

**THE POTENTIAL OF NEEM LEAF EXTRACTS USING VARIOUS
SOLVENTS TO REDUCE TERMITE ATTACKS**

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SCIENCE RESEARCH 1

by

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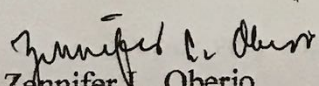
“THE POTENTIAL OF NEEM LEAF EXTRACTS USING VARIOUS
SOLVENTS TO REDUCE TERMITE ATTACKS”

Prepared and submitted by Gwyn P. Celo, Abraham N. Gastar, and Kurt Nolan B. Lopez in partial fulfillment of the requirements in Science Research 2, has been approved and is recommended for acceptance and approval.

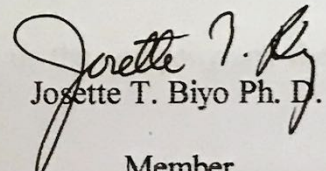
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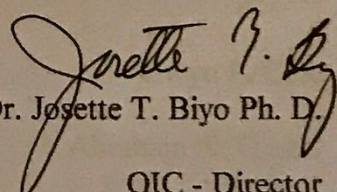

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ABSTRACT

Termites, which are wood-eating insects, are noted for the economic damage that they cause to wood products, buildings and other wood structures. Previous studies have proven that Neem has great potential in fields of pest management, environment protection and medicine. This study aimed to determine the effectivity of Neem leaf extracts as termite repellent by using various solvents (ethanol, n-hexane and water) on the weight loss of wood.

There was seven (7) treatments used in the study, namely the ethanol extract, aqueous extract, n-hexane extract, pure water, pure ethanol, pure n-hexane and untreated setup. The extracts were applied to five (5) wood blocks in every treatment. Then the treated wood blocks were randomly placed in a chosen termite mound. The weight loss was calculated by measuring weight before and after exposure to termite mound for one (1) month.

Results of the study showed that all of the wood blocks in each setup underwent a decrease in weight as shown by the percent weight losses that ranged from 1.76% to 5.04 %.

Post-hoc analysis using LSD showed that aqueous extract treatment has significantly greater percent weight loss than pure water treatment, and ethanol extract treatment has significantly greater percent weight loss than pure ethanol treatment. And it also showed that n-hexane extract treatment and pure n-hexane treatment has no significant difference between their percent weight losses.

TABLE OF CONTENTS

Content	Page
APPROVAL SHEET	<i>i</i>
ACKNOWLEDGEMENT	<i>ii</i>
ABSTRACT	<i>iii</i>
TABLE OF CONTENTS	<i>iv</i>
LIST OF TABLES	<i>v</i>
LIST OF FIGURES	<i>vii</i>
LIST OF APPENDICES	<i>viii</i>
CHAPTER I	1
INTRODUCTION	1
A. Background of the Study.....	1
B. Objectives of the Study	3
C. Hypothesis of the Study	4
D. Significance of the Study	4
E. Scope and Limitations of the Study	6
F. Definition of Terms	7
CHAPTER II	9
REVIEW OF RELATED LITERATURE	9
A. Neem Tree	9
B. Pest Control Potential Of Neem	12
C. Termites.....	14
CHAPTER III	24
METHODOLOGY	24
A. List of Materials	24
B. Research Design	24
C. Materials.....	25
D. Procedures.....	26
E. Statistical Analysis.....	28
CHAPTER IV	30
CHAPTER V	37
BIBLIOGRAPHY	39

LIST OF TABLES

Table	Page
1. Physico-chemical composition of fresh Neem Leaves.	10
2. Mean weight difference and mean percent weight loss of wood using different treatments.	31
3a. One-way analysis of variance for the difference in the weight loss of wood when untreated, treated with aqueous Extract, and Pure Water	31
3b. Post-hoc Analysis using LSD Test to determine the Significance between aqueous Extract, Pure Water, and Untreated setup.	32
3c. One-way analysis of Variance for the difference in the weight loss of wood when untreated, treated with Ethanol Extract, and Pure Ethanol.	32
3d. Post-hoc Analysis using LSD Test to determine the Significance between Ethanol Extract, Pure Ethanol, and Untreated setup.	33

- 3e. One-way Analysis of Variance for the difference in the weight loss of wood when Untreated, treated with N-hexane, and pure N-Hexane. 33
- 3f. One-way Analysis of Variance for the difference in the weight loss of wood when treated with aqueous Extract, Ethanol Extract, and N-Hexane Extract. 33
4. One-way Analysis of Variance for the difference in the weight loss of wood. 34

LIST OF FIGURES

Figure	Page
1. Effects of Various solvents used to obtain Neem extract on the weight loss of wood exposed to termite colony.	5
2. Picture of subterranean termites	16
3. Workflow diagram is for the step-by-step process of determining the effect of Neem in preventing termite attacks based on weight loss on wood.	29

LIST OF APPENDICES

CHAPTER I

INTRODUCTION

Appendix

- A. Weight of wood (in grams) before and after application of extracts, weight loss and percent weight loss on wood.

CHAPTER I

INTRODUCTION

A. Background of the Study

Termites, which are wood-eating insects, by far have the largest number of species found here in the Philippines and other tropical regions (Robert McHenry, 1992). The principal foods for termites are grass, wood and leaves, which contain cellulose. As a result, termites are noted for the economic damage that they cause to wood products, buildings and other wood structures.

Damage is severe in the tropics and warm temperate regions. (Robert McHenry, 1992). It may cause the collapsing of important buildings, which has wood as its foundations. Furniture and wooden houses would not be able to last long due to termite destruction. It is imperative therefore to look for ways in which to protect these wood products. Present practice among carpenters and wood product-makers is to impregnate the wood with pesticide prior to manufacturing, or even after manufacturing. For buildings, pesticides are injected on the ground adjacent and surrounding the foundations of the establishment. Some of the most common anti-termite solutions include Solignum and Malathion, both of which have very strong disagreeable odors which last for days (Saxena, 2001).

Pest control, as practiced today in most developing countries relies mainly on the use of imported pesticides. This dependence on importation has to be reduced. Although pesticides are generally profitable, their use often leads to the contamination of terrestrial

and aquatic environments, damage to beneficial insects and wild biota, accidental poisoning of humans and livestock, and the twin problems of pest resistance and resurgence (Saxena, 2001).

