Effect of *Grevillea robusta* Leaf extract in comparison with synthetic phytol on *Heterotermes indicola* Wasmann (Blattodea: Rhinotermitidae) and its gut flagellates

By

Shanzae Tahir

Department of Zoology

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad, Pakistan

2023

Effect of *Grevillea robusta* Leaf extract in comparison with synthetic phytol on *Heterotermes indicola* Wasmann (Blattodea: Rhinotermitidae) and its gut flagellates

A Dissertation Submitted to the Department of Zoology, Quaid-Azam University in partial fulfillment for the requirements of Degree of

Master of Philosophy

In

Zoology

(Parasitology/Entomology)

By

Shanzae Tahir

Department of Zoology

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad, Pakistan

2023

"**In the name of ALLAH, the Most Beneficent, the Most Merciful**"

This Work is dedicated

to

My family, especially my parents. They are my life. I have done nothing and will not be able to do anything even in future without having them on my back.

Declaration

The material and information contained in this thesis "**Effect of *Grevillea robusta* Leaf extract in comparison with synthetic phytol on *Heterotermes indicola* Wasmann (Blattodea: Rhinotermitidae) and its gut flagellates**" is my original work. I have not previously presented any part of this work elsewhere for any other degree.

_____

Shanzae Tahir

LIST OF CONTENTS

LIST OF ABBREVIATIONS

Sr.	Abbreviations	Full names
1	LC	Lethal Concentration
2	DDT	Dichlorodiphenyltrichloroethane
3	BHC	Benzene hexachloride
4	CAT	Catalase
5	ROS	Reactive oxygen species
6	W/V	Weight by volume
7	PYT	Phytol
8	PA	Phytanic acid
9	EO	Essential oils
10	DNA	Deoxyribose nucleic acid
11	GC-MS	Gas chromatography-mass spectromet
12	FTIR	Fourier transform infrared

LIST OF TABLES

LIST OF FIGURES

Acknowledgments

I am thankful to **Allah**, the lord of the heavens and the earth. To Him be glory throughout the heavens and the earth, and He is exalted in power, full of wisdom. Many thanks to Prophet **Muhammad** (Peace be upon him) for Whom the whole universe is shaped, who enabled us to worship only one Allah and brought us out of darkness and enlightened the way of heaven.

I am extremely grateful to my esteemed supervisor **Prof. Dr. Naveeda Akhter Qureshi** for her invaluable supervision, support, and tutelage during my MPhil degree. Their immense knowledge and plentiful experience have encouraged me in all the time of my academic research.

My gratitude extends to the **Department of Zoology**, Quaid-i-Azam University Islamabad for providing me the necessary research environment. I express my deepest gratitude to the whole faculty and all those teachers who had always been a source of motivation and inspiration for me and whose support and guidance helped me to get through all the difficulties.

I am gratefully indebted to **Prof Dr. Amina Zuberi**, Chairperson, Department of Zoology, Quaid-i- Azam University, Islamabad.

I would like to acknowledge **Dr. Muhammad Afzal** who guided me throughout my research. I would like to acknowledge my fellow lab mate **Eisha Ehsan, Tahseeen Zafar and Nabeel ur Rehman**, Senior **Sandal Miraj** for their support and cooperation throughout my research.

I gratefully acknowledge and thank my **Parents** especially my father whose contribution in our education is remarkable and cannot be explained in words, their love, care, support, encouragement, and prayers have always enlightened my way throughout every task of life. Finally, I again thank **Allah** the Almighty, the constant Guider, and the source of all good, who listened to my prayers and destined the accomplishment of this task.

Shanzae Tahir

Abstract

Heterotermes indicola (Blattodea: Rhinotermitidae) is the major wooden structural pest throughout the world except Antarctica. Pakistan is one of the foremost home of *H. indicola* having highly favorable environment. In the present study, phytol a synthetic commercial compound was experimented for its termiticidal activity along with its synergistic effect with *Grevillea robusta* ethanolic leavaf extract. *G. robusta* leaves were shade dried and their ethanolic extract was prepared via Soxhlet apparatus. LC_{50} of both Phytol and *G. robusta* was calculated and found to be 22.072 and 991.760 respectively. Five bioassays were setup to evaluate termiticidal and protozoacidal effects of Phytol (10, 20, 30, 40, 50, 100 and 150 ppm) and *G. robusta* leaf extract (100, 500, 1000, 1500 and 2000 ppm). Synergism of Phytol (50ppm) and *G. robusta* (2000ppm) along with Repellency and antifeedant activity of Phytol (10, 20, 30, 40, 50, 100 and 150 ppm) were also evaluated. Later on synergistic response of *G. robusta* and phytol on non-durable wood was experimented. A dose dependent mortality and foraging response was observed when *H. indicola* was force-fed on phytol and *G. robusta* leaf extract. The 100% mortality at maximum concentration (150 ppm) of phytol and 77% mortality at maximum concentration (2000 ppm) of leaf extract. Both phytol and *G. robusta* leaf extract showed significant decline in the population of gut protozoa where phytol caused 88% and leaf extract caused 60% reduction of hindgut protozoa count as compared to control groups ($P < 0.001$). Phytol also showed repellent and antifeedant activities against *H. indicola*. Synergistic effect of silver oak leaf extract and phytol was also observed which showed 96% mortality with 86% reduction in gut protozoa. Phytol and leaf extract were also separately and synergistically tested on non-durable woods. *H. indicola* showed significant mortality (100%) ($P < 0.001$) and decline in hindgut protozoa (92%) in all the treated groups (Phytol, leaf extract and synergism).

Graphical Abstract

INTRODUCTION

1.1 Termites

Termites are small insects that are 10% of the entire animal biomass (van Huis, 2017). Scheffrahn, (2008) reported that the biomass of termites is believed to be comparable to the biomass of vertebrates. Termites are found in all continents of the world other than Antarctica. Primarily termites are present in the subtropical and tropical regions. (Brune, 2014; Bonachela *et al*., 2015). According to data collected from various sources, the total number of termite species in the world is between 2500 to 3000 (Govorushko, 2018). Approximately 1000 species of termites are found in Africa, 435 in Asia, 400 in South America, 360 in Australia, 50 in North America, and 10 in Europe (Krishna *et al*. 2013).

Like honeybees and ants, termites are social insects and their castes consist of king, queen, workers, soldiers, and nymphs. (Watanabe *et al*., 2014). Workers are responsible for distributing food within the colony of termites. The soldier caste which is fed by workers is accountable for the defense of the colony against invaders (Franco Cairo *et al*., 2016). There are a few or only a single queen who is responsible for the production of all the offspring. Workers also forego reproduction for the beneficial activities of the colony. The life span of the queen (4-25 years) is 10-100 times more than that of workers (10-14 months) (Tasaki *et al*., 2017).

On the basis of habitat, termites can be categorized as dry wood termites which choose to live in dry wood, damp wood termites which prefer clammy wood, and subterranean termites which spend their lives under the soil (Govorushko, 2019). However, the feeding behavior of termites divides them into four categories. Those four categories are soil feeders, wood feeders, grass feeders, and fungus growing (Brauman *et al*., 2015).

1.2 History and Classification

Traditionally termites are classified into higher and lower. Lower termites have protozoa and bacteria in their intestine for the digestion of cellulose whereas higher termites have lost gut protozoa and use bacteria and fungi for cellulose digestion. Termites have seven families out of which six (Mastotermitidae,

Kalotermitidae, Termopsidae, Hodotermitidae, Rhinotermitidae, and Serritermitidae) have lower termites and one (Termitidae) has higher termites (Legendre *et al*., 2008).

