and fibers. The chitin fibers, obtained by wet-spinning of chitin dissolved in a 14% NaOH solution, can also result of blending with cellulose or silk. They are nonallergic, deodorizing, antibacterial and moisture controlling. Regenerated chitin derivative fibers are used as binders in the paper making process; addition of 10% n-isobutyl chitin fiber improves the breaking strength of paper. However, the main development of chitin film and fiber is in medical and pharmaceutical applications as wound-dressing material and controlled drug release (Rinaudo, 2006). Chitin is also used as an excipient and drug carrier in film, gel or powder form for applications involving mucoadhesivity. Another interesting application is in a hydroxyapatite–chitin–chitosan composite bone-filling material, which forms a self-hardening paste for guided tissue regeneration in treatment of periodontal bony defects. Chitin was also O-acetylated to prepare gels which are still hydrolyzed by enzyme such as hen egg white lysozyme. CM-chitin was selectively modified to obtain antitumor drug conjugates. For example, 5-fluorouracil which has marked antitumor activity and the D-glucose analog of muramyl-L-alanyl-isoglutamine, responsible for immuno-adjuvant activity were grafted on CM-chitin using a specific spacer and an ester bond. Chitin oligomers have been claimed as anticancer drugs, and the oligomer with DP ¼ 5 is active in controlling the photosynthesis of maize and soybeans (Rinaudo, 2006). Chitosan has also some important uses they are given below as Water treatment: Removal of metal ions Flocculent/coagulant (proteins, dyes, amino acids) Filtration. Pulp and paper Surface treatment: Photographic paper Carbonless copy paper. Medical Bandages, sponges, Artificial blood vessels Blood cholesterol control, Tumor inhibition Skin burns, artificial skin, Eye humor fluid, Contact lenses ,Controlled release of drugs. Cosmetics Make-up powder, Nail polish, Moisturizers, Biotechnology Enzyme/cell immobilization., Protein separation: Chromatography, Glucose electrode Agriculture Seed/leaf coating Hydroponic/fertilizer, Controlled agrochemicals release, Food Removal of dyes and acids, Preservative, Color stabilization, Animal feed additive, Membranes Solvent separation Permeability control (Bordenave, 2009).

2.2. Problems of conventional wood preservation

Wood preservatives are pesticides that protect wood against attack by fungi, bacteria, or insects. The active ingredients found in wood preservatives may include pentachlorophenol (penta or

PCP), creosote, copper, zinc, chromium, arsenic, and other compounds. Preservatives may be injected into the wood before purchase (pressure-treated wood) or applied by the user. If wood-preservative chemicals are incorporated into a paint or stain, that product is considered a pesticide Wood products used in exterior applications must be protected against bio deterioration by decay fungi, insects such as termites, and other organisms. Traditionally, wood products for residential or industrial applications have been protected by treatment with chromated copper arsenate (CCA) or older inexpensive organic biocides, but environmental and disposal concerns and governmental regulations have resulted in a rapid and dramatic worldwide shift to copper-based systems. The current development trend is towards employing totally organic biocides based on relatively benign and expensive agrochemicals, with continuing research directed towards developing non-biocidal methods to protect wood (Schultz, *et al.*, 2007)

2.3. Impregnation treatment

Impregnation modification means to impregnate the cell wall of wood with a chemical, or combined of chemicals, that then react so as to form a material that is 'locked' in to the cell wall. For this to occur, it is necessary that during the impregnation phase that the cell wall is in swollen state, so as to ensure accessibility to the impregnation. It is self-evident that the molecular components of the impregnant should be small enough that they can gain access to the cell wall interior. And it is essential that the fixed impregnant is nontoxic whilst in the cell wall under any circumstances in which it is released from the cell wall, such as disposal by incineration or composting , or due to any recycling process(Hill, 2006).

2.4. General descriptions of : *Anthocephalus chinensis (Lamk.)*

Botanical name: : *Anthocephalus chinensis (Lamk.)*

Family: Rubiaceae

Subfamily: Cinchonoideae

Synonyms: *Neolamarckia cadamba* (Roxb.) A. Rich. Ex. Walp., *Anthocephalus macrophyllus* (Roxb.) Havil, *Nauclea cadamba* (Roxb.), *Anthocephalus chinensis* (Lamk.) Bosser, *Sarcocephalus cadamba* (Roxb.) Kurz, *Anthocephalus indicus* A. Rich., *Anthocephalus*

morindaefolius Korth.In Begali (Kadam) Burmese(mau, Yemau ,maukadon,mau-lettan-she); English (common burflower, New Guinea labula)

Figure 3: *Anthocephalus chinensis (Lamk.)* a) Tree b) Leaf c) Flower

2.4.1. Tree morphology

Anthocephalus chinensis (Lamk.) is a large tree with an umbrella-shaped crown and straight cylindrical bole. The tree: may reach a height of 45 m with trunk diameters of (100-160) cm.). The branches are characteristically arranged in tiers the tree sometimes has small buttresses buttress up to 2 m high and a broad crown. The branches spread horizontally and drop at the tip .The bark is gray, smooth in young trees, rough and longitudinally fissuredin old trees. But rough and longitudinally fissured in old trees. *Anthocephalus chinensis* is closely allied to the subtribe Naucleinae (Rubiaceae) but differs from them in its placentation mode (Orwa *et al*., 2009). The fruit occurs in small, fleshy capsules packed closely together to form a fleshy yellow-orange infructescence containing approximately 8000 seeds. The seeds somewhat are trigonal or irregular shaped, not winged (Krisnawati *et al*., 2011).

2.4.2. Leaf morphology

Leaves glossy green, opposite, simple more or less sessile to petiolate, ovate to elliptical (15-50 x 8-25 cm). In young fertilised trees, the leaves are much larger, subordinate at base and acuminate at apex; the stipules are interpetiolar, narrowly triangular and deciduous. . The fruitlets are

numerous, somewhat fleshy, with their upper parts containing 4 hollow or solid structures (Orwa *et al*., 2009; Krisnawati *et al*., 2011).

2.4.3. Flower morphology

Inflorescence in clusters; terminal globose heads without bracteoles, subsessile fragrant, orange or yellow flowers; Flowers bisexual, 5-merous, calyx tube funnel-shaped, corolla gamopetalous saucer-shaped with a narrow tube, the narrow lobes imbricate in bud. Stamens 5, inserted on the corolla tube, filaments short, anthers basifixed. Ovary inferior, bilocular, sometimes 4-locular in the upper part, style exserted and a spindle-shaped stigma (Orwa *et al*., 2009).

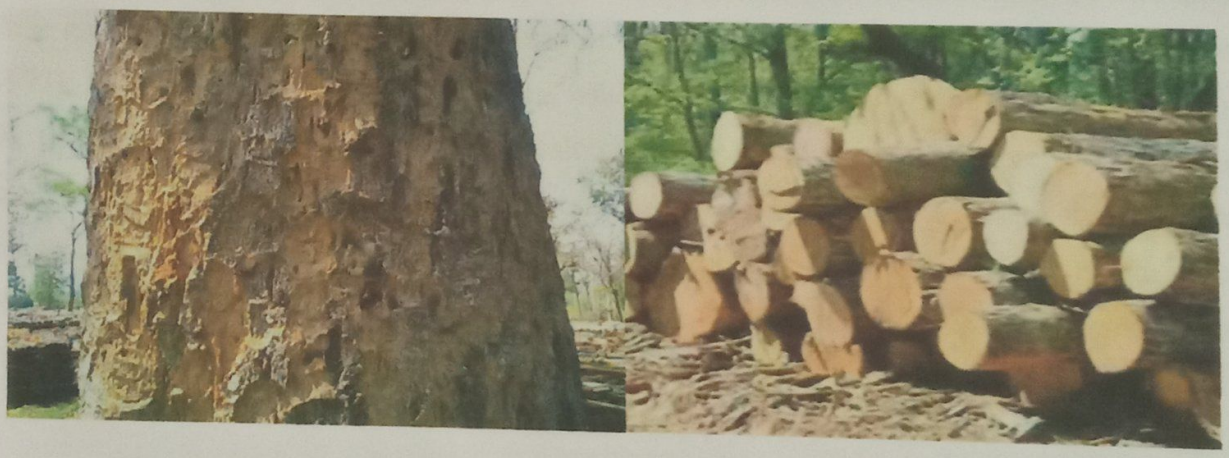

Figure 4: Stands and timber of *Anthocephalus chinensis (Lamk.)*

2.4.4. Timber value

Sapwood white with a light yellow tinge becoming creamy yellow on exposure; not clearly differentiated from the heartwood. The wood has a density of 290-560 kg/cu m at 15% moisture content, a fine to medium texture; straight grain; low luster and has no characteristic odor or taste. It is easy to work with hand and machine tools, cuts cleanly, gives a very good surface and is easy to nail. The timber is used for plywood, light construction, pulp and paper, boxes and crates, dug-out canoes, and furniture components. Kadam yields a pulp of satisfactory brightness and performance as a hand sheet. The wood can be easily impregnated with synthetic resins to

increase its density and compressive strength. Kadam is becoming one of the most frequently planted trees in the tropics (Orwa *et al*., 2009). The wood is suitable for multiple end uses, such as plywood, light construction materials, flooring, beams and rafters, boxes and crates, tea-chests, packing cases, shuttering, ceiling boards, toys, wooden shoes, bobbins, yokes, carvings, matches, chopsticks and pencils. It is also suitable for dug-outs or canoes and inexpensive furniture if properly seasoned. The pulp is sometimes mixed with other, generally long-fibred material to produce medium quality paper

2.5. Fungus selection

When testing the efficiency of a fungus, it is necessary to select sustainable strains of test fungi. Fungi are chosen on the basis of their economics importance, their resistance to disinfectants, their growth and decay rates and their ease of cultivation in the laboratory (Eaton and Hale, 1993). A large number of the white rot fungi which have been isolated are important members of the micro flora involved in natural decomposition and beneficial decay on the forest floor. On the other hand many cause decay problems of wood in storage and in service. Relatively few white rot fungi are regarded as important decay fungi of commercial timber timbers when compared to the large number of brown rot fungi (Duncan *et al*., 1965), which cause a greater degree of damage in a given period of time (Henningsson, 1967).