A World Health Organization and United Nations Environmental Program report (WHO/UNEP 1989) estimated there are 1 million human pesticide-poisoning cases each year in the world, with about 20,000 deaths, mostly in developing countries (Saxena, 2001). The problem is rendered even more difficult because few, if any, new compounds are coming to replace old insecticides. The cost of developing and registering new pesticides is staggering almost US\$ 60 million , and pesticide manufacturers are unwilling to risk investments on products whose market life could be shortened by development of pest resistance (Saxena, 2001).

For ecologically sound, equitable, and ethical pest management, there is a need for control agents that are pest-specific, nontoxic to humans and other biota, biodegradable, less prone to pest resistance and resurgence, and relatively less expensive. Among various options, Neem has been identified a source of environmentally "soft" natural pesticides (Saxena, 2001).

Neem is bitter in taste. The bitterness is due to an array of complex compounds called "triterpenes" or more specifically "limonoids". The most important bioactive principal is azadirachtin; at least 10 other limonoids possess insect growth in regulating activity. (Saxena, 2001)

The pest control potential of Neem in the Philippines has been tapped mostly on mosquito-repellants and agricultural pest control. Moreover, the identification of the

traditional use of Neem as backward gradually influenced people away from exploiting its versatility even in the most common problems (Saxena, 2001)..

It is only in the past decade, that the pest control potential of Neem, which does not kill pests but affects their behavior and physiology, has been recognized. Though subtle, Neem's effect such as repellence, feeding and ovipositor deterrence, growth inhibition, mating disruption, chemo-sterilization and many more are now considered far more desirable than a quick knock-down in integrated pest management programs as they reduce the risk of exposing pests natural enemies to poisoned food or starvation (Saxena, 2001).

In spite of the high selectivity, Neem derivatives affect 400 to 500 species of insects such as the species Isoptera where termites belong (Saxena,2003).

B. Objectives of the Study

This study aimed to determine the effectiveness of Neem extract using three kinds of solvent as termite repellent in terms of weight loss of wood exposed to the termite colony. Specifically, it

1. measured the percent weight loss of Neem extract impregnated wood using a) ethanol b) n-hexane c) water as solvent and d) pure n-hexane and e) pure ethanol and f) pure H₂O as controls when exposed to termite colony.
2. compared the effects of using a) ethanol b) n-hexane c) water as solvent and d) pure n-hexane and e) pure ethanol and f) pure H₂O as controls on percent weight loss of wood exposed to the termite colony.

C. Hypothesis of the Study

There exists no significant difference in the effectiveness of the Neem extract using the three solvents and the pure n-hexane and ethanol as controls in terms of percent weight loss of wood exposed to termite colony.

D. Significance of the Study

This study evaluated the potential of Neem tree extract as an effective termite repellent in terms of weight loss of wood when exposed to a termite colony. If the relationship between concentration and weight loss will be established and the most effective concentration will be identified, it would be useful as an alternative of controlling termite attack on anything made from wood.

The result of this study would also contribute to the growing body of knowledge on the applications or uses of Neem.

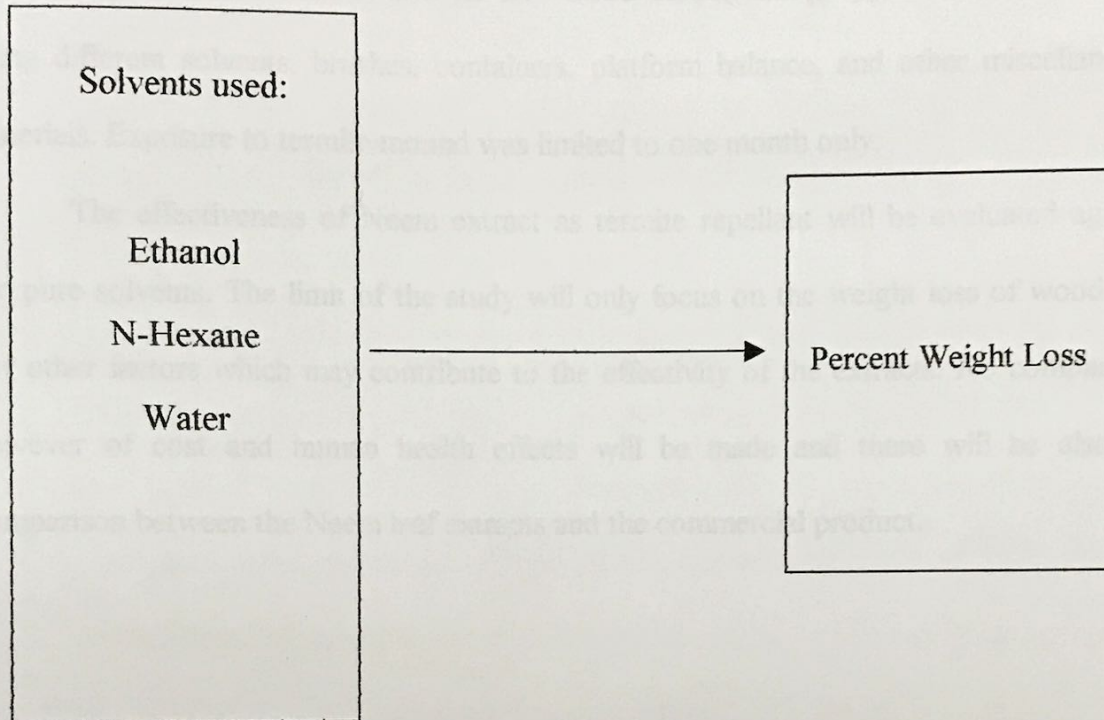
Independent Variable**Dependent Variable**

Figure 1. Effects of the various solvents used to obtain Neem extract on the weight loss of wood exposed to termite colony.

E. Scope and Limitations of the Study

The study aimed to determine the effects of the different solvent extracts of Neem on the weight loss of wood that will be exposed to a certain colony of termites. This study will use certain tools such as a 2"x2"x4" wood block(mahogany), Neem tree extracts using different solvents, brushes, containers, platform balance, and other miscellaneous materials. Exposure to termite mound was limited to one month only.

The effectiveness of Neem extract as termite repellent will be evaluated against the pure solvents. The limit of the study will only focus on the weight loss of wood and not other factors which may contribute to the effectivity of the extracts. No comparison however of cost and human health effects will be made and there will be also no comparison between the Neem leaf extracts and the commercial product.