The origin of termites is from Sub-Saharan Africa with the highest specie number in rainforests (Poulsen *et al*., 2014). The social structure, phenotypes, and diversity of diet make termites' evolution an interesting topic. Termites evolved from a group of wood-feeding cockroaches and appeared as a parallel group with *Cryptocercus* genus of cockroaches (Lo *et al*., 2000). The exact timing of termite appearance and their diversification is not completely understood, although the fossil record provides a glimpse of their evolutionary history. Cretaceous, 110-135 Ma has the first undisputed fossils of termites. These fossils are of Kalotermitidae, Mastotermitidae, Hodotermitidae, or other extinct families. Mid-Cretaceous, 98 Ma has the oldest fossils of Rhinotermitidae (Krishna and Grimaldi, 2003; Krishna *et al*., 2013). The first fossil of Termitidae is quite recent and is from the early Eocene, 50 Ma (Engel *et al*., 2011). Afterward, this family remains uncommon in the fossil record until Dominican amber, 18 Ma, where it reappears in a diversified form including some modern genera such as *Microcercerotermes, Constrictotermes,* and *Nasutitermes*. Therefore, it is suggested by the fossil record that probably termites diverged in the late Jurassic from cryptocercid roaches. Termitidae has become more abundant recently and has 75% of the modern species of termite which are a result of diversification over the last 50 Ma. Molecular clocks and supertree methods have also been used to extant termite families and to estimate the divergence dates. Ware et al. (2010) did the most comprehensive and recent analyses to estimate the divergence rates and dating of termites. According to this study, termites originated in the middle of 172 and 235 Ma while Termitidae originated somewhere amid 44 and 132 Ma. In order to get a more precise picture, more studies are needed (Bourguignon *et al*., 2015).

1.3 Invasion

Termites started spreading outside their native habitat initially via infested timber. The earliest known invasion is of *Coptotermes. formosanus* into Japan from China earlier to the 1600s (Husseneder *et al*., 2012). Before spreading to more native habitats, termites invaded environments modified by humans. The range of termites is

constantly expanding. In 1969, 17 termite species moved to new regions whereas this figure has now touched 28 species (Buczkowski and Bertelsmeier, 2017).

1.4 Ecological importance

Termites are among soil ecosystem engineers along with earthworms and ants (Lavelle *et al*. 2006). Termites are the dominant microbiota in the tropics and other areas (Bonachela *et al*. 2015). They execute various ecosystem services such as breakdown and recycling of carbon-based matter, soil loosening, soil fertility, removal of dung, soil formation, pollination, and greenhouse gas emission. As organic decomposers, termites consume all plant remains and dying wood materials (Mugerwa, 2015; Qasim *et al*. 2015). The mandibles of termites are gifted to slice plant material and their gizzard can grind it to increase its surface area for soil microorganisms hence they break down and recycle one-third of yearly dead wood production in tropical and subtropical regions (Jouquet *et al*., 2011; Harit *et al*., 2017)

Termites are of great importance in the tropical savanna when it comes to dung removal. The dung of 18 mammalian species is a food source for at least 126 termite species. In tropical regions, 33% of dung in a specific habitat is cleared by termites within a month (Freymann *et al*. 2008). Termites whether living on the soil surface or in the earth, all are involved in soil mixing which results in breaking surface crust, reducing the compaction of soil, increasing the porosity of the soil, improving the infiltration of water into the soil, and improving the water-holding capability of the soil (Lofile and Kubiniok 1996). Termites increase soil pH and hence their fertility (Li *et al*., 2017). Termites are a food source for a wide variety of animals. Forty-three species of termites are edible and are a food source for humans and livestock (Figueiredo *et al*., 2015). Termites are used as model organisms for scientific studies and also for academic studies of learning and elementary activity of the brain and for the social behavior of animals (Du *et al*., 2017). It is testified that 10 termite species are used for medical purposes (Figueiredo *et al*., 2015).

1.5 Termites as a Pest

Off all the three thousand termite species that are described only a small number is involved as agricultural pests (Brune, 2014). Termites are insect pests that are polyphagous and highly devastating. They can attack agricultural crops and plants at

any developmental stage (Qasim *et al*., 2015). Agricultural losses due to termite attacks are higher in sub-humid and semiarid tropics. Rain-fed crops are more susceptible to termite attacks than irrigated ones. Also, crops are more likely to be damaged by termites when under stress such as droughts, disease, or any physical damage. Eastern Africa is most affected by crop damage by termites (Mitchell, 2002). The activity of termites is not good for the quality of crops, it increases toxin content and reduces the market value of the crop (Jouquet *et al*., 2018). In Africa, *Microtermes* are serious pests of plants and agricultural crops (Negassa and Sileshi, 2018). Indonesia and Malaysia are palm oil producers but the agriculture in these countries is highly affected by termite activities. *C. curvignathus* is responsible for a significant loss in palm oil production in these areas (Yii *et al*., 2016).

Urban forestry and plantations are also significantly affected by termites. Some species of termites can cause the mortality of healthy trees, for example, termite related death of *Eucalyptus grandis* and *Eucalyptus camaldulensis* in Zimbabwe is reaching 100% (Rao *et al*., 2012; Rouland-Lefevre, 2011). The most malicious pest is the genus *Syntermes*, it can cause damage to debarks plant rings, roots and death of the plant. High densities of such termites in the areas of cultivation are a cause of economic loss due to increased replanting activities (Santos *et al*., 2016). The damage produced by termites and the degree of their influence on plantations is highly determined by geographical region. For example, in China, 303 tree species from 76 families have been suffering from termite attacks. In southern hilly areas of China, 40-60% of trees die due to such attacks (Li *et al*., 2010). The highest infestations and most of the losses in India are in Candolim (*Hymenolobium petraenum*), pine (Pinus sp.), jequitiba (Cariniana sp.) (Cosme *et al*., 2018).

Mainly termites feed on cellulose and its derivatives. According to research, a colony of termites that consists of 200,000 individuals can eat up to 5.4 kg of cellulose in a single year (Jones *et al*., 2015). Damage caused by termites includes cellulose content of vegetables, structures such as homes, decks, bridges, dams, surviving poles, walls, roads, insulation of underground pipes, and cables. Paper, household furniture, rubber products, plastic materials, synthetic films, and food products are also damaged by termites (Govorushko, 2019).

83 species of termites are documented to cause destruction to structures containing wood (Su and Scheffrahn, 2000). Only four termite species are the main pests in Sydney, Australia, and *C. acinaciformis* is responsible for 80% of the damage to buildings (Froggatt, 1898). Museum collections and books are usually harmed by termites. In many libraries, museums, and archives, termite infestation spread to book collections, storage, and display archives from buildings and causes serious damage (Pinniger, 2012). Personal libraries are also affected by termite attacks, for instance in South America, termites' continuous presence made it difficult to find books older than 50 years (Govorushko, 2019).

1.6 Economic loss

Termites cause significant economic loss. The figure of harmful species of termites varies in different regions of the world. Nine Harmful termite species are found in North America, 24 in Africa, 16 in Australia, 26 in the Indian subcontinent, and 17 in West Indies and Central America.

The genera *Coptotermes* has the most pest species of termites among which 28 species are known pests to cause damage (Su & Scheffrahn, 2000). Globally, the economic loss instigated by termites is estimated as 22-40 billion USD annually (Afzal *et al*., 2019). Table 1.1 shows the economic loss caused by termites in different areas of the world (Govorushko, 2019).

Table 1.1 Estimation of economic loss caused by termites.