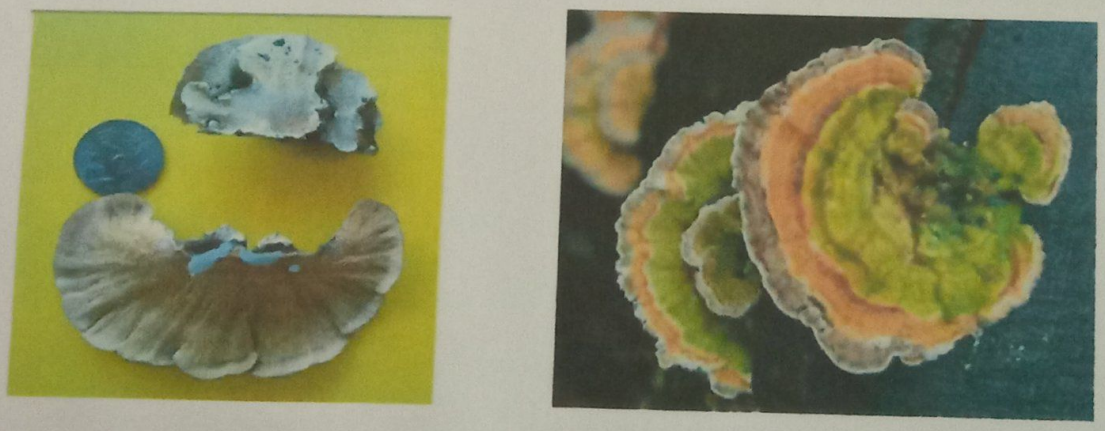

Figure 5: *Trametes versicolor*

2.5.1. General description of *Trametes versicolor*

Wood decaying fungi possess considerable ability to decompose lignified cells of coarse woody materials using enzymatic and non-enzymatic reactions. These fungi also cause serious damage to roots and standing tree trunks and finally destroying the mechanical strength of wood. *T. versicolor,* a colorful cap shaped white-rot basidiomycete, found throughout temperate and subtropical zones of all continents to infect and infest a variety of species in nearly all hardwood tree genera and many conifer species. Caps are sessile normally 1.6 - 6.8 cm wide and 1-3mm in thickness, grows singly, sometimes overlapping either in a row or a rosette, with multicolored concentric zoning on the upper surface (Gautam, 2013).

Hyphae are trimitic: generative hyphae are thin-walled with clamps, 1.5-2.5 um in diameter; skeletal hyphae thick-walled, nonseptate, 3.0 - 4.0 um in diameter; binding hyphae are also thick-walled, nonseptate, heavily branched, 1.5 - 2.0 um in diameter. Although, the major use of these fungi in pulp and paper industry is based on this approach but, they also cause serious damage to roots and standing tree trunks through posing urban environmental stresses i.e. general weakening of the tree defense system to frequent injuries on branches and roots, allowing the wood-rotting agents to gain entry through wounds and making serious loss of wood mechanical strength finally (Gautam, 2013)

Chapter 3

Materials and Method

3.1.1 Preparation of wood sample

The wood sticks of : *Anthocephalus chinensis (Lamk.)* (kadam,) sapwood of 20cm ×20cm cross section and 100cm were collected from Khulna region. The wood samples straight grained and free from knots, were converted to dimension of 20 mm x 20 mm x 5 mm (radial x tangential x longitudinal) (Ashaduzzaman *et al.*, 2013). After sanding to a remove adhering fibres, the sample, the green weight and green dimension were measured. Then the samples were put into an oven at 105°C for 24 hour. The sample were re-weighted and re-measured the dimension. The moisture content of the samples were determined according to standard procedure. The moisture content of : *Anthocephalus chinensis (Lamk.)* was measured 15-16%.

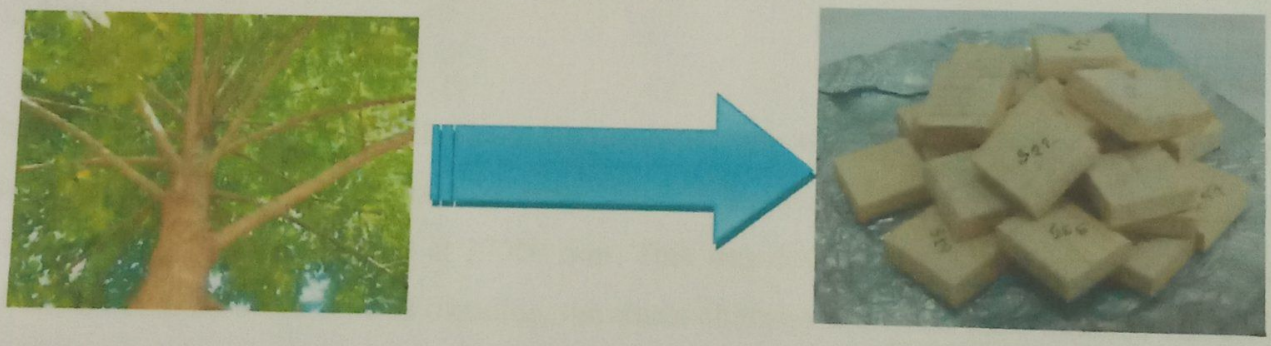

Figure 6: *Neolamarckia cadamba* (Roxb.) tree and wood sample

3.1.2. Preparation of chitin-chitosan nanoparticle

In this study, prawn shell wastes were used to produce chitin-chitosan nanofibre. The tiger shrimp (*Peneus monodon*) waste were collected from a commercial shrimp shell processor of Khulna region. Shells were then further sun dried for a period of 24 hours or longer until

completely dried. Dried ground shells were placed in opaque plastic bags and stored at ambient temperature until used. To obtain a uniform size product, the dried shell was grounded into course particles through a blender. The particles were 3 to 5 mm in size. The grounded prawn shells (100g) were demineralized with 10% hydrochloric acid (HCl) at ambient temperature with a solid to solvent ratio of 1:10 (w/v) in an acid resistant vessel and stirred for 22 hours. Then washed it about 4-6 hours. This Process was done two times sequentially for better removal of minerals.

The demineralized shells were deproteinized with 6% sodium hydroxide (NaOH) solution for 24 hrs at 60° C at a solid to solvent ratio of 1:15 (w/v). Samples were then washed with tap water and decolorized with 200gm acetone (Ethanol). The resultant product was chitin (Nessaa *et al.* 2010; Shinsuke Ifuku, 2014). To remove some of acetyl groups from chitin and to get a mixture of chitin and chitosan 40% sodium hydroxide was added at 60°C for 8 hours. The cause of preparing the mixture of chitin and chitosan nanoparticle were chitin alone have small amount of antifungal properties which was the main aims of the research (to test the antifungal properties) it is also soluble in water (Rinaudo M. , 2006) but the chitosan have strong antifungal properties but are not soluble in water it is highly soluble in acetic acid (Rinaudo *et al.*, 2015) (Sashiw *et al.*, 2004).

These chitin-chitosan were not fibrillated and unable to mix with water until blending. So after a washing of 6 hour the nano material were blended by using high speed blender (Vita-Mix Blender, Osaka Chem. Co. Ltd.) at 37000 rpm. This was done around 10-12 minute to prepare 1% chitin solution. In the time of blending, the chitin-chitosan and water ratio must be 1:99(w/v) it ensure the proper fibrillation and reduce the fiber breakage and also ensure the proper distribution of chitin-chitosan particle in to the solution .

3.1.3. Preparation procedure of chitin-chitosan nanoparticle from prawn Shell

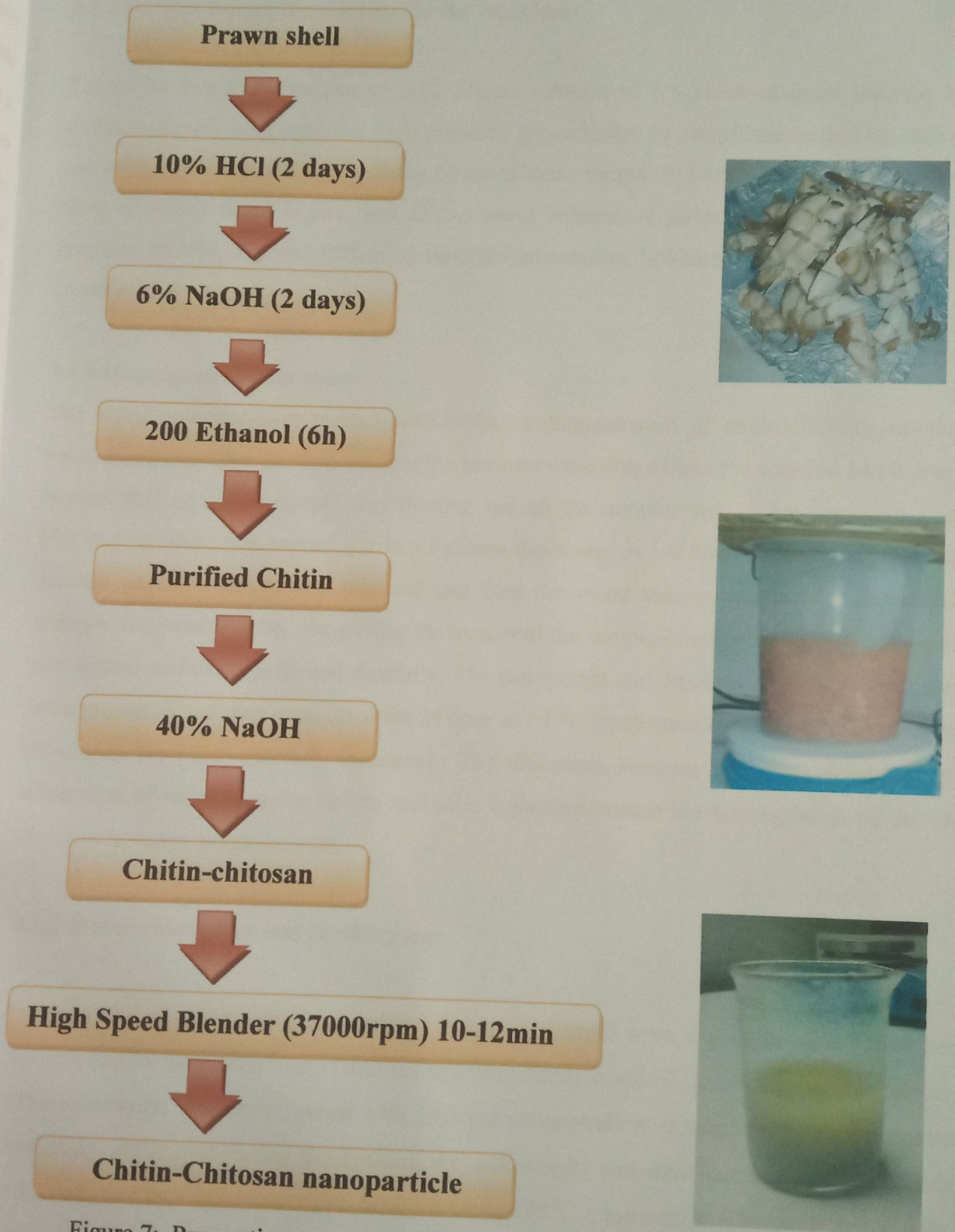

Figure 7: Preparation procedure of chitin-chitosan nanoparticle from prawn shell.