F. Definition of Terms

aqueous solution – a solution with a solvent as water.

ethanol – (C_2H_5OH) A colorless liquid, miscible with water, boiling point $78.32^{\circ}C$; used as a reagent or solvent. In this study Ethanol will be used as a solvent.

extract – a substance or part of plants removed by solvents and used in drug preparations. In this study, extract will specifically refer to the active ingredients in Neem leaves extracted using water as solvent.

neem tree – a large East Indian tree whose trunk exudes a tenacious gum and has a bitter bark used as a tonic and whose fruit and seeds yield medicinal aromatic oil.

n-hexane – (C_6H_{14}) water insoluble, toxic, flammable, colorless liquid with faint aroma; a straight-chain compound boiling at $68.7^{\circ}C$ and used as a solvent, paint diluent, alcohol denaturant, and polymerization-reaction medium. In this study, n-hexane will be used to extract Neem oils from the leaves.

solvent extraction – method of separation in which a solid or solution is contacted with a liquid solvent to transfer one or more components. In this study this refers to the methods of extracting the active ingredients from the leaves of Neem using various solvents.

termite – any of numerous pale-colored, soft-bodied, chiefly tropical, social insects of the order Isoptera that feed on wood, some being highly destructive to buildings, furniture and other wood materials.

termite repellent – wood preservative that protects wood from attack by termites and wood borers.

triterpenes – one of a class of compounds having molecular skeletons containing 30 carbon atoms, and theoretically composed of six isoprene units; numerous and widely distributed in nature, occurring principally in plant resins and sap.

weight loss of wood – the difference between the weight of the wood before and after exposure to the termite colony.

CHAPTER II

REVIEW OF RELATED LITERATURE

This chapter consists of three topics namely (1) Neem Tree, (2) Pest Control of Neem, (3) Termites.

A. Neem Tree

The Neem is being considered to be one of the most promising trees of the 21st century. It has great potential in the fields of pest management, environment protection and medicine. It is now known to help control diseases like Malaria, Cancer and AIDS, combat desertification and deforestation, reduce excessive global temperature and even help in population control. Two generations of Indian Neem was known to provide protection from disease. Neem is a tropical tree with wide adaptability and is especially suited to semi-arid conditions. Currently it is grown in many Asian countries and in the tropical regions of the western hemisphere (Saxena, 2001).

The Neem tree appears to be a biochemical factory producing a mixture of over 135 biologically active compounds. As a pesticide, the oil from Neem seeds are believed to break the life cycle of pests and deters them from feeding and hatching. Studies have shown that active compounds in the oil inhibited the secretion of hormones into the blood inhibiting the molting and reproductive function in insects (Saxena, 2001).

Neem is bitter in taste. The bitterness is due to an array of complex compounds called "triterpenes" or more specifically "limonoids". The most important bioactive

principal is azadirachtin; at least 10 other limonoids possess insect growth in regulating activity. (Saxena, 2001)

Table 1 below lists the physico-chemical composition of Neem leaves. The Table implicates that Neem leaves are composed mostly of moisture, making water a suitable solvent for the extract. And also it shows that the leaf composition does not contain any substance that can be hazardous to health.

Table 1. Physico-chemical composition of fresh Neem leaves.

Moisture	59.4 %	Vitamin C	218 Mg/100g
Proteins	7.1 %	Carotene	1998 Microgram/100g
Fat	1.0 %	Calorific Value	1290 Kcal/Kg
Fibre	6.2 %	Glutamic acid	73.30 Mg/100g
Carbohydrates	22.9 %	Tyrosine	31.50 Mg/100g
Minerals	3.4 %	Aspartic acid	15.50 Mg/100g
Calcium	510 Mg/100g	Alanine	6.40 Mg/100g
Phosphorous	80 Mg/100g	Proline	4.00 Mg/100g
Iron	17 Mg/100g	Glutamine	1.00 Mg/100g

A.1. Neem twigs

Neem twigs are sometimes used as toothbrushes to combat teeth decay. Its extracts have a powerful pesticidal activity and are used by both households and farmers to control a wide variety of pests (insects, fungi, bacteria, viruses, nematodes, rodents etc.) (Saxena, 2001). These extracts have considerable antiseptic affects and are used as a skin care agent in soaps and shampoos. The leaves are often mixed with rice and

consumed as a cure all and prophylactic against bacterial and helminthic infection (Saxena, 2001). Neem leaf pastes are used to repair scarred skins arising from the effects of chickenpox. The pesticidal and medicinal properties of extracts from the Neem tree have been exploited for at least the last 2500 years (Saxena, 2001).

A.2. Neem extracts

Sanskrit texts dating back to the sixth century BC, document the microbicidal and prophylactic effects of Neem extracts. Charaka in the 6th Century BC recommended the oral consumption of Neem extracts to ward off pimples, leprosy and edema. Neem extracts have also been shown to be effective against nematode pests. Neem cake, the by-product from Neem seed processing appears to be effective on nematodes, snails and certain fungi (Saxena, 2001). The Neem tree and its extracts surprisingly appear to be benign to bees and other nectar feeding insects. Seed extracts are not known to have any toxic effect on plants, mammals and birds and in fact in studies by the US EPA, no LD-50₁ could be established even at high doses (Saxena, 2001).

Neem oil is known to be active on over 400 insect pests. It has for example been found to be effective against fleas, head lice, ticks, termites, plague locusts, mosquitoes and sheep blow flies. It is believed to be particularly active against chewing and sucking insects such as caterpillars and beetle larvae. These remarkable properties have attracted considerable interest from both researchers and pharmaceutical companies. This renewed interest in Neem created no more than amusement in India where the beneficial properties of Neem have been known for countless generations (Saxena, 2001).

A.3. Neem-leaf smoke

Sushruta in the 5th century BC recommended the use of Neem-leaf smoke for fumigation and maintenance of general hygiene. He also recommended it as a "krimihara", an agent effective against insects, grubs and maggots and detailed the ability of Neem leaves to cure gangrenous and otherwise difficult to cure wounds (Saxena, 2001).

B. Pest Control Potential Of Neem

B.1. Blood sucking pests

Ascher and Meisner have reviewed the effects of Neem on hematophagous insects affecting humans and livestock. Application of a paste made from Neem leaves and turmeric in 4:1 proportion to the skin cured 97% of the patients suffering from scabies caused by the mite *Sarcoptes scabiei* in 3-15 days. Monthly sprays of ethanolic extracts of Neem or weekly bathing in azadirachtin-rich aqueous 1:20 'Green Gold' controls the bush tick, *Ixodes holocyclus*, and the cattle tick, *Boophilus microplus* in Australia, but were less effective against the brown dog tick, *Rhipicephalus sanguineus* (Saxena, 2001).