Region	**Loss per year**	**Source**
Australia	$ 1.5 billion	Staunton 2012
China	$ 0.3 billion	Junhong & Bingrong 2004
China	$ 1 billion	Lenz *et al*. 2013b
China	$ 217 million (only forestry)	Li *et al*. 2010
Indonesia	$ 200-300 million	Yusuf, 2004
Japan	$ 0.8-1.0 billion	Tsundoda & Yoshumura, 2004
Malaysia	$ 4-5 million (only control)	Lee 2002
Thailand	$ 500 million	Vongkaluang 2004
Southwestern USA	$ 1.5 billion	Su & Scheffrahn 1990
USA	$ 2-3 billion	Lenz et a*l*. 2013b
USA	$ 11 billion	Su 2002
USA	$ 5 billion	Tvedten 2005
World	$ 15-20 billion	Jones *et al*. 2015
World	$ 20 billion	Ye *et al*. 2004
World	$ 40 billion	Rust & Su 2012

(Govorushko, 2019)

1.7 *Heterotermes indicola*

Heterotermes indicola is a subterranean termite and a notorious pest of wood and other cellulosic materials. It can cause significant economic losses wherever it is found. In Pakistan, genus *Heterotermes* is of significant importance and is attributed as the most damaging group among subterranean termites in the region (Manzoor and Mir 2010). Warm neotropics such as Australia and Indian subcontinenet is the main habitat of this genus (Saljoqi *et al*., 2012; Misbah ul haq *et al*., 2015). *H. indicola* Wasmann

(Blattodea; Rhinotermitidae) is present primarily in tropical and sub-tropical regions of Afghanistan, China, Pakistan, and India (Malti, 2006). In Pakistan, the maximum damage instigating termite species causing significant economic loss are *C. heimi* (Wasmann), *H. indicola* (Wasmann), *Odontotermes obesus* (Rambur), *and Microtermes obesi* (Holmgren). *H. indicola* is the major structural pest in Pakistan and has been placed among the most vicious termite species of Lahore (Manzoor, 2010). As the most notorious lower termite, it attacks various trees including: Akk (*Calotropis procera*), Mulberry (*Morus alba*), Lokat (*Erioborrya japonica), Populus euramericana, Dalbergia sissoo, Melia azedarach*, *Acacia spp*. and dead woods (Chaudhry and Ahmad, 1972). Other than wooden structures, *H. indicola* can infest other cellulosic materials. Colonies of *H. indicola* build mud ducts to travel from soil to the cellulosic material in connection. This process facilitates the transfer of moisture to wooden or cellulosic materials which results in greater attacks of termites. The infestation of *H. indicola* inside houses is judged by mud tubes on walls and ceilings. It is observed that it only consumes the softer part of the wood and always keeps contact with the ground soil which is its breeding place (Manzoor, 2010).

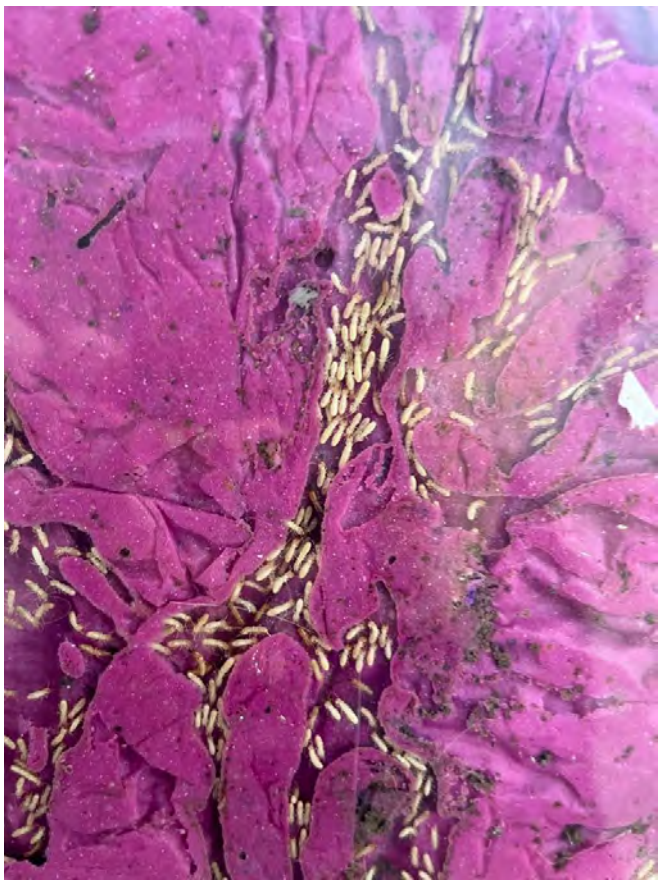

Fig1.1: Tunnels of *Heterotermes indicola*

1.8 Termite Gut flagellates

Termites have strong mutualistic relationships with various endomicrobes (bacteria, flagellates, and archea). These endomicrobes live in the hindgut of termites and make them capable to digest the cellulose they eat. Among these hindgut-inhibiting microbes, mainly flagellates produce cellulase enzyme which helps in the digestion of plant cellulose and makes it suitable for assimilation (Breznak, 2000). This mutualistic relationship makes us believe that logically termites cannot survive without their gut flagellate fauna. By focusing on their gut symbionts, we can effectively control termite infestations (Stingl *et al*., 2004, 2005; Ikeda *et al*., 2007; Ohkuma, 2008) as their removal can make the termites starve to death (Honigberg, 1970; Brune, 2009).

1.9 Termite control

According to an estimation, annually billions of dollars are expended to control termites globally (Tsunoda, 2003). Different kinds of management systems for termites such as physical and chemical control methods have been used against damaging termites. The unavailability of satisfactory control methods against termites is because of their long life, cryptic nature, giant colony size, and wide range of feeding places (Salihah *et al*., 1994). Liquid termiticides as chemical control have been used to treat soil. Treating the foraging areas of termites with baiting systems and dust has been used as a cost-effective and long-lasting method, but in post chlordane era, termite control seemed to be very difficult even with new technologies and chemicals (Potter, 2002). New insecticides against formation termites have been examined in various studies (Delgarde and Rouland-Lefevre, 2002; Shelton and Grace, 2003). However, the toxicity of insecticides for *H. indicola* is less known. The effectiveness of any insecticide against termites is mainly dependent on its toxicity and the mode of action of its compounds. Some other factors which define the effectivity of termiticides are termite susceptibility to that insecticide, formulation applied, application protocol, and soil properties such as soil group, pH, particle size, compactness, and organic matter (Osbrink *et al*., 2001).

Mainly termite control is centered on synthetic insecticides. Insecticides are divided into main three categories: repellent, toxic, and non-repellent or non-repellent with tardy toxicity. Insecticides of the repellent category work by repelling the foraging termites and thus protect structures. Also, some repellent insecticides such as pyrethroid in sub-lethal concentrations inhibit the tunneling activity of termites. Toxic and non-repellent insecticides work as killing termites by contact. The action (speed of killing) of slow-acting insecticides varies with the insecticide concentrations (Raj and Rust, 2014). Non-repellent insecticides have a delayed mode of action and their use has increased over the past many years. Termites are incapable to detect the treated soil and continue their foraging activity (Renato *et al*., 2007). Basically, all insecticides are designed to stop the termite from penetrating the soil either by repelling or killing them (Yeoh and Lee, 2007). Any successful method for the control of *H. indicola* has not been discovered yet. The reason for this is the absence of information about the organization of its colonies as they do not construct mounds or any other structure that

is easily detectable (Zubair *et al*., 2007). Non-repellent insecticides are potentially harmful as they transfer horizontally. This has made termite control an important paradigm (Neil *et al*., 2008). Chlorinated hydrocarbons such as Chlordane, Aldrin, Dieldrin, DDT, Heptachlor, and BHC have been effectively used against termites in the past but now health and environmental concerns have phased out their use (Rust and Saran, 2006).