3.1.3 Preparation of the solution for the treatment

The wood samples were treated with acquas solution of 1% chitin-chitosan solution 200gm solution of each concentration were prepared sequentially. 10 sample are treated by each of the treatment and the sum of the volume of all of these sample is 34-36 cm^3 and the solution was more or less 5 times higher than of the wood volume. A series of treatment solution were prepared as 20%, 305 and 40% of chitin-chitosan solution. Solution with 05 chitin-chitosan was considered as control.

3.1.4 Impregnation treatment

The wood samples were treated with different concentration of chitin-chitosan solution by vacuum impregnation method. At first the samples were dipped into the solution and it was been ensured that no single sample was floating and all the samples were submerged into solution. The impregnation was carried out in a vacuum desiccator at (–0.9MPa) vacuum for 30min. the vacuum pressure was slowly released and then the wood samples were kept under ambient pressure for 90min. After completing the treatment the samples were removed from the solution and soaked surface slightly and carefully. The wet weight and dimension of the treated samples were measured and then oven dried for 24 hour at 105^0C temperature. The oven dried weight and dimension were also carefully measured. The difference between the ovens dried weight and dimension of wood samples before and after treatment ensure the impregnation of the chitin-chitosan in to the wood samples.

3.1.5 Water absorption and leaching test

For the water absorption and leaching test the wood samples were soaked in water for 24 hour at ambient temperature. The treated and control (untreated) wood of samples were submerged into water separately. The treated wood with different concentration of solution were also submerged separately. After completing the soaking the wet weight and dimension of the wood samples were recorded and dried into oven for 24 hour at 105^0C temperature. The oven dry weight and dimensions were also recorded. Each of test was done by using 5 replicates.

3.1.6 Decay resistance test

3.1.6.1. Sterilization of laboratory glass wares

The jars, beakers and fluxes etc. were sterilized by autoclaving for 20 minutes at 15 p.s.i. at 121^0c temperatures. Other laboratory equipment's such as knives, forceps, scalpels etc. were also sterilized by dipping in rectified spirit and then flaming over spirit lamp.

The chamber (Laminar Airflow) in which the sterilized inocula were placed and other transferring works were done was also sterilized by using rectified spirit. The hands were wiped with cotton soaked in rectified spirit up to the elbow.

3.1.6.2. Preparation of culture media

The selection of satisfactory media for stimulating growth and sporulation of particular fungus is important. In this study 2% Malt Agar (MA media was used). For the preparation of 2% MA media 20gm of malt, 20gm agar powder and 1 litter water were used.

At first the malt was added into the water and boiled until dissolved. Then agar powder was added into the suspension and boiled with constant stirring using glass rod till dissolved. The medium was then autoclaved for 20 minutes at 15 p.s.i at 12^0C temperatures in an autoclave.

3.1.6.3. Planting inocula

After cutting the inocula from the pure culture, the inocula were placed on the sterilized media that was previously poured and solidified into the sterilized laminar airflow. The inocula were put on to the media by sterilized forceps about one cm from edge of the jars. About 4 to 5 inocula were inoculated into each jar. The jars were then wrapped with brown paper and incubated in an incubator at temperature 25 ± 2^0c, which is appropriate for fungal growth.

3.1.6.4. Observation of inocula

After two days the inoculated petridishes were observed for fungal growth. The inocula yielding fungal growth were carefully observed and then further followed at two days interval up to ten days, which is the optimum range for maximum fungal growth after inoculation.

3.1.6.5. Agar block test

Treated and untreated wood sample of *Anthocephalus chinensis* were place into the petri dish. In each dish 2 sample were placed. The petridishes were then transformed to incubator (25±5^{0}C, 65±5 RH) for 8 weeks. After the exposure to fungus, the wood samples were removed from petridishes and wood samples were wilted to remove fungal mat. The oven dry weight before and after decay test was used to determine the mass loss because of decaying.

3.2. Calculation

3.2.1. Determination of moisture content percentage

Moisture content of the wood has been determined by the formula given below:

$$\text{Moisture Content \%} = \frac{\text{Green weight} - \text{Oven dry weight}}{\text{Oven dry weight}} \times 100$$ (Amusant *et al.*, 2005)

3.2.2 Determination of weight percent gain (WPG)

Weight percent gain (WPG) of the chitin-chitosan treated wood samples was determined by using the following formula:

$$(WPG)\% = [(W_2 - W_1)/W_1] \times 100$$ (Pandey *et al.*, 2009)

Where, W_2 is the oven dry weight of chitin-chitosan treated and W_1 is the oven dry weight before the chitin treatment of wood sample.

3.2.3. Determination of specific gravity %

Specific Gravity (SG_{OD}) before and after chitin-chitosan` treated wood samples were determined by using following formula:

$SG_{OD} = [(W_2/V_2)/1\ gcm^{-3}]$

Where,

W_2 = Oven dry weight of (chitin-chitosan treated or untreated) wood sample.

V_2 = Corresponding Oven dry volume of (chitin-chitosan treated or untreated) wood sample.

3.2.4. Determination of void volume %

Void volume before and after chitin-chitosan treatment was determined by using following formula:

Void volume (%) = $[1-(SG_{OD}/1.50)] \times 100$ (Stamm, A. J., 1938)

Where,

SG_{OD} = Oven dry specific gravity of (chitin-chitosan treated or untreated) wood sample and 1.50 is the constant for the specific gravity of the wood cell wall material.

3.2.5. Determination of leachability %

The leach ability of the impregnated chitin was determined from the following formula:

Leachability (%) = $[\{(W_2-W_3)-CL\}/(W_2-W_1)] \times 100$

Where,

W_1 = Oven dry weight of untreated wood sample.

W_2 = Oven dry weight of chitin-chitosan treated wood before the leaching test.

W_3 = Oven dry weight of chitin-chitosan treated wood after leaching test.

CL = Control losses for the individual species based on the average of the control leached block.

3.2.6. Determination of dimensional stabilization %

Dimensional stabilization was determined by the following way. The volumetric swelling co-efficient of the chitin-chitosan treated wood was determined from the following formula:

$S_{TOD} = [(V_{WST} - V_{TOD})/ V_{TOD}] \times 100$ (Pandey *et al.*, 2009)

Where,

V_{WST} = Water saturated volume chitin-chitosan treated wood sample.

V_{TOD} = Oven dry volume of chitin-chitosan treated wood

.

And the volumetric swelling co-efficient of the untreated wood as determined from the following formula:

S_{UtOD} (%) = $[(V_{WSUt}-V_{ODUt})/ V_{ODUt}] \times 100$

Where,

V_{WSUt} = Water saturated volume of untreated wood sample.

V_{ODUt} = Oven dry volume of untreated wood sample.

The anti-swelling efficiency of the chitin-chitosan treated wood sample was determined from the following formula:

ASE (%) = $[(S_{UtOD}-S_{TOD})/ S_{UtOD}] \times 100$ (Pandey *et al.*, 2009)

Where,

S_{UtOD} = Volumetric swelling co-efficient of untreated wood sample.

S_{TOD} = Volumetric swelling co-efficient of chitin-chitosan treated wood sample

3.2.7. Determination of water absorption and water repellent efficiency %

The water absorption (WA) of the chitin-chitosan treated and untreated wood were determined by using the following formula:

$WA\ (\%) = [(W_{WS}-W_{OD})/W_{OD}] \times 100$ (Rowell and Banks, 1985)

Where,

W_{WS} = Water saturated weight of the (treated or untreated) wood samples.

W_{OD} = Oven dry weight of (treated or untreated) wood samples.

The water repellent efficiency (WRE) of chitin-chitosan treated wood sample was determined by the following formula:

$WRE\ (\%) = [(WA_{Ut}-WA_T)/WA_T] \times 100$

Where,

WA_{Ut} = Water absorption of the untreated (control) wood samples.

WA_T = Water absorption of the chitin-chitosan treated wood samples.

3.2.8. Determination of mass loss %

The Weight loss of wood due to fungal attack was determined using following formula

Mass loss (%) in wood = $\{(M_0-M)/M_0\} \times 100$ (Amusant *et al.*, 2005; Pandey *et al.*, 2009; Ali, T. *et al.*, 2014)

Where,

Mo = Oven dry weight of wood before decay test.

M = Oven dry weight of wood after decay

3.2.9. Analysis of the data

The data were analyzed by SPSS 16.0 software. ANOVA (Analysis of Variance) and LSD were done for analyzing the data. The other calculation were done by using Microsoft Excel-13.

Chapter 4

Result and Discussion

4.1. Weight percent gain (WPG)

The WPG of : *Anthocephalus chinensis (Lamk.)* wood blocks treated with chitin-chitosan represent in the figure 8. The weight percent gain of treated wood blocks with 20%, 30% and 40%, chitin-chitosan represent 1.32%, 1.14% and 1.24% respectively.