In Jamaica, Neem kernel extract controls ticks on cattles and dogs. Neem products repel and affect the development of mosquitoes. Two percent Neem oil mixed in coconut oil, is applied to exposed body parts of human volunteers, provided complete protection for 12 h from bites of all anophelines. Kerosene lamps containing 0.01-1% Neem oil, lighted in rooms containing human volunteers, reduced mosquito biting activity as well as catches of mosquitoes resting on walls in the rooms; protection was greater against *Anopheles* than against *Culex* (Saxena, 2001).

Effectiveness of mats with Neem oil against mosquitoes has also been demonstrated; the vaporizing repelled mosquitoes for 5-7 h at almost negligible cost. The sandfly, *Phlebotomus argentipes*, also was totally repelled by Neem oil, mixed with coconut or mustard oil, throughout the night under field conditions in India (Saxena, 2001).

B.2. Crop Pest

Neem has a long history of use primarily against household and storage pests and to some extent against crop pests in the Indian sub-continent. It is a common practice in rural India to mix dried Neem leaves with grains meant for storage. Mixing of Neem leaves (2-5%) with rice, wheat and other grains is now practiced in some parts of India and Pakistan. Also, as early as 1930, Neem cake is applied to rice and sugarcane fields against stem borers and white ants. Some innovative farmers in Karnataka and Tamil Nadu states in India today "puddle" green twigs and leaves in rice nursery beds to produce robust seedling and simultaneously ward-off attack by early pests-leafhoppers, planthoppers, and whorl maggots. Controlled experiments confirmed that rice seedlings raised from seed treated with Neem kernel extract or cake is vigorous and resistant to rice leafhoppers and planthoppers. Early observations that Neem leaves were not attacked by swarming locusts were also confirmed in laboratory studies and attributed to Neem's antifeedant activity against locusts (Saxena, 2001).

B.3. Pest of stored products

Postharvest losses are notoriously high in developing countries. Worldwide annual losses in store reach up to 10% of all stored grain, i.e. 13 million tons of grain lost

due to insects or 100 million tons to failure to store properly.(Saxena, 2001) Dr. Ramesh Saxena, Senior principal scientist, is the former head of the Integrated Pest Management Section at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, has recently reviewed the potential of Neem against pest of stored products grain legumes, maize, sorghum, wheat rice and paddy, potato tubers. At farm level storage and warehouses, the application of Neem derivatives to bags and stored grains has provided protection against insect pests. Powdered Neem seed kernel mixed with paddy (1 to 2%) significantly reduced infestation and damage to grain during a 3 month storage period; the effectiveness capacity jute bag (100 x 60 cm) controlled 80% of the population of major insects and checked the damage to wheat up to 6 months. The Neem seed extract treatment was as effective as that of 0.0005% primiphos methyl mixed with the grain. Using this technology in Sind, Pakistan, high benefit-cost ratios were obtained by small, medium, and large-scale farmers (Saxena, 2001).

C. Termites

Subterranean termites are the most common and economically important wood-destroying organisms in the United States. Termites feed on materials that contain cellulose, primarily dead wood and wood by-products. Subterranean termites are closely associated with the soil habitat where they excavate a network of tunnels through the soil to reach water and food. These termites need moisture to survive (Columbus,1991).

Biology

Subterranean termites are social insects that live in colonies that may contain hundreds of thousands of individuals. Termite colony members are dispersed throughout the soil and can extend underground tunnels tens to hundreds of feet to reach feeding sites (Columbus,1991).

Each termite colony contains three forms or castes, which are the workers, soldiers, and reproductives. These castes are physically distinct and perform different tasks in the termite society (Columbus, 2002)

Workers are about 1/8 inch long and are blind, wingless, soft-bodied, creamy white to grayish-white with a round head. Workers are the most numerous individuals in a termite colony, and they are the termite caste that actually eats the wood. These sterile individuals forage for food and water, construct and repair shelter tubes, feed and groom other termites, care for eggs and young, and participate in colony defense (Columbus,1991).

Soldiers are also wingless and resemble workers except that they have a large, rectangular, yellowish-brown head with large mandibles (jaws). The soldiers' primary function is colony defense (Columbus,1991).

Male and female reproductives can be winged (primary) or wingless (neotenic). Each can produce new offspring. Winged primary reproductives are called alates or swarmers. However, they shed their wings soon after flight. Their body color varies by species from black to yellow-brown.

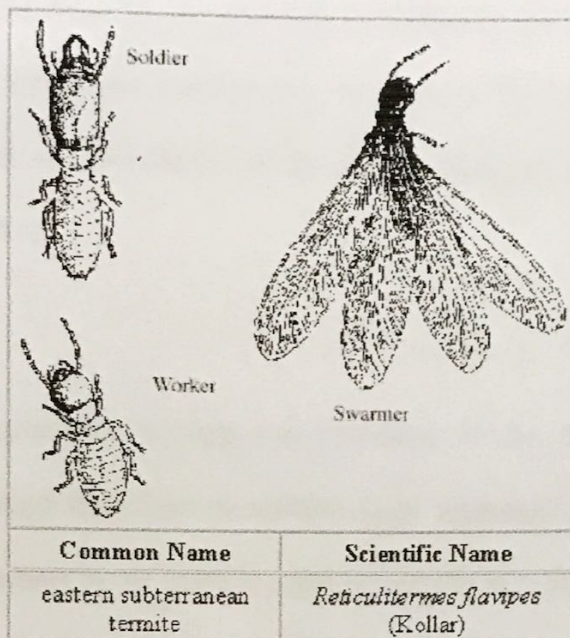


Figure 2. Picture of subterranean termites.

The eastern subterranean termite, *Reticulitermes flavipes*, is the most common termite in Ohio and its alates are black and about 0.4 inch long, with pale or grayish, translucent wings. A pair of primary reproductives that heads a colony is called the king and queen. Neotenic reproductives often serve as replacements if something happens to the king and queen. Neotenic reproductives are generally yellow or mottled black and the female's abdomen may be distended due to developing eggs or mottled black and the female's abdomen may be distended due to developing eggs (Columbus, 1991).

Detection of Termites

It is important for homeowners to recognize the signs of a subterranean termite infestation. Subterranean termites may be detected by the sudden emergence of winged termites (alates or swarmers), or by the presence of mud tubes and wood damage (Columbus, 1991).

The appearance of winged or swarming termites is often the first indication of a problem. Although the swarm poses little or no immediate danger to the structure, it gives warning of termites in, or in close vicinity, and the homeowner should make an effort to locate the source from which they are emerging (Columbus, 1991).