Traditionally highly repellent insecticides with long residual effects were used to treat soil in order to flood the targeted area. This method is ineffective, expensive, and environmentally unfriendly (Su *et al*., 1993). On the other hand, insecticides have slow action and are safe and palatable. Termites carry these chemicals with them while foraging and hence bring them back to the entire colony (Sattar, 2002).

The termites should penetrate the treated soil in order to check the efficiency of termiticides (Su *et al*., 1997). Repellent compounds are therefore being replaced by non-repellent compounds. For instance, non-repellent termiticides imidacloprid (Premise), fipronil (Termidor), and Chlorfenapyr (Phantom) are prioritized over Cadusafos and Bifenthrin. Non-repellent termiticide will kill the termite and would not allow it to cause any structural damage after allowing it to enter the treated soil (Kubota *et al*., 2007).

Chemical treatments which are highly effective for the treatment of subterranean termites are available to control infestations but the frequent use of such fast-acting insecticides for termite control has created numerous environmental and biological hazards in soil, air, water, and food. Moreover, these treatments are costly and require special techniques. Conventional pesticides have the disadvantage of killing the beneficial insects which consume insect pests. A single spray can disturb the balance between beneficial predators and pests. Synthetic chemicals can stay in animal bodies and respective environments causing multiple problems for years. Resistance to pests against conventional pesticides has led us to make more powerful insecticides (Parihar and Singh, 1992).

Fipronil is an insecticide that is used in the public health sector and to protect agricultural crops. In laboratories, 5-ply plywood is manufactured by adding fipronil to

the formulation of phenol-formaldehyde which is a resin. Fipronil loading is very effective against termites on plywood samples (Smith and Rust, 2007).

Many synthetic termiticides have been eradicated from markets these years due to environmental and toxicological concerns (Little *et al*., 2010). In this era, the environment is becoming increasingly sensitive towards recalcitrant chemicals which is forcing manufacturers to replace these toxic insecticides with environmentally friendly and biodegradable alternatives. Botanical biocides are a considerable approach that is present in the heartwood of natural plant species. The heartwood and bark of many wood species contain polyphenolic compounds which are strong antioxidants (Chang *et al*., 1990). Studies have revealed the presence of phenolic compounds such as flavonoids, tannins, stilbenes, and lignans in the heartwood. These compounds have toxic properties which protect the plant from termite attacks. These toxic compounds present in the heartwood have antioxidant or free radical scavenging properties (Doi *et al*., 2001; Sroka and Cisowski, 2003; Morimoto *et al*., 2006; Ragon *et al*., 2008; Little *et al* 2010). Antioxidant enzymes present in insects include catalase (CAT), superoxide dismutase, esterase, glutathione reductase, and glutathione transferase. The antioxidants present in plants act as pro-oxidants and can effectively reduce the antioxidant enzyme system of insects (Lukasik, 2007). This reduction will cause an increase in reactive oxygen species (ROS). These ROS are cytotoxic in nature and can form lesions in the lumen of the insect gut and ultimately death can occur (Barbehenn, 2002).

1.10 Chronological study

The termiticidal potential of four insecticides Chlorfenapyr, Imidacloprid, Bifenthrin Fipronil, and Cadusafos at various concentrations was tested against *H. indicola* for their repellency and toxicity. Results of this study revealed that Imidacloprid, Chlorfenapyr, Fipronil, and Bifenthrin were toxic against *H. indicola*. Bifenthrin was repellent at every tested concentration while Fipronil was repellent against termites only above 25 ppm. Chlorfenapyr was a non-repellent termiticide (Manzoor *et al*., 2012).

Heartwood extractives of *Tectona grabdis* (L.f), *Cedrus deodara* (Roxb.), *Dalbergia sissoo* (Roxb.), and *Pinus rocburghii* (Sarg.) were tested for their antioxidant potential against *Heterotermes indicola* (Wasmann). According to the results, *D. sissoo* showed maximum antioxidant activity and it was independent of the concentrations for all the extractives. *T. grandis and D.sissoo* extractives significantly reduce the glutathione-s-transferase activity in the gut of termite workers whereas, *P. roxburghii* extractives reduce esterase activity. Catalase activity is not altered by any of the used extractives (Hassan *et al*., 2018).

The synergistic effect of heartwood extracts from *Dalbergia sissoo, Tectona gradis, pinus roxburghii,* and *Cedrus deodara* with linseed oil was studied for its termiticidal potential against *H. indicola*. Results show that solvent-extracted heartwood is less effective than non-extracted heartwood for all the durable plant species. Also heartwood extracts combined with linseed oil have a synergistic effect and cause higher termite mortality compared to both used alone and control group. Non-durable wood blocks treated with extracts and oil were less likely to lose mass than all other groups when exposed to *H. indicola* (Hassan *et al*., 2020).

The entomocidal effect of three medicinal plants was evaluated against *H. indicola*. Aqueous leaf extracts of *Carica papaya, Bougainvillea glabra,* and *Helianthus annus* were prepared and their various concentrations were applied to the termite. The results of this study revealed that all these extracts are effective and can be used against *H. indicola* (Aihetasham *et al*., 2017).

Heartwood extracts of two *Morus* species (*Morus nigra* and *Morus alba*) were tested for their toxicity, natural resistance, and repellency against *H. indicola*. Results indicate that both *Morus* species are resistant against the termite but *Morus alba* is more resistant than *Morus nigra*. The non-durable wood of *Populus deltoides* vacuum-pressure treated with *Morus* species also showed resistance while more mortality of *H. indicola* was observed where Morus alba was used. It can be concluded that *Morus alba* can be used to make environment-friendly termiticides (Hassan *et al*., 2019).

The medicinal plant of Ocimum sanctum was used for its toxicity and repellency against *H. indicola*. Crude extracts of inflorescence, root, leaf, and stem of the plant were made in different solvents such as chloroform, hexane, methanol, butanol, water,

and ethyl acetate were studied. According to the results, maximum mortality of termite was observed in ethyl acetate leaf extract whereas, minimum mortality was noted in stem extract made in water. Maximum repellency was observed in methanol root extract while minimum repellency was shown by water extracts (Manzoor, 2011).

The three insecticides regent (Fipronil), tracer (Spinosad), and Match (Lufenuron) were tested as slow toxicants against *H. indicola.* 0.000312, 0.000156, 0.00078, 0.00039 and 0.000195%. concentrations were made. Among these three insecticides, maximum mortality and avoidance were observed in the group treated with regent (Fipronil). Even before 10 days of the experiment, it showed 100% mortality in all concentrations and hence was considered as highly toxic. Lesser concentrations of tracer (Spinosad) also caused 100% mortality and hence can be regarded as a slow-acting toxicant. The first concentration of match (Lufenuron) could cause 80 % mortality on the 10^{th} day (Saljoqi *et al*., 2014).

The termiticidal potential of five plant extracts garlic (*Allium sativum*), black tea (*Camellia sinensis*), turmeric (*Curcuma longai*), ginger (*Zingiber officinale*), and green chilies (*Capsicum annum*) were tested against *H. indicola*. 1:2 ratios were used for garlic, green chilies, and ginger whereas, 1:4 (W/V) was used for turmeric and tea. Garlic causes 100% mortality after one day. After garlic, green chilies, turmeric, ginger, and black tea were effective sequentially. The behavioral response of *H. indicola* was also accessed and results showed that the speed of termite workers was maximum in turmeric as compared to others, with passing time, workers became excited (Saljoqi *et al*., 2012).