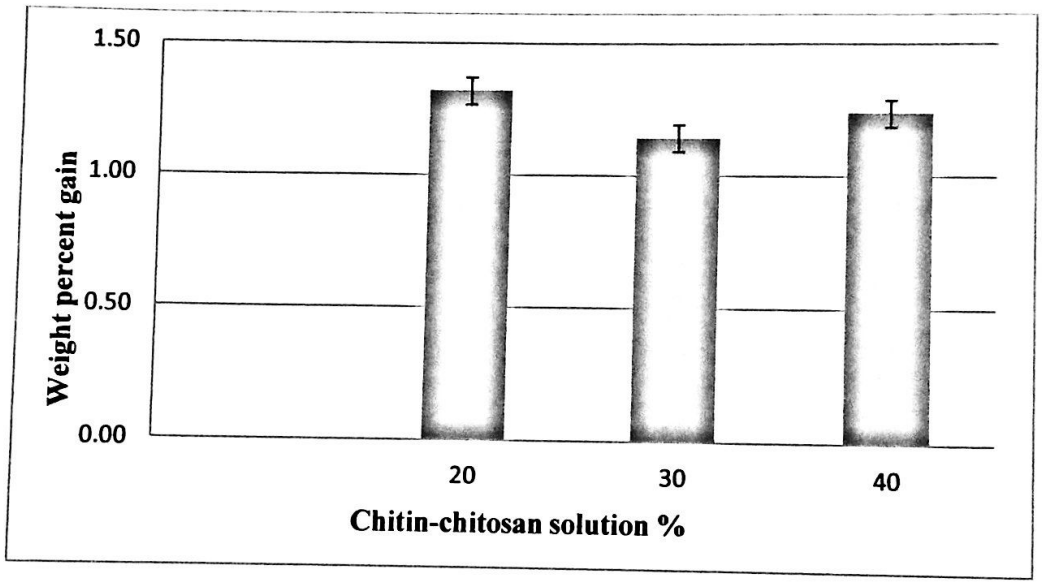

Figure 8: Mean weight percent gain (WPG) of chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood.

From the ANOVA (Analysis of Variance) it was found that there was significant difference ($p < 0.05$) among the different concentration of chitin-chitosan solution treated wood. The LSD (Least Significant Difference) test revealed that there was no significant difference between the other three treatments (Appendix.9).

The results of WPG of treated wood revealed that the chitin-chitosan nanofibre was penetrated into wood but the increase of concentration didn't have any effect on the treatment. It can be assumed that a very small amount of chitin-chitosan (1%) was impregnated into wood which only increase around 1.0% WPG.

4.2. Specific gravity

The result of the difference between the specific gravity of control and chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood was founded in the following figure 9. The specific gravity of control was lower (0.37) than the treated wood blocks, whereas the specific gravity of treated block was higher. The specific gravity of treated wood block with 20%, 30% and 40% chitin-chitosan showed similar specific gravity.

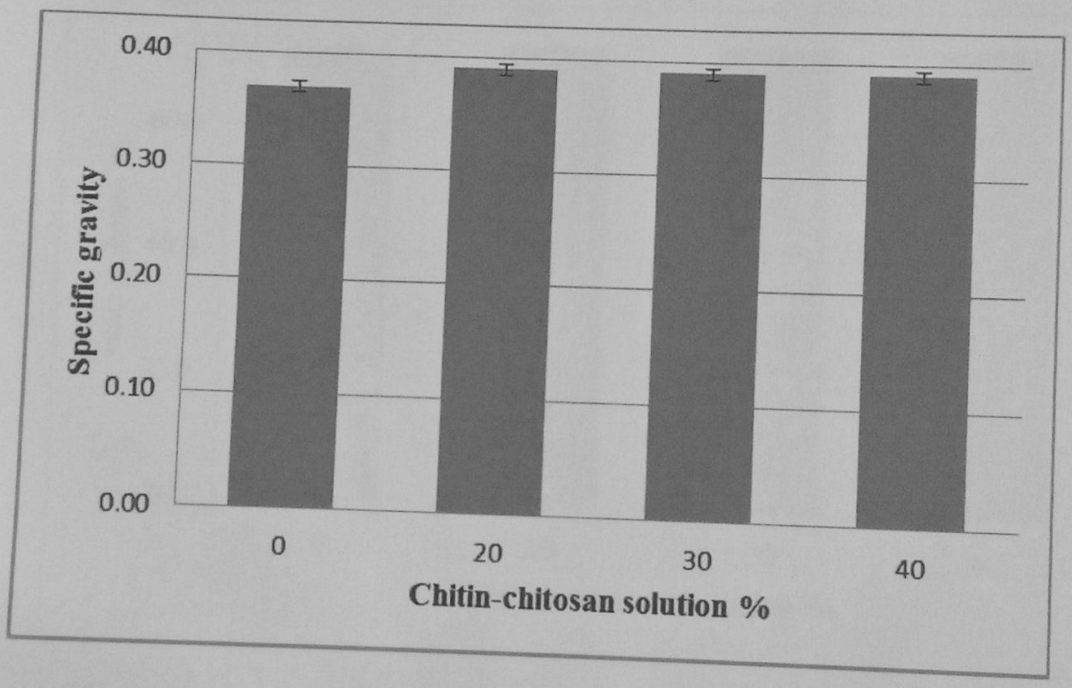

Figure 9: Mean specific gravity of chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood.

From the ANOVA (Analysis of Variance) it was found that there was no significant difference ($p > 0.05$) among the different treatment of chitin-chitosan solution treatment solution (Appendix.10).

Thus, it can be assumed that the chitin-chitosan nanofibre may not effective to increase the specific gravity of the treated wood.

4.3. Void volume

The result of void volume of treated : *Anthocephalus chinensis (Lamk.)* sapwood was increases because of chitin-chitosan treatment. Figure 10 showed the void volume of control was 72.7% and the void volume of treated wood blocks were 73.5%, 73.9% and 74.1% for chitin-chitosan treatment 20%, 30% and 40% respectively.

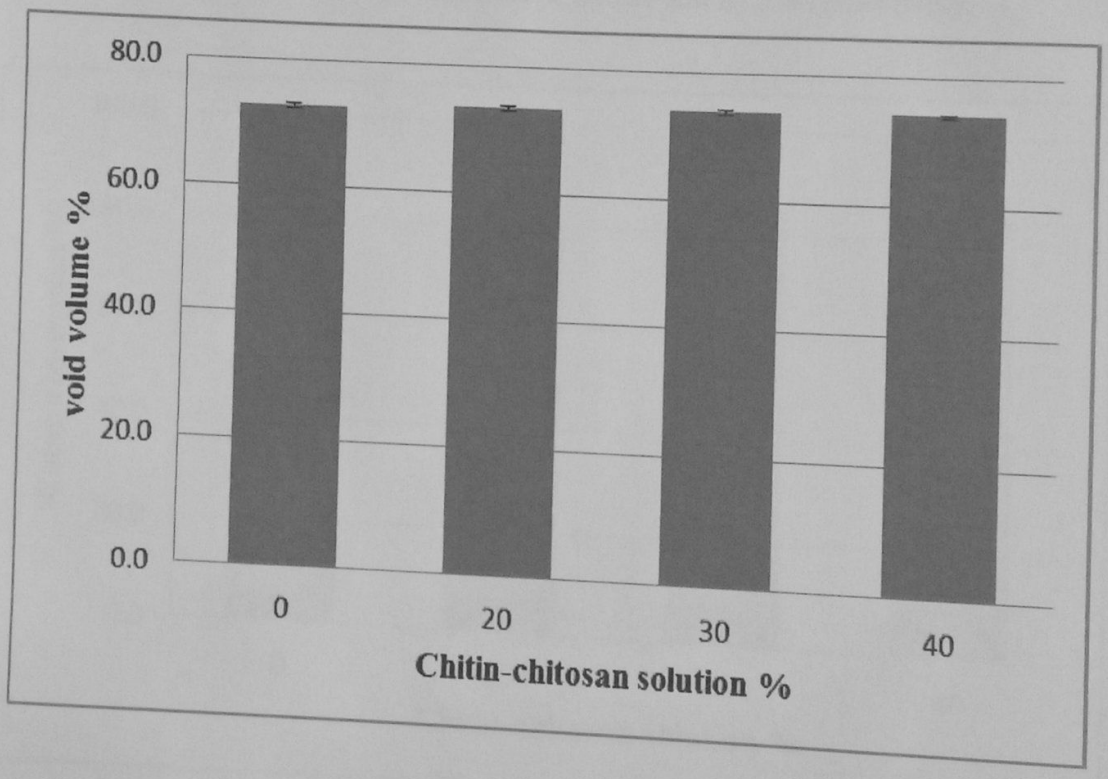

Figure 10: Mean void volume % of chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood.

From the ANOVA (Analysis of Variance) it was found that there was no significant difference ($df = 3$, $p > 0.05$) among the different chitin-chitosan solution treatment (Appendix.11).

It is also obvious that the amount of chitin-chitosan entered into wood didn't have impact on the change on the voidvolume of wood. In general, the void volume represent the macro and micro void present in wood cell lumen and cell wall. Thus small amount and light weight nano-material can't change the cell wall properties.

4.4. Water absorption %

The water absorption tendency of chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood are presented in the figure-13. From the figure 11, it has been found that the water absorption percentage of control was 90.2% and higher than the treated blocks i.e. 20%, 30% and 40% chitin-chitosan treated blocks showed 81.4%, 84.8% and 84.5% respectively.