Earthen shelter tubes, constructed over surfaces of foundation walls, or sometimes directly connecting the soil and the structure, are another readily visible sign of termite infestation. These tubes are between 1/4" and 1/2" or more wide. If it is in use, the inside is moist and there may be white workers present. Also, if a section is broken, the workers will repair it as soon as possible because it is used as a passageway between the wood and the soil from which they obtain essential moisture. The tubes also protect the termites from the drying effects of direct exposure to air (Columbus, 1991).

Without the external evidence of winged termites or visible shelter tubes, it is more difficult to determine whether or not termites are present in a building. The first place to be checked is the wood which is near or rests on the ground. Weather boarding, wood supports, basement window frames, door casings, sills, etc., can be checked for soundness by being tapped with a hammer and probed with a screwdriver. If hollow

wood is found, and it is the result of a termite infestation, the soft portions of the wood will be eaten leaving the hard sections (Columbus,1991).

Winged Termites

Large numbers of winged termites swarming from wood or the soil often are the first obvious sign of a nearby termite colony. Swarming occurs in mature colonies that typically contain at least several thousand termites. A "swarm" is a group of adult male and female reproductives that leave their colony in an attempt to pair and initiate new colonies (Columbus,1991).

Alate emergence is stimulated when temperature and moisture conditions are favorable, usually on warm days following rainfall. In Ohio, swarming typically occurs during daytime in the spring (March, April, and May), but swarms can occur indoors during other months. However, swarming occurs during a brief period (typically less than an hour), and alates quickly shed their wings. Winged termites are attracted to light, and their shed wings in windowsills, cobwebs, or on other surfaces often may be the only evidence that a swarm occurred indoors. The presence of winged termites or their shed wings inside a home should be a warning of a termite infestation (Columbus,1991).

Termite swarmers have straight, bead-like antennae; a thick waist; and two pair of long, equal-length wings that break off easily. Winged termites can be differentiated from winged ants, which have elbowed antennae, a constricted waist, and two pair of unequal-length wings (forewings are larger than hind wings) that are not easily detached. Ants also generally are harder-bodied than termites (Columbus,1991).

Mud Tubes

Other signs of termite presence include mud tubes and mud protruding from cracks between boards and beams. Subterranean termites transport soil and water above ground to construct earthen runways (shelter tubes) that allow them to tunnel across exposed areas to reach wood. Shelter tubes protect them from the drying effects of air and from natural enemies, such as ants. These tubes usually are about 1/4 to 1 inch wide, and termites use them as passageways between the soil and wood. To determine if an infestation is active, shelter tubes should be broken or scraped away and then monitored to determine whether the termites repair them or construct new ones (Columbus, 1991).

Feeding

Termites feed primarily on wood which contains a high proportion of cellulose. The lower termites don't possess the enzymes to break down the cellulose themselves, instead they live in a mutualistic relationship with one or more protozoan flagellates which live inside the termites guts and digest the cellulose in the wood fibres ingested by the termites. The Protozoa get a stable environment and a constant supply of food and the termites are believed to get acetic acid and other simple organic acids which they can metabolize. The Termites loose their intestinal flora every time they moult and have to reinoculate themselves from the anal secretion of another member of the nest (proctodeal feeding).

The higher termites (Termitidae) do not possess these protozoans but they do possess anaerobic (living only in the absence of oxygen) bacteria in their guts instead. Though it is believed they do not play as important a role in cellulose digestion as the Protozoa do in the lower termites, and it may be that the Termitidae can secrete cellulase

(the enzyme that breaks down cellulose) themselves. A number of species of the higher termites in the Hodotermitidae forage outside the nest, *Odontotermes latericus* in South Africa collects green grass and seeds which it stores in granaries inside its nest. *Nasutitermes triodiae* in Australia store dry grass in special chambers in the walls of their termitaria, while *Hospitalitermes monocerus* the black termite of Ceylon sends out large foraging columns of workers which are guarded by soldiers to collect the lichens on which it feeds its young. Some species (i.e. the Macrotermtinae (Termitidae) grow fungi (*Termitomyces* sp.) inside their nests on piles of faecal pellets, the fungus is used for food and each termite species has its own species of fungi, these fungi are not found anywhere outside of the termites nests.

Termite Homes

Most primitive termites live in dead wood, their homes are just the tunnels created while they are acquiring food, and their nests have no real structure. Species of *Rhinotermes*, *Reticulotermes* and *Captotermes* are what is known as 'subterranean termites' in America and have their nest below ground but attack above ground wooden structures. In order to avoid the dryness of the open air they construct covered walkways to allow themselves access to otherwise unreachable above ground wood. The largest and most complex termitaria are built by the more advanced Termitidae in Africa and Australia. These termitaria are built of either soil excavated in course of digging underground tunnels or from soil and sand collected on the surface mixed with saliva and faeces. *Nasutitermes triodiae* of Northern Australia builds huge termitaria up to 8 metres in height while another Australian Termite *Omitermes meridionalis* is known as the Compass Termite

because it builds its 3 metre high termitaria with one long axis and one short axis, the long axis always runs North/South and the short axis East/West. The result of this is that the termitaria has a large surface area facing the sun in the morning and the afternoon and very small surface area receiving the sun in the middle of the day thus helping produce a steady temperature for the longest possible time. Other Termites nest in the ground and feed on plant roots and leaf litter while still others build rounded nests in trees that look a bit like the nest of Vespid wasps.

Wood Damage

Termite damage to the wood's surface often is not evident because termites excavate galleries within materials as they feed. Wood attacked by subterranean termites generally has a honeycombed appearance because termites feed along the grain on the softer spring growth wood. Their excavations in wood often are packed with soil, and fecal spotting is evident. When inspecting for termites, it is useful to probe wood with a knife or flat blade screwdriver to detect areas that have been hollowed. Severely damaged wood may have a hollow sound when it is tapped. Subterranean termites do not reduce wood to a powdery mass, and they do not create wood particles or pellets, as do many other wood-boring insects (Columbus, 1991).

Prevention

Preventive practices are a critical aspect of termite management. Prevention of subterranean termite infestation of wooden structures centers upon disrupting their ability to locate moisture, food (wood), and shelter. OSU Extension Fact Sheet HSE-1000-00

lists measures that can be employed to reduce the risk of termite infestation (Columbus,1991).

Avoid moisture accumulation near the foundation, which provides water needed for termite survival. Divert water away from the foundation with properly functioning downspouts, gutters, and splash blocks. Soil needs to be graded or sloped away from the foundation in order for surface water to drain away from the building (Columbus,1991).