The leaf extract of silver oak (*Grevillea robusta*) was also checked for its termiticidal effect against *H. indicola*. The ethanol-solvent system was used to make the extract via the Soxhlet apparatus. 1-20 mg/mL was the selected dose range to evaluate the mortality, foraging response, tunneling activity, and gut protozoa count. Results showed maximum mortality of termites at 20 mg/ml along with the reduction in the population of gut protozoa. The tunneling activity was also significantly affected compared to negative controls and untreated groups. The non-durable wood vacuum pressure treated with the extract showed resistance and 100% mortality was observed

at the highest concentration. Hence, silver oak leaf extract can be used for the development of botanical insecticides (Afzal *et al*., 2019).

1.11 *Grevillea robusta*

Silver oak (*Grevillea robusta*) A. Cunn. Is native to Australia and belongs to the plant family Proteacea. This plant is also introduced to parts of Southern Europe, Asia, Africa, and the United States where it spreads invasively. Its normal length is up to 15-30m. *G. robusta* is a deciduous tree that is used in agroforestry and landscaping in order to conserve soil in the areas of East Africa and South Asia (Harwood and Booth, 1992). The roots and leaves of this plant contain allelopathic compounds which restrict the surrounding soil to grow other plants (Smith *et al*., 1998). The timber of this has ordinary strength and is used for packing cases, flooring, furniture, paneling, window frames, pencils, musical instruments, and baskets (Skolmen, 1974). In Sri Lanka and Kenya use this plant as a shade species for perimeter in tea plantations (Baggio *et al*., 1997). In past studies, the leaves of Silver oak have been used against the pests of stored grain, *Sitophilus oryzae* and *Callosbruchus chinesis* L. (Bhuvaneswari *et al*., 2014); Waqas *et al*., 2011). Other studies revealed the antibacterial, antifungal, antioxidant, antileishmanial, and larvicidal properties of Silver oak bark extracts (Cock, 2008; Cock and Ruebhart,2008; Samarth and Krishna, 2007; Takahashi *et al*., 2004; Ullah *et al*., 2014).

1.12 Phytol (PYT; 3,7,11,15-tetramethylhexadec-2-en-1-ol)

Phytol occurs abundantly in nature. It is produced almost in all organisms such as Plants (Ischebeck *et al*., 2006), algae (de Souza and Nes, 1969), and bacteria (cyanobacteria (Proteau, 1998). which can perform photosynthesis as a part of chlorophyll molecules. Ruminant animals also produce phytol as a metabolite during catabolism. As a result, phytol is regarded as the most abundant acyclic isoprenoid present in the Earth's biosphere (Rontani and Volkman, 2003). Phytol is released in the gut of ruminants as a result of the digestion of plant material. This phytol is first converted into phytanic acid (PA) then it is stored in the body as fat (Islam *et al*., 2015; Van den Brink and Wanders, 2006).

Initially, it was used as a fragrant but now its biological significance has been recognized. Its possible applications in the field of biotechnology and pharmaceutical has drawn attention recently. Many experiments have been conducted to recognize the metabolic role of phytol, and its pathophysiological contributions but its biological actions are still not sufficiently understood. Recent investigations about Phytol have revealed its metabolism-modulating, anxiolytic, cytotoxic, apoptosis, and autophagy including, antioxidant, anti-inflammatory, antinociceptive, immune-modulating, and antimicrobial effects (Islam *et al*., 2018).

Essential oils (EO) derived from plants have phytol as a major constituent and a lot of research is going on to confirm that the cytotoxic or antimicrobial activity of these EOs is because of phytol content in them. Generally, the antimicrobial potential of these essential oils is well documented (Murbach Teles Andrade *et al*., 2014; Prabuseenivasan *et al*., 2006), but the exact mechanism is not well understood. According to a hypothesis, the antimicrobial activity of these lipophilic molecules works by crossing the cell membranes and exerting inhibitory action on many targets simultaneously. The antimicrobial activity of phytol is reported against *Escherichia coli* (Ghaneian *et al*., 2015) and *Pseudomonas aeruginosa* (Pejin *et al*., 2015). EOs having phytol dysregulate the function of eukaryotic cells in multiple ways such as depolarizing the mitochondrial membrane and disrupting the permeability of the membrane (Bakkali *et al*., 2008). Consequently, ion channels are highly affected along with the proton pump and ATP pool. All these actions lead to the leakage of cytochrome C, radicals, diverse proteins, and Ca^{2+}. These conditions are similar to bioenergetics failure and oxidative stress. Also, the permeabilization of the mitochondrial membranes causes cell death by necrosis or apoptosis. Later prooxidative actions cause cell death. The effects define the action of phytol against *Aspergillus niger* and *Candida albicans* (Ghaneian *et al*., 2015). When EOs are applied at higher concentrations, they instantaneously cross the membranes. The oxidative effect of essential oils causes harm to macromolecules such as DNA, lipids, and proteins (Faix *et al*., 2007). Similarly, high concentrations of phytol cause cytotoxicity activity in *Schistosoma mansoni* (de Moraes *et al*., 2014). *Lacistema pubescent* leaves have a phytol-rich hexane fraction and hence it exerts anti-promastigote activity against both amastigote and promastigote forms of *Leishmania amazonensis* (da Silva *et al*., 2015). Phytol cytotoxicity has been studied a

lot to date for instance, in *S. mansoni* (de Moraes *et al*., 2014) and against lymphoid leukemia Molt 4β cells (Komiya *et al*., 1999). Phytol can also be used as a drug candidate against lung carcinoma (Islam *et al*., 2018).

Insecticidal properties of phytol are not studied properly. Benelli *et al*. studied the essential oil of *Stevia rebaudiana* (Asteraceae) for its insecticidal potential against the aphid *Metopolophium dirhodum* which is a pest of cereals. The chemical composition of EO was analyzed by gas chromatography-mass spectrometry (GC–MS). Results showed that phytol was the most effective aphicide (Benelli *et al*., 2020). Ashraf *et al*. studied the plant extract of *Grevillea robusta* against subterranean termites *Heterotermes indicola* and *Odontotermes obesus*. FTIR analysis of the plant extract reveals the presence of phytol in it along with other phytochemicals (Ashraf *et al*., 2020).

Aim

- Termite control through natural and synthetic chemicals

Objectives

- Termiticidal and Protozocidal potential of leaf extract and synthetic chemical
- Synergistic effect of both leaf extract and synthetic chemical.

MATERIALS AND METHODS

2.1 Collection of termites

Active workers of the termite *H. indicola* were collected from the infested storeroom in the basement of the Biological Science Department at Quaid-i-Azam University Islamabad, Pakistan. Plastic bottles containing moist toilet tissues and molasses (phagostimulant) were used as baits and were placed half-buried in the dark storeroom. After 15 days, baits were removed after inspection, and active and healthy workers were kept in Petri dishes at 27±1ºC. Relative humidity of 80±5% was maintained using damp cardboard. Termites were forced-fed on Whatman No. 2 filter paper for 15 days prior to conducting the experiment in order to remove wood particles and debris.