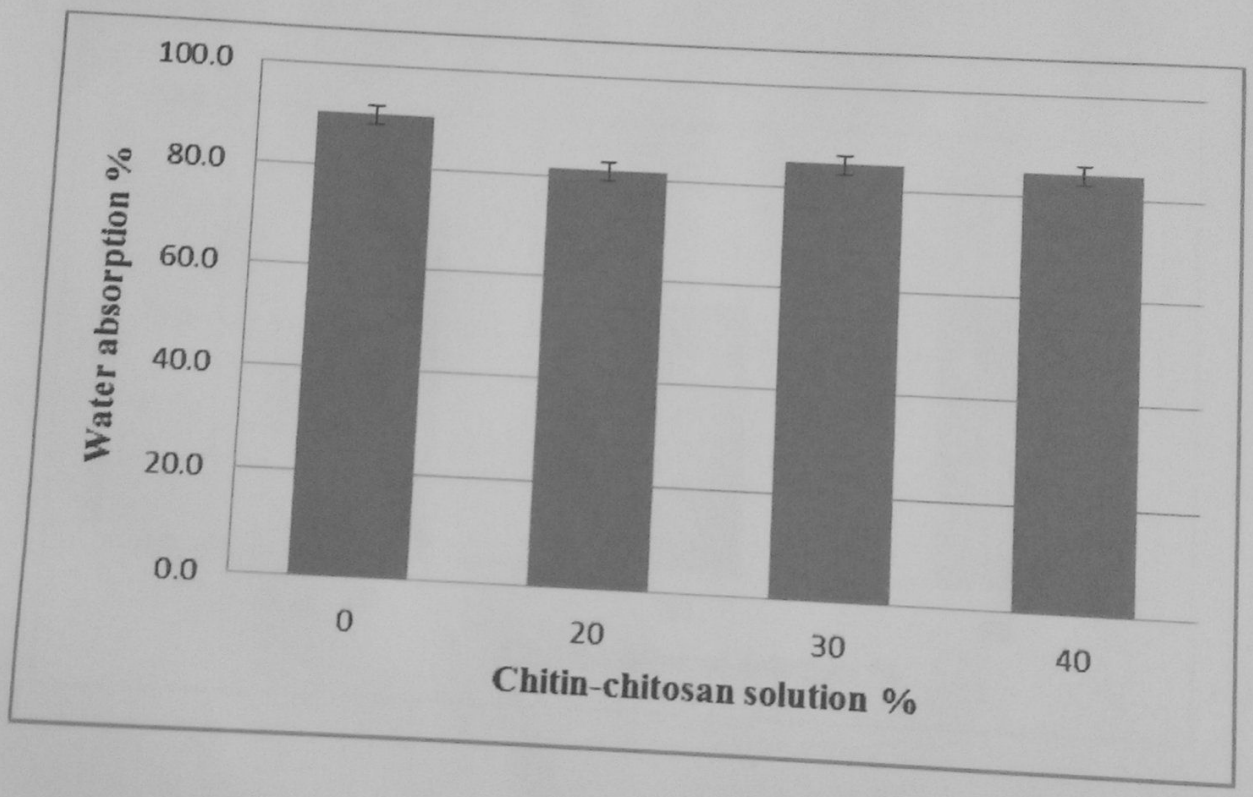

Figure 11: Mean water absorption % of chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood.

From the ANOVA (Analysis of Variance) it was found that there was significant difference ($p < 0.05$) among the chitin-chitosan solution treatment solution(Appendix.12).

Thus, from water absorption results it can be revealed that the chitin-chitosan has some influence on the water absorption properties of treated wood as it reduce the water absorption capacity of treated wood as compared with control.

4.5. Water repellent efficiency (WRE) %

The water repellent efficiency (WRE) among the chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood are presented into the figure 12. The result showed that the water repellent efficiency 9.8% of 20% chitin-chitosan treated wood is higher (7.7) than the other two treatment i.e. 30% and 40% chitin treated wood blocks were 6.0% and 6.3 % respectively.

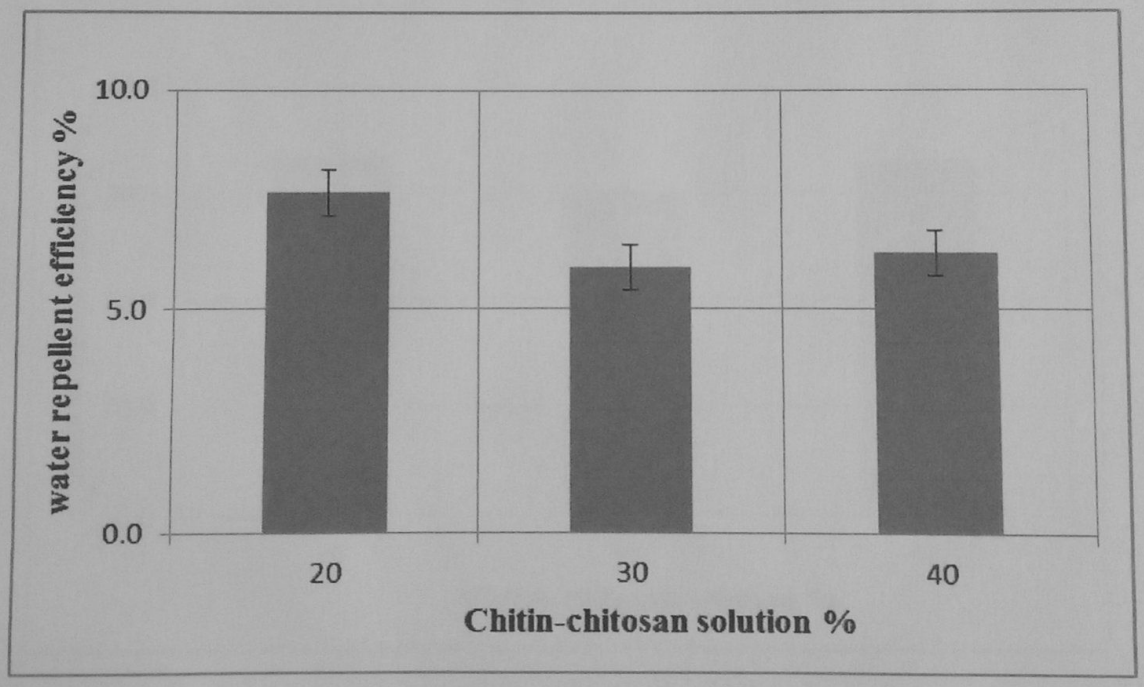

Figure 12: Mean water repellent efficiency (WRE) % of chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood.

The statistical/ ANOVA (Analysis of Variance) analysis showed ($p > 0.05$) there was no significant difference among the chitin-chitosan treatment (Appendix.13).

It is found that the water repellent efficiency of chitin chitosan treated wood was improved around 6-7 %.

4.6. Anti-swelling efficiency (ASE) %

The chitin-chitosan treated wood blocks of were submerged into water for about 24 hours to know the anti-swelling efficiency of the treated wood. The result showed into figure 13 and it was found that the anti-swelling efficiency of 20%, 30% and 40% chitin-chitosan solution treated wood blocks were 33.4%, 30.1% and 32.9% respectively.

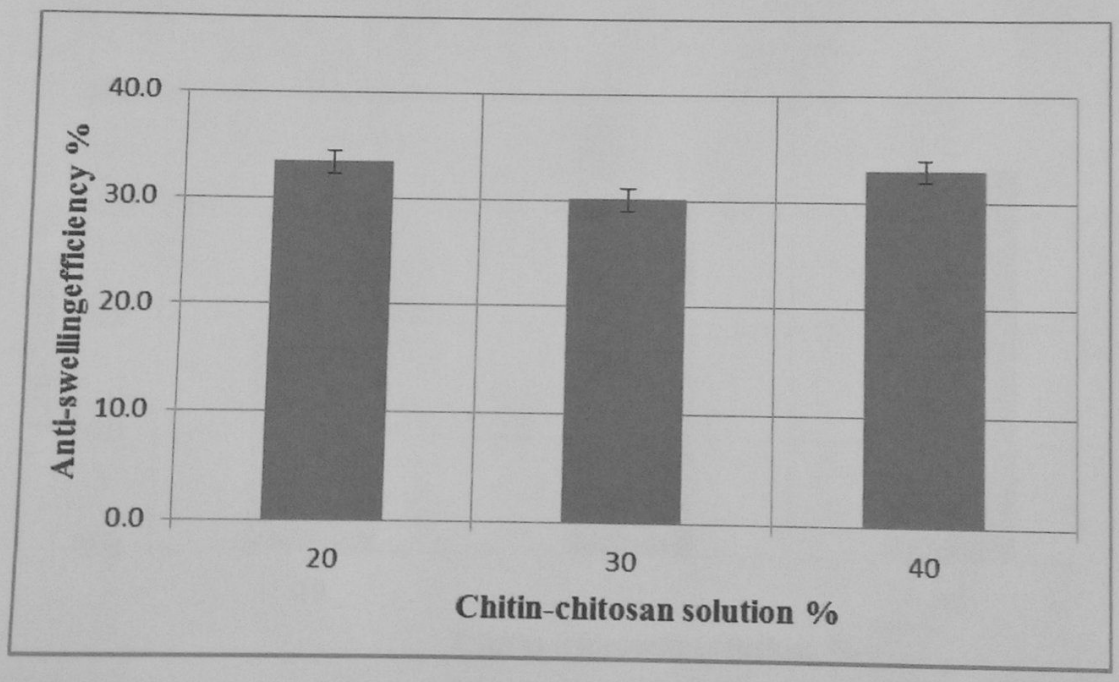

Figure 13: Mean anti-swelling efficiency (ASE) % of chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood.

The ANOVA (Analysis of Variance) showed that ($p > 0.05$) there was no significant difference among the anti-swelling efficiency of chitin-chitosan treated wood blocks (Appendix.14).

It is revealed that the anti-swelling efficiency of chitin-chitosan treated wood was improved to around 30% due to treatment which is evident for all treatment concentration but the increase of concentration did not poses any effect.

4.7. Leachability%

For the leachability test the control loss (0.0032g) was determined and the leachability of chitin-chitosan treated is presented into the figure14. From the figure it has been found that the leachability of 20%, 30% and 40% chitin-chitosan treated wood blocks were 29.2%, 30.6% and 34% and the leachability of 40% chitin-chitosan treated wood blocks was higher than other two treatments.