Cellulose (wood, mulch, paper, etc.) that is in contact with soil provides termites with ready and unobservable access to food. It is very important to eliminate any contact between the wooden parts of the house foundation and the soil. Maintain at least 6 inches between the soil and porch steps, latticework, door or window frames, etc. Never stack or store firewood, lumber, newspapers, or other wood products against the foundation or within the crawl space. Prevent trellises, vines, etc. from touching the house. Before and during construction, never bury wood scraps or waste lumber in the backfill, especially near the building. Be sure to remove wooden or cellotex form boards, grade stakes, etc. used during construction. Remove old tree stumps and roots around and beneath the building. Avoid or minimize use of wood mulch next to the foundation (Columbus,1991).

Control Measures

Termites feed slowly so there is no need to panic if they are discovered in one's home. A few weeks or months may be needed to decide on a course of treatment, which typically requires employing a professional pest management firm. Homeowners seldom have the experience, availability of pesticides, and equipment needed to perform the job effectively (Columbus,1991).

Termite Woodborers

Termites and beetles are the primary concern because they are much more destructive. They thrive where cellulose and high moisture exist, therefore it is essential that moisture drains away from your dwelling and wood products are up and away from the finish grade.

Termites can have colonies of over 1,000,000 and can eat one pound of wood per day. Termites appear similar to winged ants except they have non-segmented bodies, four wings of equal length and straight antennas (not elbowed like ants). Power Post Beetles enter a home already embedded inside wood members (usually large timbers in older homes). They too can be devastating to your homes structural integrity. They kick out their frass (sawdust like powder) from small 1/16 inch holes they create in the wood.

CHAPTER III

METHODOLOGY**A. List of Materials**

- neem leaves
- wood blocks
- 750 ml ethanol
- 750 ml n-hexane
- 750 ml water
- burner
- beaker
- mortar and pestle
- filter paper
- protective net

B. Research Design

This study aimed to know the effectiveness of Neem leaves as a termite repellent. It also aimed to compare the percent weight loss of wood in three different extracts using three different solvents, pure ethanol, pure water and pure n-hexane as controls, and if no termite repellent was applied. There were five replicates in each set of termite repellent. The independent variable is the termite repellent which is Neem Leaf extracts and the pure solvents, while the dependent variable is the percent weight loss in the wood. A completely randomized design was used in the study.

There was one set up for the experiment, although there were six(6) different termite repellants, all the wood blocks with different treatment were placed around the termite mound. The different treatments were as follows:

	Solvent	Neem Leaves
A	500 mL ethanol	100 g
B	500 mL n-hexane	100 g
C	500 mL distilled water	100 g
D	250 mL n-hexane	None
E	250 mL ethanol	None
F	None	None
G	250 mL Distilled H ₂ O	None

C. Materials

The leaves were obtained from PSHS-WVC. Only the dark green mature leaves were used in the study. They were obtained manually. The ethanol were bought from local drugstores here in Iloilo City. The n-Hexane was provided by PSHS-WV. Red mahogany wood blocks sized 2"x2"x4" and bought from local suppliers were used for the termite resistance test. All other equipment and materials needed for the study were obtained from PSHS-WV. This study was conducted in PSHS-WVC, Brgy. Biton, Jaro, Iloilo City. The period of the study was from April of the 2nd week up to June of the 1st week.

D. Procedures

Aqueous extraction

One hundred grams of fresh Neem leaves was macerated using a mortar and pestle. After that the leaves with the 500 ml distilled water was boiled for 10 minutes using a burner and a beaker. The resulting extract was filtered using filter paper and then the filtrate was set aside under room condition until it is ready for testing.

Extraction w/ Alcohol

One hundred grams of fresh Neem leaves were macerated using a mortar and pestle, then it were boiled together with the 500 ml of pure Ethanol for 10 minutes using a beaker and a burner.

The resulting extract was filtered using filter paper and then the filtrate was set aside under room condition until it is ready for testing.

Extraction w/ n-Hexane

One hundred grams of fresh Neem leaves were macerated with five (5) ml of pure n-Hexane to produce a paste using a mortar and pestle. Then the remaining 495 ml of n-Hexane was added. After that the mixture was boiled for 10 minutes using a beaker and a burner. The resulting extract was filtered using filter paper and then the filtrate was set aside under room condition until it is ready for testing.

Preparation of Termite Mound

The termite mound was chosen in a random manner. It was covered by a protective net with a shape of a cube and a side measuring one(1) meter. The mound is located near the walls of Philippine Science High School Western Visayas, slightly visited, and shaded by a tree. It has plants around it. The mound is undisturbed by humans, but is vulnerable to insects. Before the mound was chosen, it was first checked if it has live termites by opening a small portion using a wooden stick

Test Organisms

Termites needed in the experiment came from local termite mounds. Termites living on mounds are known as subterranean termites.

Application and Testing of the Extracts

After extraction, all of the extracts were set aside for five (5) minutes before it was applied to the wood blocks. Two hundred (200) ml of the extracts in each solution was used in the treatment and was applied evenly in each wood block. The extracts of each termite repellent was brushed to the wood blocks for the application. Before exposure to termites, each treated wood block were weighed. Wood blocks were randomly placed around the mound. This set-up was

observed for one month. Then the wood blocks were cleaned and weighed to calculate the percent weight loss.

The effectiveness of the treatments was measured in percent weight loss after exposure to the termite colonies.

$$\text{Percent Weight loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100\%$$

E. Statistical Analysis

The means of the percent weight loss per treatment was calculated and compared to describe the effects of the various treatments. One-way Analysis of Variance (ANOVA) was used to determine if there is a significant difference in the effectivity of the various treatments in terms of the average percent weight loss in each wood block.