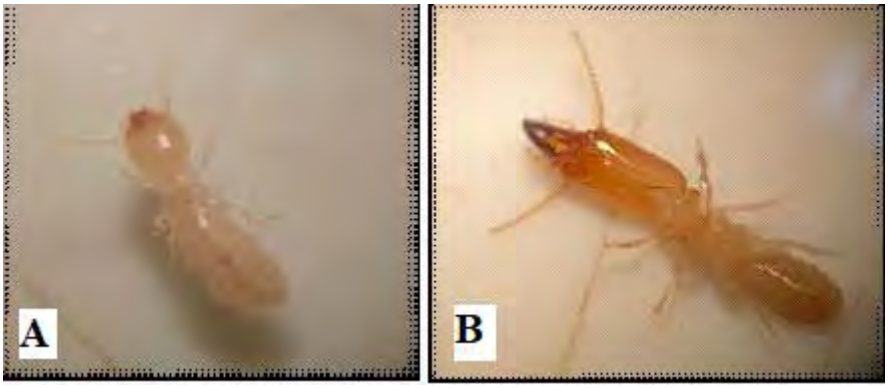

Fig 2.1: *Hetrotermes indicola* (A) worker (B) soldier

2.2 Leaf extract preparation

Grevillea robusta leaves were collected from Quaid-i-Azam University Islamabad, Pakistan. Leaves were shade dried in the temperature range of 27-37ºC for 20 days. An electric blender was used to make the powder of leaves. 30 g of leaf powder was used to make an extract in ethanol (300 ml) using the soxhlet apparatus (Shanghai Heqi, China). The resulting extract was further concentrated by evaporating the solvent using a rotary evaporator (R-300). To calculate the yield/gram, resultant aliquots were weighted after drying (Ordonez *et al*., 2006). A stock solution of 10,000 ppm was prepared by re-dissolving 1g of crude extract in 100 ml of distilled water. Five concentrations of 100, 500, 1000, 1500, and 2000 ppm were prepared by applying the formula:

$$C_1V_1=C_2V_2$$

Where C1: stock solution; C2: required ppm; V2: required volume and V1: volume to be applied.

Fig 2.2 *Grevillea robusta* leaves

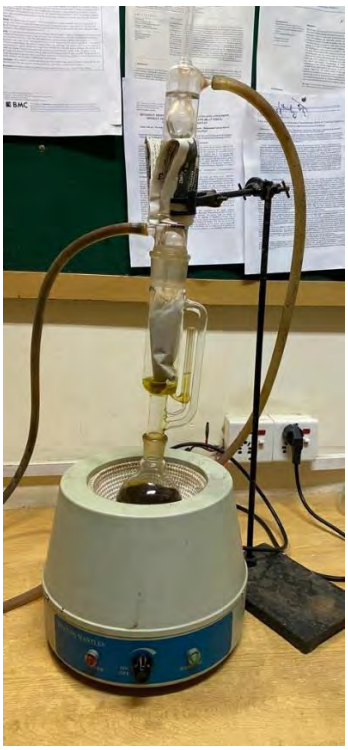

Fig 2.3 Soxhelet apparatus

2.3 Phytol

Phytol (97% solution of isomers) was purchased from Sigma-Aldrich (Munich, Germany). A stock solution of 300 ppm was prepared by dissolving 30µl phytol in 100 ml of distilled water. Seven concentrations of 10, 20, 30, 40, 50, 100, and 150 ppm were prepared by applying the following formula:

$$C_1V_1=C_2V_2$$

2.4 No-choice bioassay

The termiticidal effect of synthetic phytol was compared with the leaf extract of silver oak by conducting a no-choice feeding essay. For the substrate, we use filter paper (Whatman No. 1, 9.0 cm diameter) (Cheng *et al*., 2007; Elango *et al*., 2012). The filter paper was weighted before conducting the experiment and treated with 1.5 ml of each concentration (100, 500, 1000, 1500, and 2000 ppm) of plant extract and phytol (10, 20, 30, 40, 50, 100 and 150 ppm). The control group was treated with distilled

water only. After that, the treated filter paper was air dried at room temperature and positioned in Petri dishes (3"x3"). 30 termite workers and 10 soldiers were added to each petri dish containing treated filter paper. To maintain moisture, 0.5 ml of distilled water was periodically added to the bottom of all the petri dishes. Petri dishes were kept in the dark at 27±1ºC and 80±5 RH. All the experiments were conducted in replicates. After 15 days, percentage mortality was calculated for every concentration by applying the following formula:

$$\% \text{ Mortality} = \text{ODP} \div \text{TP} \times 100$$

Where: ODP=observed dead population of termites; TP=total population of termites.

The treated filter paper was cleared, oven-dried (60 °C for 12 h), and reweighed after 15 days. The percent weight loss for each treated filter paper available to termites was calculated using the formula (Sotannde *et al*., 2011).

$$\% \text{ Weight loss} = \frac{W_b - W_a}{W_a} \times 100$$

Where: W_b= before experiment weight of filter paper; W_a= after experiment weight of filter paper.

2.5 Repellency and antifeedant bioassays

The repellent effect of synthetic phytol against *H. indicola* was checked according to the method used by Hassan *et al*., (2018). A filter paper (9 cm in diameter) was taken. It was cut into two equal halves. One half was treated with 1.5 ml of each concentration of phytol (10, 20, 30, 40,50, 100, and 150 ppm) while the other half was used as a control and was treated with distilled water only. After treatment, these two halves were air-dried and rejoined using adhesive tape and were kept in a petri dish (3.5 x 3.5 diameter). 30 workers and 10 soldiers of *H. indicola* were placed in each Petri dish. After equal time intervals (every two hours), the number of termites on each half was noted and repellency (%) was calculated using the following formula (Kadir *et al*., 2014).

$$\text{Repellency (\%)} = 100 \times \frac{(NC-NT)}{(NC+NT)}$$

Where, NC: number of termites in control area; NT: number of termites in treated area.

To check the antifeedant activity of synthetic phytol, an estimation of the antifeedant indices based on the loss of filter paper weight of both treated and control halves was made. The following formula was used to calculate the antifeedant indices (Dungani *et al*., 2012; Hassan *et al*., 2018).

$$\text{Antifeedant indices (A)} = 100 \times \frac{(CC-TT)}{(CC+TT)}$$

Where CC: weight loss of the control area; TT: weight loss of the treated area.

Results were compared with the repellency and antifeedant bioassay of *G. robusta* against *H. indicola* by Afzal *et al*., (2019).

2.6 Synergistic effect

The synergistic effect of plant extract and synthetic phytol was checked by applying the effective concentrations i.e. 2000 ppm plant extract and 50 ppm phytol on the same filter paper in another no-choice bioassay using 50 termite workers and 10 soldiers. Both of these concentrations were also separately used in the same experiment for comparison. The control group was treated with distilled water only. Protozoa count, percentage mortality and percent weight loss of the treated filter paper was noted after 15 days and the results of the experiment were compared with both the separate concentrations for synergism.

2.7 Wood assay

The synergistic effect of leaf extract of *Grevillea robusta* and synthetic phytol against *H. indicola* was further tested on non-durable commercial wood species i.e. *Pinus roxburghii* (Chir pine), and *Populus deltoides* (cottonwood) by conducting no-choice bioassay. Wood blocks (1x1cm) were pre-weighted and dipped in 5ml of each concentration (2000 ppm leaf extract, 50 ppm phytol and both of these together for

synergism) overnight. Control was treated with distilled water. Wooden blocks were air-dried under a fume hood and each was placed in a separate petri dish (3.5 x 3.5 diameter). 50 termite workers and 10 soldiers were released in each petri dish. 0.5 ml of distilled water was periodically applied at the bottom of all the petri dishes to maintain moisture. All the petri dishes were placed in the dark at 27±1ºC and 80±5 RH. Protozoa count, percentage mortality, and percent weight loss of the treated wood was checked after 15 days and the results of the experiment were compared with both the separate concentrations for synergism.

2.8 Protozoa count

The comparison of the effect of synthetic phytol and leaf extract on the hindgut flagellates and the synergism of both was evaluated using the method by Hassan *et al*., (2017). Workers of *H. indicola* were fed on the filter paper and wood treated with various concentrations of phytol and extract accompanied by control of distilled water for 14-15 days as described in no-choice bioassays. Starvation control was also conducted by depriving the workers of their food source. After 15 days, five workers from each treatment were taken and their hindguts were removed using sterilized forceps and needles. Contents of five hindguts were pooled and considered as a single sample for each treatment. That sample was homogenized in 250µl of 0.2% saline solution.