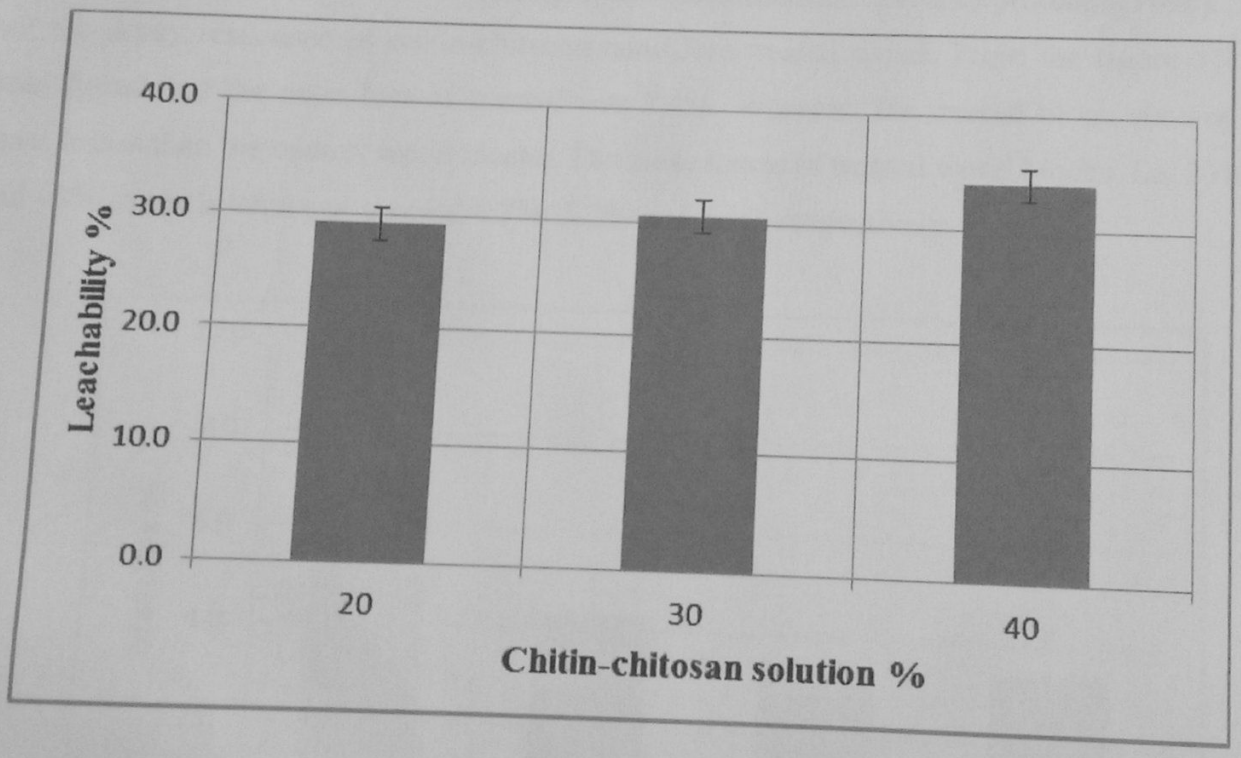

Figure 14: Mean leachability% of chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood.

The ANOVA (Analysis of Variance) analysis showed ($p > 0.05$) there was no significant difference among the leachability of chitin-chitosan treated wood blocks (Appendix.15).

For a small 24 hours leaching test it has been found that a considerable amount (around 30%) of impregnation i.e chitin-chitosan was leached out. But the leaching of control was blocks indicates the losses of soluble hemicelluloses and extractives (if any presented). Thus it can be assumed that the leaching of chitin-chitosan accompanied with the water soluble fragments of wood.

4.8. Decay resistance

The chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood were exposed to white rot fungus *Trametes versicolor* according to European Standard EN-113 protocol (1996). to find out the decay resistance of chitin-chitosan nanofibre treated wood. From the figure 15, it has been found that the mass loss of control was 7.4%. Whereas, the treated block showed lower mass losses than the control wood blocks. The mass losses of treated wood blocks, i.e. 20%, 30% and 40% of chitin-chitosan showed 4.3%, 3.2% and 3.4% respectively.

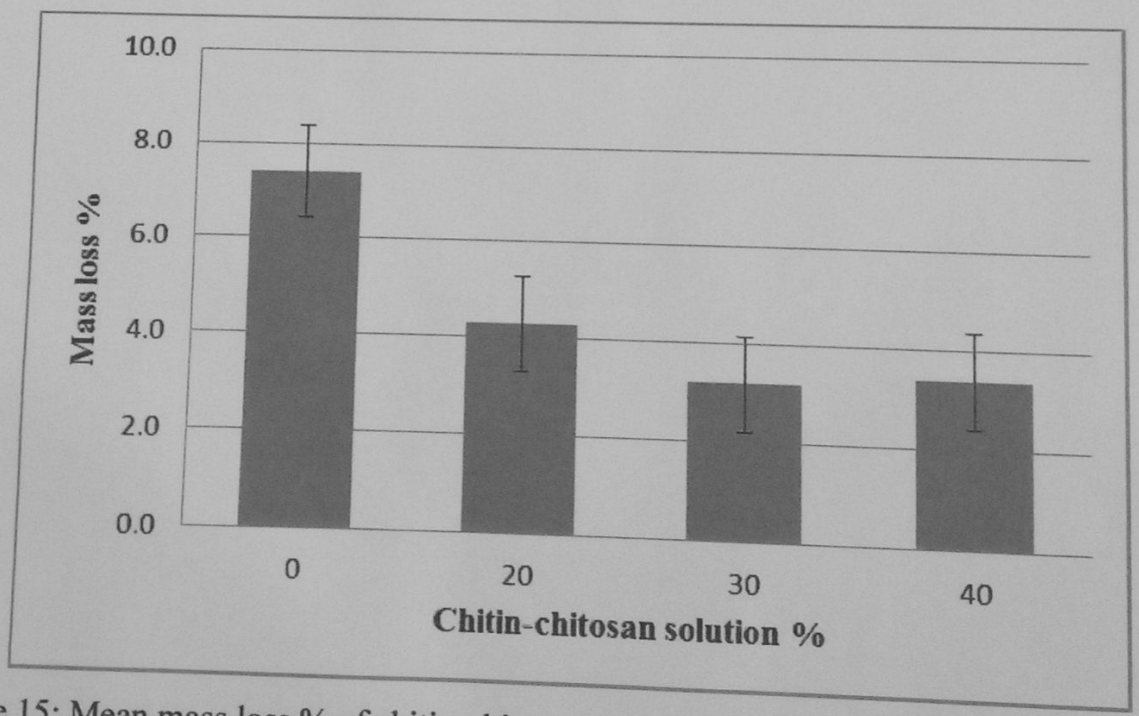

Figure 15: Mean mass loss % of chitin-chitosan treated : *Anthocephalus chinensis (Lamk.)* sapwood.

From the ANOVA (Analysis of Variance) it was found that there was significant difference ($p<0.05$) among the different chitin-chitosan solution treatment. The LSD (Least Significant Different) test revealed that the control treatment was significantly different from the other three treatment but, there was no significant difference between the solutions (Appendix.16).

It is evident that control wood blocks (untreated) were vulnerable to decay in exposure to white rot fungus. But, the decay resistance was imparted to the wood due to the treatment with chitin-chitosan nanofibre. So it can be concluded that the chitin-chitosan has antifungal properties which may effective on the improvement of decay resistance of wood.

Chapter 5

Conclusion

In this study, weight percent gain (WPG), specific gravity, void volume%, water absorption%, water repellent efficiency (WRE) %, anti-swelling efficiency (ASE), leachability and decay resistance ability was tested of the chitin-chitosan treated wood. All of the properties without specific gravity were improved.. In this study, we have not fully succeeded to fibrillate chitin-chitosan nanofibres by existing process. Further study is required to develop technich to fibrillate chitin-chitosan nanofibres and enhance its penetration to wood samples.

References

Temiz, A., Alfredsen, G., Yildiz, U. C., Gezer E. D. and Kose G. (2014). Leaching and decay resistance of alder and pine wood. *Maderas. Ciencia y tecnología*, 16(1): 63-76.

Amusant N., Moretti, C., Richard, B., Prost, E., Nuzillard, J.M., and Thevenon, M. (2005). Chemical compound from eperua falcata and Eperua grandifolora heartwood and their biological activies against wood destroying fungus (*Coriolus versicolor*). International Research group on wood preservation. Document No. IRG/WP 05-30373.

Duncan, C. G., and Lombard, F. F. (1965). Fungi associated with principal decays in wood products in the United States. *U.S. Forest Service Paper,* WO-4, Washingtone

European standard EN 113. (1996). Wood preservatives- Method of test for determining the protective effectiveness against wood destroying basidiomycetes- determination of the toxic values. European Committee for Standardization (CEN), Brussels, Belgium.

Nessaa, F., Masum S. M., Asaduzzaman M., Roy S. K., Hossain M. M. and. Jahan M. S. (2010). A Process for the Preparation of Chitin and Chitosan from Prawn Shell Waste. *Bangladesh Journal of Scientific and Industrial Research*, 45(4): 323-324.

Gautam, A. K. (2013). Notes on wood rotting fungi from India (1): Trametes versicolor - The Turkey. *Journal on New Biological Reports*, 2(2): 67-70.

Henningsson, B. (1967). Changes in the impact bending strenth, weight and alkali solubility following fungal attack on birch wood. *Studia Forestalia Suecica,* 41: 1-21.

Hill, C. A. S. (2006). *Wood Modification: Chemical, Thermal and Other Process*. John Wiley & Sons Ltd., England.

Sashiwa H., and Aiba, S. (2004). Chemically modified chitin and chitosan as biomaterials. *rogress in Polymer Science*, 29: 887-902.

Eaton, R. A. (1993). Wood: decay,pests and protection. 2-6 Boundary Row LandonSE1 8HN, UK: Chapman and Hall.

Younes I. and Rinaudo M. (2015). Chitin and chitosan preparation from marine sources. structure, properties and applications. *Marine Drugs*, 13: 1136-1156.

Pandey, K. Jayashree and. Nagaveni, H. C. (2009). Study of dimensional stability and light stability of phenylisothiocyanate modified rubber wood. *BioResources*, 4(1): 257-267.

Krisnawati, H., Kallio, M. and Kanninen, M. (2011). *Anthocephalus cadamba* Miq.Ecology, silviculture and productivity. *Center for International Forestry Research*, Bogor, Indonesia. 1-3.

Lebow, S. T. (2010). *Wood preservation.*Wood handbook: wood as an engineering material chapter 15 Madison, Wisconsin, USA.

Ashaduzzaman, M., Halel, M. D. Tverezovskiy V. and Ormondroyd, G. A. (2013). Effect of bio-resin from cashew nut shell liquid (CNSL) on decay resistance properties of wood. *The International Research Group on Wood Protection*, Document No. IRG/WP 13-40649, 4-5.