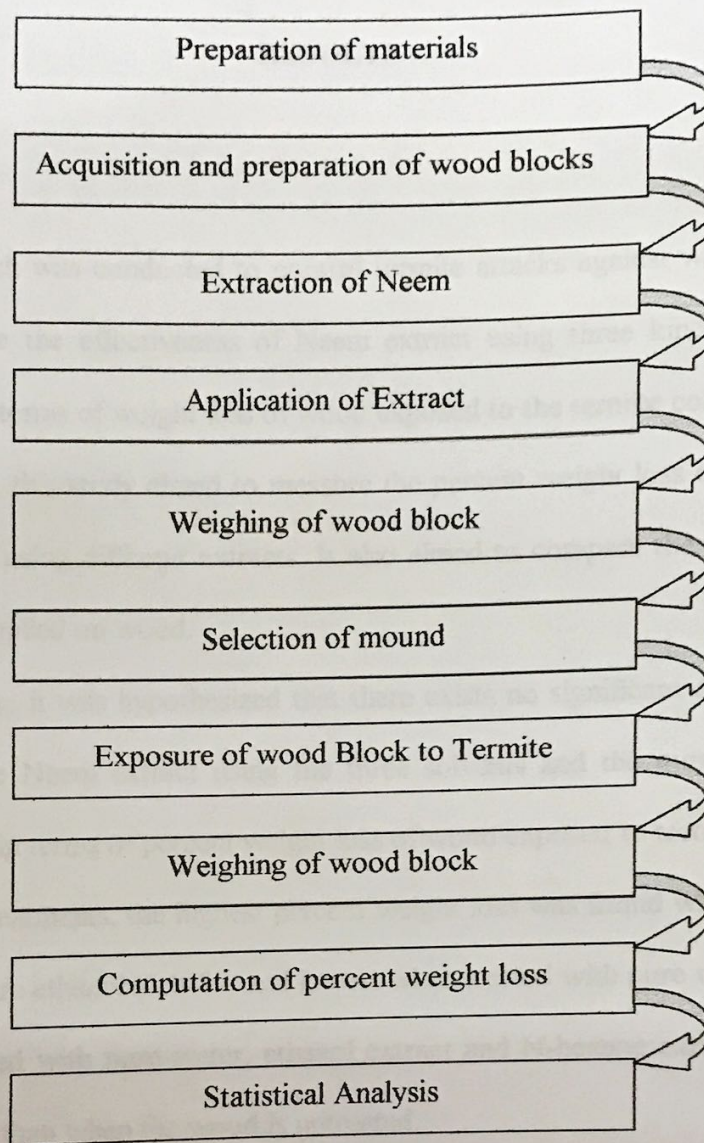


Figure 3. Workflow diagram for is for the step-by-step process of determining the effect of Neem in preventing termite attacks based on weight loss on wood.

CHAPTER IV

RESULTS

A. Results

This research was conducted to control termite attacks against wood. This study aimed to determine the effectiveness of Neem extract using three kinds of solvent as termite repellent in terms of weight loss of wood exposed to the termite colony.

Specifically, this study aimed to measure the percent weight loss of Neem extract impregnated wood using different extracts. It also aimed to compare the effects of using different extracts applied on wood.

In this study, it was hypothesized that there exists no significant difference in the effectiveness of the Neem extract using the three solvents and the pure n-hexane and ethanol as controls in terms of percent weight loss of wood exposed to termite colony.

Of all the treatments, the highest percent weight loss was found when wood block was treated with pure ethanol (5.04%) and lowest when treated with pure water (1.75%).

Wood treated with pure water, ethanol extract and N-hexane extract have lower percent weight loss than when the wood is untreated.

Table 2 shows the results.

Table 2. Mean weight Difference and Mean percent weight loss of wood using different Treatments.

Treatment	Mean weight before	Mean weight after	Mean weight difference	Mean % weight loss \pm SD
Aqueous Extract	105.6	99.7	3.9	3.834 \pm 1.272411
Pure Water	105.04	103.3	2.08	1.758 \pm 1.46259
Ethanol Extract	112.32	109.17	3.15	2.634 \pm 1.083596
Pure Ethanol	120.72	114.34	5.98	5.04 \pm 1.889418
N-Hexane Extract	112.8	108.76	4.04	3.32 \pm 1.713914
Pure N-Hexane	112.3	108.22	4.14	3.912 \pm 1.60559
Untreated	99.22	95.52	3.5	3.734 \pm 0.671513

There is a significant difference in the weight losses of woods treated with pure water, aqueous extract and the untreated wood ($p=0.028$, $\alpha=0.05$).

Table 3a shows the results

Table 3a. One-way Analysis of Variance for the difference in the weight loss of wood when untreated, treated with Aqueous Extract, and Pure Water ($\alpha=0.05$).

	Sum of Squares	Df	Mean Square	F	Sig	Interpretation
Between Groups	13.712	2	6.856	4.887	.028	Significant
Within Groups	16.835	12	1.403			
Total	30.546	14				

Post-hoc analysis showed that no significant difference in the weight losses was found when the three treatments (aqueous, pure water, and untreated) were compared with each other.

Table 3b shows the results.

Table 3b. Post-hoc Analysis using LSD Test to determine Significant difference in weight loss between Aqueous Extract, Pure Water, and Untreated setup ($\alpha=0.05$).

Grp	Grp	Mean difference	Significance	Interpretation
Aqueous Extract	Pure Water	2.0766	0.051	Not Significant
Aqueous Extract	Untreated	.1006	.991	Not Significant
Pure Water	Untreated	-1.9760	0.064	Not Significant

There is a significant difference in the weight loss of wood blocks treated with pure ethanol, ethanol extract and the untreated wood ($p=0.042$, $\alpha=0.05$).

Table 3c shows the results.

Table 3c. One-way Analysis of Variance for the difference in the weight loss of wood when untreated, treated with Ethanol Extract, and Pure Ethanol ($\alpha=0.05$).

	Sum of Squares	Df	Mean Square	F	Sig	Interpretation
Between Groups	14.507	2	7.254	4.189	.042	Significant
Within Groups	20.780	12	1.732			
Total	35.287	14				

There is a significant difference in the weight loss of wood blocks treated with ethanol extract and pure ethanol.

Table 3d shows the results.

Table 3d. Post-hoc Analysis using LSD Test to determine Significant difference in the weight loss between Ethanol Extract, Pure Ethanol, and Untreated setup ($\alpha=0.05$).

Grp	Grp	Mean Difference	Significance	Interpretation
Ethanol Extract	Pure Ethanol	-2.0460	.042	Significant
Ethanol Extract	Untreated	-1.1000	.442	Not Significant
Pure Ethanol	Untreated	1.3060	.326	Not Significant

There is no significant difference in the weight loss of wood blocks treated with n-hexane extract, pure n-hexane and the untreated wood ($p=0.796$, $\alpha=0.05$).

Table 3e shows the results.

Table 3e. One-way Analysis of Variance for the difference in the weight loss of wood when Untreated, treated with N-Hexane Extract, and Pure N-Hexane ($\alpha=0.05$).

	Sum of Squares	Df	Mean Square	F	Sig	Interpretation
Between Groups	.923	2	.461	.232	.796	Not Significant
Within Groups	23.865	12	1.989			
Total	24.788	14				

There is no significant difference in the weight loss of wood blocks treated with ethanol extract, n-hexane extract and aqueous extract of neem leaves ($p=0.414$, $\alpha=0.05$).

Table 3f show the results.

Table 3f. One-way Analysis of Variance for the difference in the weight loss of wood when treated with Aqueous Extract, Ethanol Extract, and N-Hexane Extract ($\alpha=0.05$).