Ten microliters of the resulting contents of the gut were taken and loaded on a Neubauer hemocytometer. The mean numbers of the protozoa were calculated by counting them in four squares of each chamber using a digital biological trinocular microscope. All flagellate species were included in the count and the method used to calculate the protozoa was according to Lewis and Forschler (2014);

$$X = \frac{\left(No.\ of\ cells\ counted \times volume\ of\ saline\ \frac{soltion}{sample}\right)}{\left(Volume\ of\ homocytometer \times No.\ \frac{termites}{samples}\right)}$$

Results were compared with the control to check the fall in the population of gut flagellates.

Fig 2.4. Gut of *Heterotermes indicola*

2.9 Statistical analysis

One-way ANOVA was used in each bioassay to determine the significance of the effects of various concentrations of silver oak leaf extract and phytol on the response (termite and its gut protozoa), Tukey' analysis was also used to evaluate the significance among groups. The LC_{50} value of various concentrations of silver oak leaf extract and phytol was evaluated using probit analysis and for all statistical evaluations software SPSS 2021 version was used.

RESULTS

3.1 No-choice bioassay

In no-choice bioassay, both plant extract and synthetic phytol greatly affected termite behavior and induced significant mortality. All the treated groups of *G. robusta* leaf extract and phytol show significant mortality compared to the control group ($F (df) = 216.900 (41)$; $P < 0.001$). Only 100 ppm concentration of plant extract gives mortality which was not significantly different from the control group. The LC_{50} value for *G. robusta* leaf extract and phytol was 991.760 ppm and 22.072 ppm respectively. In the case of *G. robusta* leaf extract, treatments of 1500 ppm and 2000 ppm showed more than 50% termite mortality, and maximum mortality was 77% in 2000 ppm treatment on the 15th day. Whereas, in the case of phytol, all treatments give more than 50% mortality except 10 and 20 ppm treatments. 85% mortality was observed on the 15th day in 50 ppm treatment while 100 ppm and 150 ppm concentrations give 98% and 100% mortality on the 15th and 5th day respectively. Hence, the termiticidal activity of phytol was time and dose-dependent.

All the termites of the treated groups consumed a significantly lower percentage of filter paper compared to the control group other than 100 ppm leaf extract treatment ($F (df) = 237.456 (41)$; $P < 0.001$). fivefold reduction in filter paper consumption was observed in higher concentrations of leaf extract and phytol compared to the control treatment. The reduction in the filter paper weight was concentration dependent. Treatments of higher concentrations of leaf extract and phytol consumed less filter paper. A negative correlation was observed in filter paper weight loss and termite mortality ($r = -0.666$; $P < 0.001$).

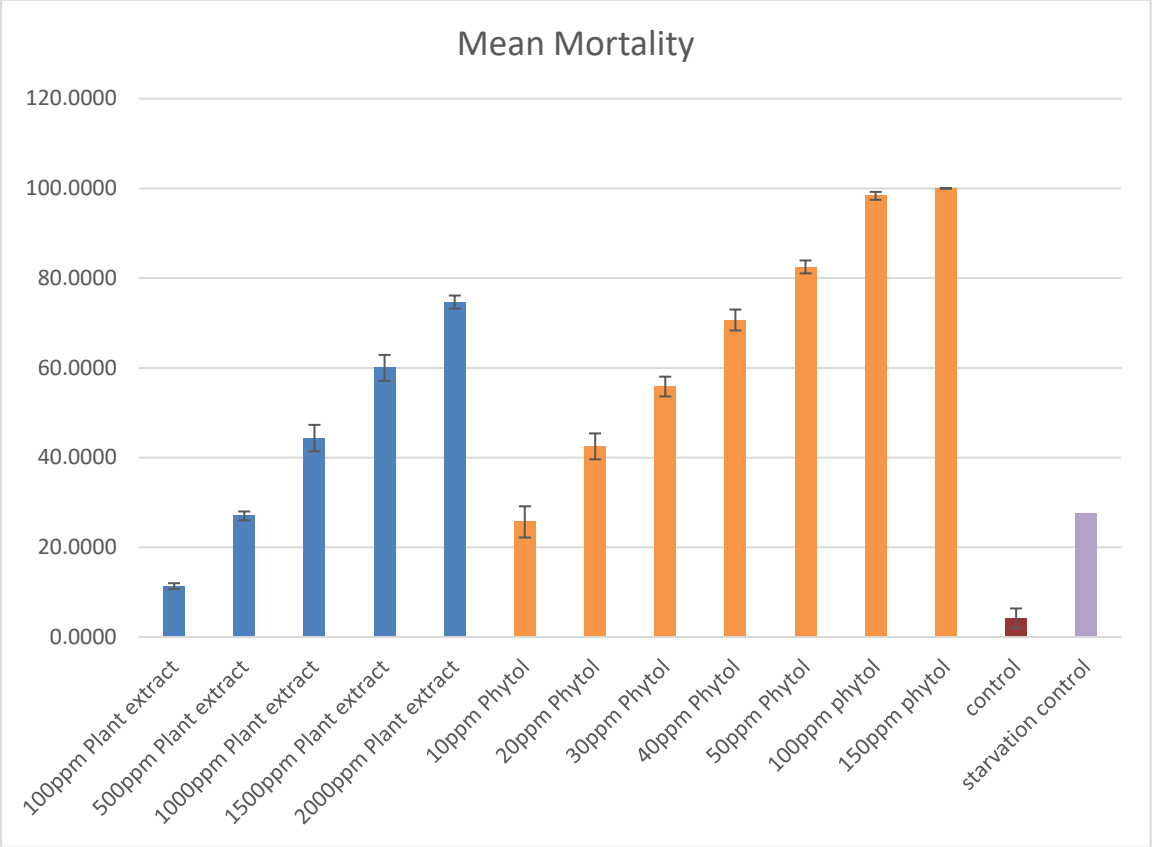

Fig 3.1 Mean termite mortality at various concentrations of *G. robusta* leaf extract and phytol.

3.2 Protozoa count

All the treatments in no-choice bioassay showed a protozoacidal effect on the 15th day, which was significantly different from the control and starved treatment other than 100 ppm leaf extract treatment ($F (df) = 240.214 (41)$; $P < 0.001$). This in vivo effect of silver oak leaf extract and phytol was dose-dependent and maximum reduction occur at 2000 ppm plant extract concentration (60%) and 100 ppm phytol (88%). Starved treatment showed an 87% reduction in gut protozoa while mortality was 27%.

The number of gut protozoa in all the treatments showed a negative correlation with mortality ($r = -0.801$; $P < 0.001$). It can be concluded that reduction in gut protozoa causes termite mortality as groups with high termite mortality had lesser gut protozoa. Also, protozoa count and filter paper weight loss are positively correlated ($r = 0.780$; $P < 0.001$), which means an increase in concentration has caused high mortality, less consumption of filter paper, and a reduction in gut protozoa.