Mincea, M. Negrulescu, A. and Ostafe V. (2012). Preparation, modification, and applications of chitin nanowhiskers: A review. *Advance Study Centre Co. Ltd.* 30: 225-242.

Maram T. H., Kana, A., Radi M. and Maher, Z. E. (2013). Wastewater treatment with chitosan nano-particles. *International Journal of Nanotechnology*. 3 (2): 39-50.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Anthony, S. (2009). Agroforestry tree database: atree reference and selection guide version 4.0.(website: http://www.worldagroforestry.org/treedb2/.)

Dutta P. K., Dutta, J. and Tripathi V. S. (2004). Chitin and chitosan: chemistry, properties and application. *Journal of Scientific and Industrial Research, 63*: 20-31.

Queen, H. A. (2006). Electrospinning chitosan-based nanofibres for biomedical application. *Thesis,* 1-71

Jayakumara, R., Prabaharanb, M., Kumara, S. P. T., Naira, S.V. and Tamurac, H. (2011). Biomaterials based on chitin and chitosan in wound dressing applications. *Biotechnology Advances,* 29: 322–337.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in polymer science*, 31: 603-632.

Ifuku, S. (2014). Chitin and chitosan, nanofibers:preparation and chemical modifications. *Molecules, 19:* 67-90.

Shinsuke Ifuku, Zameer Shervani and Hiroyuki Saimoto. (2013). Chitin Nanofibers, Preparations and Applications.85-96 (Website): http://dx.doi.org/10.5772/57095

Schultz T. P., Nicholas D.D. and Preston, A.F.(2007). A brief review of the past, present and future of wood preservation. *Pest Manag Sci.* 63(8): 784-8.

Stamm, A. J. (1938). Calculations of the void volume in wood. *Industrial & Engineering Chemistry*, 30: 2-4

Rowell, R. M. and Banks, W. B. (1985). Water repellency and dimensional stability of wood. *Department of Agriculture*, 24: 4-26.

Xia, Y., Yang, P., Sun, Y., Wu, Y., Mayers, B., Gates, B., Yin, Y., Kim, F. and Yan, H. (2003). Synthesis, characterization, and applications. *One-dimensional nanostructures, 15*, 353–389.

Bordenave, D. N. (2009). *Chitin and Chitosan.* (Website: nbordena@purdue.edu).

Appendix

Appendix 1: Mean weight percent gain (WPG) of chitin-chitosan treated *Neolamarckia cadamba* (Roxb.) sapwood.

Treatment (Chitin-Chitosan Solution)	weight percent gain (WPG)
20%	1.32 (0.13)
30%	1.14 (0.08)
40%	1.24 (0.12)

Note: Value in parenthesis indicates Standard Error (n=10).

Appendix 2: Mean specific gravity of chitin-chitosan treated *Neolamarckia cadamba* (Roxb.) sapwood.

Treatment (Chitin-Chitosan Solution)	Density (gcm^{-3})
Control (0%)	0.37 (0.01)
20%	0.39 (0.01)
30%	0.39 (0.01)
40%	0.39 (0.01)

Note: Value in parenthesis indicates Standard Error (n=10).

Appendix 3: Mean void volume % of chitin-chitosan treated *Neolamarckia cadamba* (Roxb.) sapwood.

Treatment (Chitin-Chitosan Solution)	Void volume %
Control (0%)	72.7 (0.58)
20%	73.5 (0.51)
30%	73.9 (0.36)
40%	74.1 (0.41)

Note: Value in parenthesis indicates Standard Error (n=10).

Appendix 4: Mean water absorption % of chitin-chitosan treated *Neolamarckia cadamba* (Roxb.) sapwood.

Treatment (Chitin-Chitosan Solution)	Water absorption %
Control (0%)	90.2 (2.74)
20%	81.4 (0.90)
30%	84.8 (2.51)
40%	84.5 (1.26)

Note: Value in parenthesis indicates Standard Error (n=5).

Appendix 5: Mean water repellent efficiency (WRE) % of chitin-chitosan treated *Neolamarckia cadamba* (Roxb.) sapwood.

Treatment (Chitin-Chitosan Solution)	Water repellent efficiency (WRE) %
20%	9.76 (1.00)
30%	5.95 (2.78)
40%	6.28 (1.40)

Note: Value in parenthesis indicates Standard Error (n=5).

Appendix 6: Mean anti-swelling efficiency (ASE) % of chitin-chitosan treated *Neolamarckia cadamba* (Roxb.) sapwood.

Treatment (Chitin-Chitosan Solution)	Anti-swelling efficiency (ASE) %
20%	33.4 (3.90)
30%	30.1 (3.34)
40%	32.9 (6.91)

Note: Value in parenthesis indicates Standard Error (n=5).

Appendix 7: Mean Leach ability% of chitin-chitosan treated *Neolamarckia cadamba* (Roxb.) sapwood.

Treatment (Chitin-Chitosan Solution)	Leach ability%
20%	29.2 (2.79)
30%	30.6 (2.79)
40%	34.0 (2.20)

Note: Value in parenthesis indicates Standard Error (n=5).

Appendix 8: Mean mass loss % of chitin-chitosan treated *Neolamarckia cadamba* (Roxb.) sapwood.

Treatment (Chitin-Chitosan Solution)	Mass loss %
Control (0%)	7.43 (0.68)
20%	4.37 (0.63)
30%	3.21 (0.34)
40%	3.48 (0.29)

Note: Value in parenthesis indicates Standard Error (n=5).

Appendix 9: Weight percent gain (WPG)

Descriptives

VAR00002	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0% chitin-chitosan solution	9	.0000	.00000	.00000	.0000	.0000	.00	.00
20% chitin-chitosan solution	11	1.1998	.44145	.13310	.9032	1.4964	.00	1.72
30% chitin chitosan solution	10	1.1436	.24086	.07617	.9713	1.3159	.74	1.47
40% chitin chitosan solution	10	1.2383	.36692	.11603	.9758	1.5008	.40	1.70
Total	40	.9254	.59211	.09362	.7360	1.1148	.00	1.72

ANOVA

VAR00002	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.991	3	3.330	32.555	.000
Within Groups	3.683	36	.102		
Total	13.673	39			

Multiple Comparisons

Dependent Variable: VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	0% chitin-chitosan solution	20% chitin-chitosan solution	-1.19979*	.14375	.000	-1.4913	-.9082
		30% chitin chitosan solution	-1.14359*	.14695	.000	-1.4416	-.8456
		40% chitin chitosan solution	-1.23829*	.14695	.000	-1.5363	-.9403
	20% chitin-chitosan solution	0% chitin-chitosan solution	1.19979*	.14375	.000	.9082	1.4913
		30% chitin chitosan solution	.05620	.13975	.690	-.2272	.3396
		40% chitin chitosan solution	-.03850	.13975	.785	-.3219	.2449
	30% chitin chitosan solution	0% chitin-chitosan solution	1.14359*	.14695	.000	.8456	1.4416
		20% chitin-chitosan solution	-.05620	.13975	.690	-.3396	.2272
		40% chitin chitosan solution	-.09470	.14303	.512	-.3848	.1954
	40% chitin chitosan solution	0% chitin-chitosan solution	1.23829*	.14695	.000	.9403	1.5363
		20% chitin-chitosan solution	.03850	.13975	.785	-.2449	.3219
		30% chitin chitosan solution	.09470	.14303	.512	-.1954	.3848

* The mean difference is significant at the 0.05 level.

Appendix 10: Specific gravity

Descriptives

VAR00002	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0% chitin-chitosan solution	9	.3889	.01965	.00655	.3738	.4040	.36	.42
20% chitin-chitosan solution	11	3900	02098	00632	3759	.4041	.36	44
30% chitin chitosan solution	10	3950	01900	00601	.3814	.4086	.38	.43
40% chitin chitosan solution	10	.3930	.02983	.00943	.3717	.4143	.37	.46
Total	40	3918	02206	.00349	3847	.3988	.36	.46

ANOVA

VAR00002	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.146	.931
Within Groups	019	36	001		
Total	019	39			

Multiple Comparisons

Dependent Variable VAR00002							
	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confid ence Interval	
						Lower Bound	Upper Bound
LSD	0% chitin-chitosan solution	20% chitin-chitosan solution	- 00111	.01026	.914	-.0219	0197
		30% chitin chitosan solution	- 00611	01049	564	- 0274	0152
		40% chitin chitosan solution	- 00411	01049	697	-.0254	0172
	20% chitin-chitosan solution	0% chitin-chitosan solution	.00111	.01026	914	- 0197	.0219
		30% chitin chitosan solution	- 00500	.00997	.619	- 0252	0152
		40% chitin chitosan solution	- 00300	.00997	765	- 0232	0172
	30% chitin chitosan solution	0% chitin-chitosan solution	.00611	.01049	564	-.0152	.0274
		20% chitin-chitosan solution	.00500	.00997	.619	-.0152	0252
		40% chitin chitosan solution	.00200	.01021	846	- 0187	0227
	40% chitin chitosan solution	0% chitin-chitosan solution	.00411	01049	697	- 0172	0254
		20% chitin-chitosan solution	.00300	00997	765	- 0172	0232
		30% chitin chitosan solution	- 00200	.01021	846	- 0227	0187

Appendix 11: Void volume %

Descriptives

VAR00002	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0% chitin-chitosan solution	10	72.6858	1.84346	.58295	71.3671	74.0046	68.72	74.64
20% chitin-chitosan solution	10	73.4505	1.59925	.50573	72.3064	74.5945	70.40	75.71
30% chitin-chitosan solution	10	73.8947	1.12752	.35655	73.0881	74.7013	71.68	74.79
40% chitin-chitosan solution	10	74.0876	1.31074	.41449	73.1499	75.0252	71.21	75.63
Total	40	73.5296	1.53703	.24303	73.0381	74.0212	68.72	75.71

ANOVA

VAR00002	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.629	3	3.876	1.733	.178
Within Groups	80.507	36	2.236		
Total	92.136	39			

Multiple Comparisons

VAR00002 LSD						
(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confid ence Interval	
					Lower Bound	Upper Bound
0% chitin-chitosan solution	20% chitin-chitosan solution	-.76465	.66878	.260	-2.1210	.5917
	30% chitin-chitosan solution	-1.20889	.66878	.079	-2.5652	.1475
	40% chitin-chitosan solution	-1.40174*	.66878	.043	-2.7581	-.0454
20% chitin-chitosan solution	0% chitin-chitosan solution	.76465	.66878	.260	-.5917	2.1210
	30% chitin-chitosan solution	-.44424	.66878	.511	-1.8006	.9121
	40% chitin-chitosan solution	-.63709	.66878	.347	-1.9934	.7193
30% chitin-chitosan solution	0% chitin-chitosan solution	1.20889	.66878	.079	-.1475	2.5652
	20% chitin-chitosan solution	.44424	.66878	.511	-.9121	1.8006

	40% chitin-chitosan solution	-.19285	.66878	.775	-1.5492	1.1635
	0% chitin-chitosan solution	1.40174*	.66878	.043	.0454	2.7581
40% chitin-chitosan solution	20% chitin-chitosan solution	.63709	.66878	.347	-.7193	1.9934
	30% chitin-chitosan solution	.19285	.66878	.775	-1.1635	1.5492
*. The mean difference is significant at the 0.05 level.						