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3.625	2	1.812	.949	.414
Within Groups	22.923	12	1.910		
Total	26.547	14			

There exists a significant difference percent weight loss comparing all the setups performed in the experiment.

Table 4 shows the results.

Table 4. One-way Analysis of Variance for the Difference in the weight loss of wood when treated with various extracts using leaves of *Azadirachta indica*, the pure solvents and the untreated wood.

	Sum of Squares	Df	Mean Square	F	Sig.	Interpretation
Between Groups	32.572	6	5.429	2.626	.038	Significant
Within Groups	57.875	28	2.067			
Total	90.446	34				

B. Discussion

It is known that the seeds and other parts of the neem tree (*Azadirachta indica* and related species) contain natural pesticidal compositions. But the seeds is the most effective part of a neem tree. The main active pesticidal composition is azadirachtin which is a tetranortriterpenoid that causes feeding inhibition and growth inhibition in a variety of organisms including insects, mites and nematodes. It is possible that there are a number of similar insecticidal compounds present in neem extracts that partition with the azadirachtin. As used in this specification, the term azadirachtin is taken to include all insecticidal terpenoids present in neem extracts that partition with azadirachtin (Brushett, 1999).

After the experiment, it was found out that neem extracts was not effective against termites. Since most neem extraction processes only involves seeds and the most effective part of a neem is its seeds and not he leaves. Since leaves was used in the experiment, there is a possibility that results will show that neem leaf extracts is not effective.

Based on normal feeding activity of termites, it takes three to eight years to cause appreciable damage. There have been some predictions that, under ideal conditions, a termite colony of 60,000 workers may consume a one-foot length of 2" x 4" pine in 118 to 157 days. In Nebraska, the extent of damage may be different because of reduced feeding activity during the cold season (Shripat, 2002).

It takes a long time before a termite can cause a significant damage into wood. Therefore, a period of one month for exposure is not enough for the termites to eat the wood.

After extraction, extracts were applied to wood blocks and dried for about three (3) hours. The wood blocks were dried under the sunlight. And the process of drying might not have thoroughly dried the wood blocks and greatly affected the percent weight losses.

Placement of wood blocks around the mound might have caused some change in the weight loss in wood. At some point of the exposure, it had rained several times should have added moisture to the wood blocks. Moreover, the position of the wood blocks is not that perfect that each wood block gets equal amount of moisture.

After exposure to the termite mound, each wood blocks are removed from its unwanted dirt and mud. The wood blocks were scraped vigorously to eliminate the unwanted mud that may cause a change in weight loss. The process may not be accurate since there is a possibility that parts of the wood blocks had been scraped off causing a change in the weight loss.

Measured weight losses cannot be attributed to termites because wood blocks have no traces of any termite infestation. The percent weight losses might be caused of all the inaccurate processes (extraction of neem, application of extracts, drying of wood blocks, exposure of wood blocks, and cleaning of wood blocks after exposure) that had been performed.

CHAPTER V

CONCLUSION

This research study was conducted to find an alternative way of controlling destruction caused by termites. This study aimed to determine the effectiveness of Neem extract using three kinds of solvent as termite repellent in terms of weight loss of wood exposed to the termite colony.

Specifically, it aimed to measure the percent weight loss of Neem extract impregnated wood using a) ethanol b) n-hexane c) water as solvent and d) pure n-hexane and e) pure ethanol and f) pure H₂O as controls when exposed to termite colony.

It also aimed to compare the effects of using a) ethanol b) n-hexane c) water as solvent and d) pure n-hexane and e) pure ethanol and f) pure H₂O as controls on percent weight loss of wood exposed to the termite colony.

In this study, it was hypothesized that there exists no significant difference in the effectiveness of the Neem extract using the three solvents and the pure n-hexane and ethanol as controls in terms of percent weight loss of wood exposed to termite colony.

This study was able to establish the following findings:

1. Of all the treatments, the highest percent weight loss was found when wood block was treated with pure ethanol (5.04%) and lowest when treated with pure water (1.75%).

2. There is no significant difference between the three setups specifically the ethanol extract, N-hexane extract and aqueous extract.

3. The percent weight loss on wood when treated with aqueous extract is significantly greater than when wood is treated with pure water. The percent weight loss on wood when treated with ethanol extract is significantly greater than when wood is treated with pure ethanol.

Conclusion:

The results of the study is inconclusive of the potential of neem leaf extracts to prevent termite attacks because of some inaccuracies in data gathering and the conduct of experiment.

Recommendations:

Future studies which is similar to this study is recommended to determine the most suitable part of Neem tree to be used for extraction, try other extraction methods and solvents that may be used for the extraction processes, lengthen the time of exposure of wood to termites, and look for a better place to perform the experiment.

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APPENDIX A

Extract	Before	After	Weight Loss	%Weight Loss
Ethanol				
1	135.6	130.2	5.4	3.98
2	85.7	84.5	1.2	1.4
3	121.2	117.2	4	3.3
4	88.9	87.4	1.5	1.69
5	130.2	126.55	3.65	2.8
N-Hexane				
1	87.3	85.6	1.7	1.95
2	125.9	119.4	6.5	5.16
3	86.5	85.4	1.1	1.27
4	134.5	128	6.5	4.83
5	129.8	125.4	4.4	3.39
Aqueous				
1	84.5	79.4	5.1	6.03
2	128.2	124.1	4.1	3.19
3	125.8	122.3	3.5	2.78
4	99.2	95.6	3.6	3.63
5	90.3	97.1	3.2	3.54
Pure Ethanol				
1	90.3	86.9	3.4	3.76
2	149.5	139.4	10.1	6.75
3	83	76.9	6.1	7.35
4	141.5	137.1	4.4	3.11
5	139.3	133.4	5.9	4.23
Pure Water				
1	135.5	132.4	3.1	2.29
2	129.1	124	5.1	3.95
3	80.7	80.4	0.3	0.37
4	95.5	95	0.5	0.52
5	84.4	82.8	1.4	1.66
Pure N-Hexane				
1	129.9	124.8	5.1	3.92
2	87.3	84.1	3.2	3.66
3	81	75.7	5.3	6.54
4	124.1	120.1	4	3.22
5	139.2	136.1	3.1	2.22
Untreated				
1	83	79.7	3.3	3.97
2	86.6	84.2	2.4	2.77
3	113	108.9	4.1	3.62
4	125	120.4	4.6	3.68
5	88.5	84.4	4.1	4.63

Weight
 of wood
 (in grams)
 before and
 after
 application of
 extracts,
 weight loss and
 percent weight
 loss on wood.