Appendix 12: Water absorption %

Descriptives

VAR00002	N	Mean	Std. Deviation	Std. Error	95% Confide nce Interval for M ean		Minimum	Maximum
					Lower Bound	Upper Bound		
0%	5	90.2111	6.13695	2.74453	82.5911	97.8312	81.33	96 79
20%	5	81.4073	2.02270	.90458	78 8958	83 9188	78 48	83 10
30%	5	84 8400	5 60538	2.50680	77 8800	91.8000	75 83	89 64
40%	5	84.5439	2.81498	1.25890	81.0486	88.0391	82.41	89 44
Total	20	85.2506	5.25467	1.17498	82.7913	87.7099	75.83	96.79

ANOVA

VAR00002	Sum of Squares	df	Mean Square	F	Sig
Between Groups	200 229	3	66 743	3 292	048
Within Groups	324 391	16	20 274		
Total	524.620	19			

Multiple Comparisons

VAR00002 LSD						
(I) VAR 00001	(J) VAR 00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confid ence Interval	
					Lower Bound	Upper Bound
0%	20%	8.80381*	2.84777	.007	2.7668	14.8408
	30%	5.37112	2.84777	.078	-.6659	11.4081
	40%	5.66728	2.84777	.064	-.3697	11.7043
20%	0%	-8.80381*	2.84777	.007	-14.8408	-2.7668
	30%	-3.43269	2.84777	.246	-9.4697	2.6043
	40%	-3.13653	2.84777	.287	-9.1735	2.9005
30%	0%	-5.37112	2.84777	.078	-11.4081	.6659
	20%	3.43269	2.84777	.246	-2.6043	9.4697
	40%	.29616	2.84777	.918	-5.7408	6.3332
40%	0%	-5.66728	2.84777	.064	-11.7043	.3697
	20%	3.13653	2.84777	.287	-2.9005	9.1735
	30%	-.29616	2.84777	.918	-6.3332	5.7408

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

Appendix 13: Water repellent efficiency (WRE) %

Descriptives

VAR00002	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0 % chitin-chitosan solution	5	.0000	.00000	.00000	.0000	.0000	.00	.00
20 % chitin-chitosan solution	5	7.6662	3.41324	1.52645	3.4281	11.9043	2.57	11.99
30 % chitin-chitosan solution	5	5.9539	6.21362	2.77882	-1.7613	13.6692	.63	15.95
40 % chitin-chitosan solution	5	6.2822	3.12043	1.39550	2.4077	10.1568	.86	8.65
Total	20	4.9756	4.66390	1.04288	2.7928	7.1584	.00	15.95

ANOVA					
VAR00002					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	173.302	3	57.767	3.851	.030
Within Groups	239.986	16	14.999		
Total	413.288	19			

Multiple Comparisons						
VAR00002 LSD						
(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confid ence Interval	
					Lower Bound	Upper Bound
0 % chitin-chitosan solution	20 % chitin-chitosan solution	-7.66621*	2.44942	.006	-12.8587	-2.4737
	30 % chitin-chitosan solution	-5.95394*	2.44942	.027	-11.1465	-.7614
	40 % chitin-chitosan solution	-6.28224*	2.44942	.021	-11.4748	-1.0897
20 % chitin-chitosan solution	0 % chitin-chitosan solution	7.66621*	2.44942	.006	2.4737	12.8587
	30 % chitin-chitosan solution	1.71227	2.44942	.495	-3.4803	6.9048
	40 % chitin-chitosan solution	1.38397	2.44942	.580	-3.8086	6.5765
30 % chitin-chitosan solution	0 % chitin-chitosan solution	5.95394*	2.44942	.027	.7614	11.1465
	20 % chitin-chitosan solution	-1.71227	2.44942	.495	-6.9048	3.4803
	40 % chitin-chitosan solution	-.32830	2.44942	.895	-5.5208	4.8642
40 % chitin-chitosan solution	0 % chitin-chitosan solution	6.28224*	2.44942	.021	1.0897	11.4748
	20 % chitin-chitosan solution	-1.38397	2.44942	.580	-6.5765	3.8086
	30 % chitin-chitosan solution	.32830	2.44942	.895	-4.8642	5.5208
*. The mean difference is significant at the 0.05 level.						

Appendix 14: Anti-swelling efficiency (ASE) %

Descriptives

VAR00004	N	Mean	Std. Deviation	Std. Error	95% Confide nce Interval for M ean		Minimum	Maximum
					Lower Bound	Upper Bound		
20%	5	33.4382	8.72000	3.89970	22.6109	44.2655	22.60	46.28
30%	5	30.0720	7.46848	3.34001	20.7987	39.3453	22.52	40.20
40%	5	32.8578	15.44133	6.90557	13.6849	52.0307	23.21	59.90
Total	15	32.1227	10.39707	2.68451	26.3650	37.8804	22.52	59.90

ANOVA

VAR00004	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32.381	2	16.191	.131	878
Within Groups	1481.005	12	123.417		
Total	1513.387	14			

Multiple Comparisons

VAR00004 LSD						
(I) VAR 00001	(J) VAR 00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confid ence Interval	
					Lower Bound	Upper Bound
20%	30%	3.36620	7.02615	.640	-11.9425	18.6749
	40%	.58040	7.02615	.936	-14.7283	15.8891
30%	20%	-3.36620	7.02615	.640	-18.6749	11.9425
	40%	-2.78580	7.02615	.699	-18.0945	12.5229
40%	20%	-.58040	7.02615	.936	-15.8891	14.7283
	30%	2.78580	7.02615	.699	-12.5229	18.0945

Appendix 15: Leach ability %

Descriptives

VAR00 005								
	N	Mean	Std. Deviation	Std. Error	95% Confide nce Interval for M ean		Minimum	Maximum
					Lower Bound	Upper Bound		
20%	5	29.1746	6.23684	2.78920	21.4305	36.9187	22.70	35.78
30%	5	30.5972	6.24026	2.79073	22.8489	38.3455	21.11	38.39
40%	5	33.9690	4.90931	2.19551	27.8733	40.0647	26.72	39.45
Total	15	31.2469	5.78417	1.49347	28.0438	34.4501	21.11	39.45

ANOVA

VAR00005					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	60.632	2	30.316	.892	.435
Within Groups	407.762	12	33.980		
Total	468.393	14			

Multiple Comparisons

VAR00005 LSD						
(I) VAR 00001	(J) VAR 00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confid ence Interval	
					Lower Bound	Upper Bound
20%	30%	-1.42260	3.68674	.706	-9.4553	6.6101
	40%	-4.79440	3.68674	.218	-12.8271	3.2383
30%	20%	1.42260	3.68674	.706	-6.6101	9.4553
	40%	-3.37180	3.68674	.378	-11.4045	4.6609
40%	20%	4.79440	3.68674	.218	-3.2383	12.8271
	30%	3.37180	3.68674	.378	-4.6609	11.4045

Appendix 16: Decay resistance %

Descriptives

VAR00002	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0% chitin-chitosan solution	5	7.4346	1.52457	.68181	5.5416	9.3276	5.80	9.87
20 % chitin-chitosan solution	5	4.2731	.96533	.43171	3.0745	5.4717	3.66	5.97
30 % chitin-chitosan solution	5	3.2127	.76114	.34039	2.2676	4.1578	2.45	4.37
40 % chitin chitosan solution	5	3.3756	.65367	.29233	2.5639	4.1872	2.61	4.21
Total	20	4.5740	1.98503	.44387	3.6450	5.5030	2.45	9.87

ANOVA

VAR00002	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	57.815	3	19.272	18.084	.000
Within Groups	17.051	16	1.066		
Total	74.866	19			

Multiple Comparisons

VAR00002 LSD						
(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confid ence Interval	
					Lower Bound	Upper Bound
0% chitin-chitosan solution	20 % chitin-chitosan solution	3.16155*	.65290	.000	1.7775	4.5456
	30 % chitin-chitosan solution	4.22191*	.65290	.000	2.8378	5.6060
	40 % chitin chitosan solution	4.05903*	.65290	.000	2.6749	5.4431
20 % chitin-chitosan solution	0% chitin-chitosan solution	-3.16155*	.65290	.000	-4.5456	-1.7775
	30 % chitin-chitosan solution	1.06036	.65290	.124	-.3237	2.4445
	40 % chitin chitosan solution	.89748	.65290	.188	-.4866	2.2816
30 % chitin-chitosan solution	0% chitin-chitosan solution	-4.22191*	.65290	.000	-5.6060	-2.8378
	20 % chitin-chitosan solution	-1.06036	.65290	.124	-2.4445	.3237
	40 % chitin chitosan solution	-.16288	.65290	.806	-1.5470	1.2212
40 % chitin chitosan solution	0% chitin-chitosan solution	-4.05903*	.65290	.000	-5.4431	-2.6749
	20 % chitin-chitosan solution	-.89748	.65290	.188	-2.2816	.4866
	30 % chitin-chitosan solution	.16288	.65290	.806	-1.2212	1.5470

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