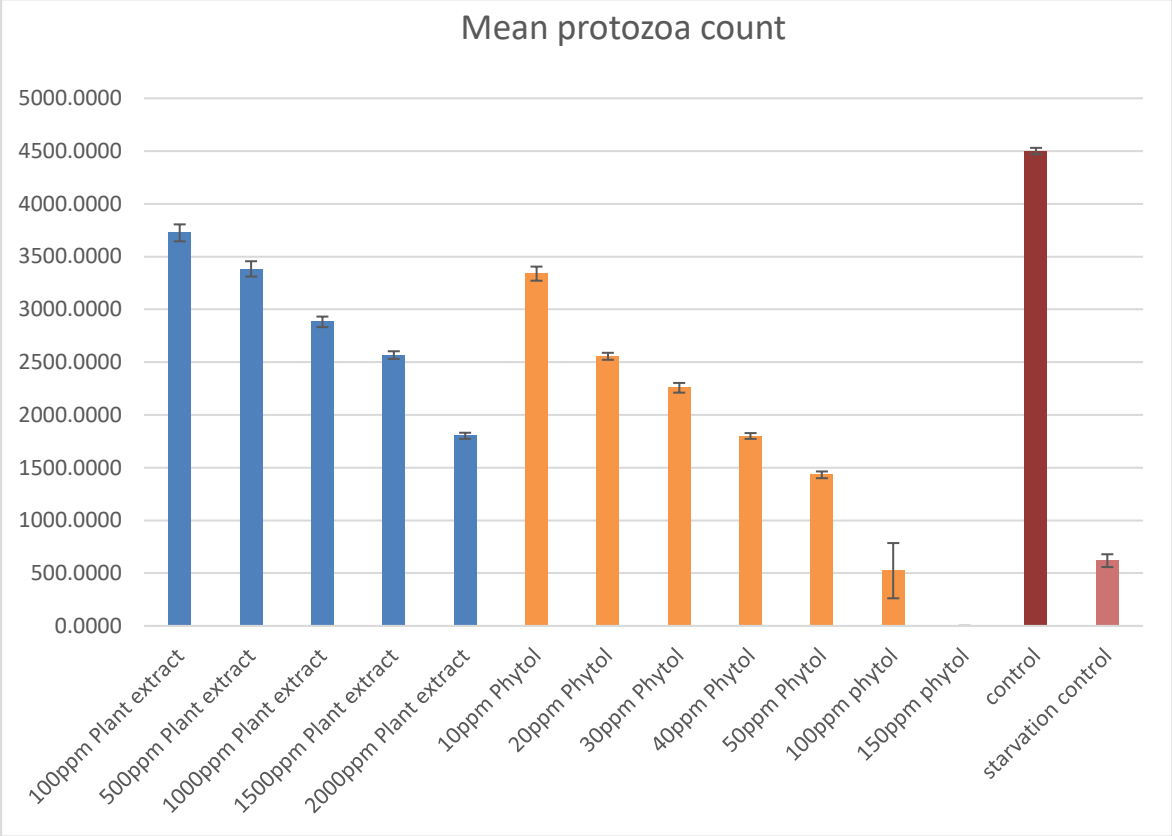

Fig 3.2 Mean protozoa count at various concentrations of *G. robusta* leaf extract and phytol.

Table 3.1 Correlation coefficient (r), P values of Protozoa count and filter paper weight loss in comparison with mortality and protozoa count.

	Protozoa count (r)	Filter paper weight loss (r)
Mortality	-0.80	-0.67
Sig	0.00	0.00
Protozoa count	NA	0.78
Sig	NA	0.00

Table 3.2 Mean mortality, protozoa count, and loss in filter paper weight (± SE) at different concentrations of *G. robusta* leaf extract and phytol.

Concentration	Mean mortality	Mean protozoa count	Mean loss in filter paper weight
100 ppm leaf extract	11.00 ± 0.67	3725.00 ± 80.36	4.23 ± 0.12
500 ppm leaf extract	27.00 ± 1.00	3383.33 ± 72.65	3.43 ± 0.07
1000 ppm leaf extract	44.33 ± 2.96	2881.67 ± 49.78	2.20 ± 0.12
1500 ppm leaf extract	60.00 ± 2.89	2566.67 ± 36.32	1.43 ± 0.07
2000 ppm leaf extract	74.67 ± 1.45	1801.67 ± 28.92	0.83 ± 0.12
10 ppm phytol	25.67 ± 3.48	3338.33 ± 66.92	0.45 ± 0.00
20 ppm phytol	42.50 ± 2.89	2555.00 ± 33.29	0.46 ±0.00
30 ppm phytol	55.83 ± 2.20	2256.67 ± 46.04	0.46 ± 0.00
40 ppm phytol	70.67 ± 2.33	1800.00 ± 27.54	0.46 ± 0.00
50 ppm phytol	82.50 ± 1.44	1431.67 ± 31.93	0.46 ± 0.00
100 ppm phytol	98.33 ± 0.88	523.33 ± 261.81	0.47 ± 0.07
150 ppm phytol	100.00 ± 0.00	NA	0.13 ± 0.07
Control	4.17 ± 2.20	4500.00 ± 30.31	4.5 ± 0.29
Starvation	27.50 ± 1.44	618.33 ± 60.58	NA

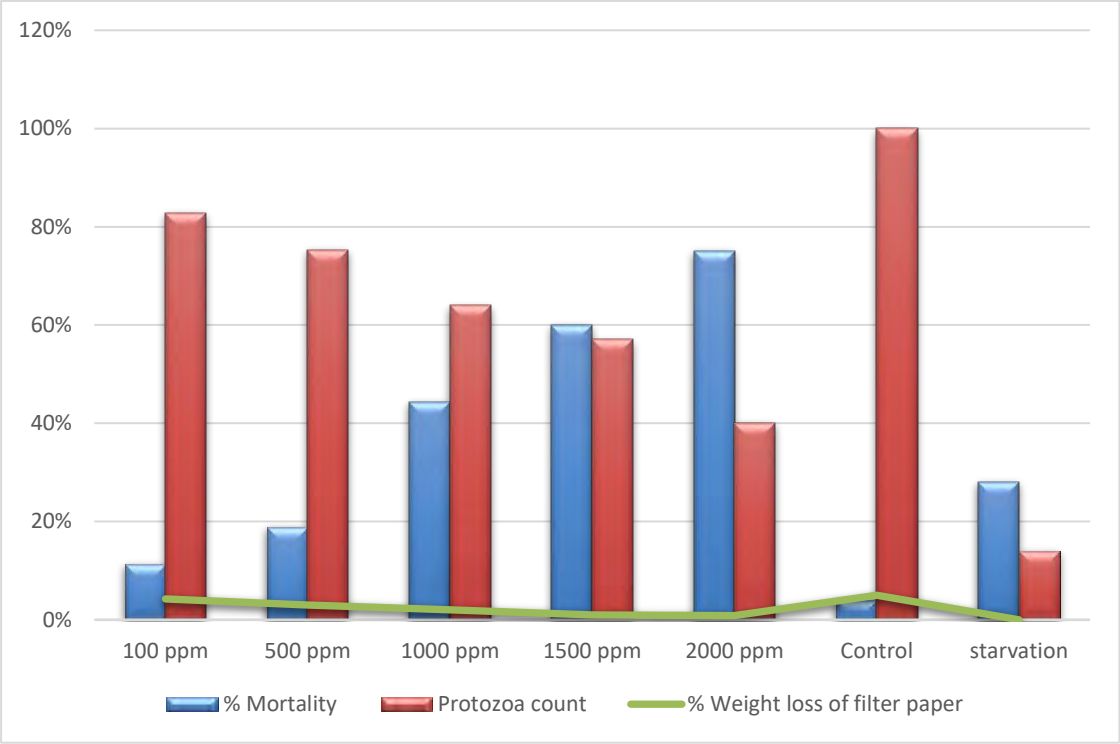

Fig. 3.3 Termite mortality (%), Protozoa count (%) per termite, and loss in filter paper weight (%) at different concentrations of *G. robusta* plant extract after 15 days.

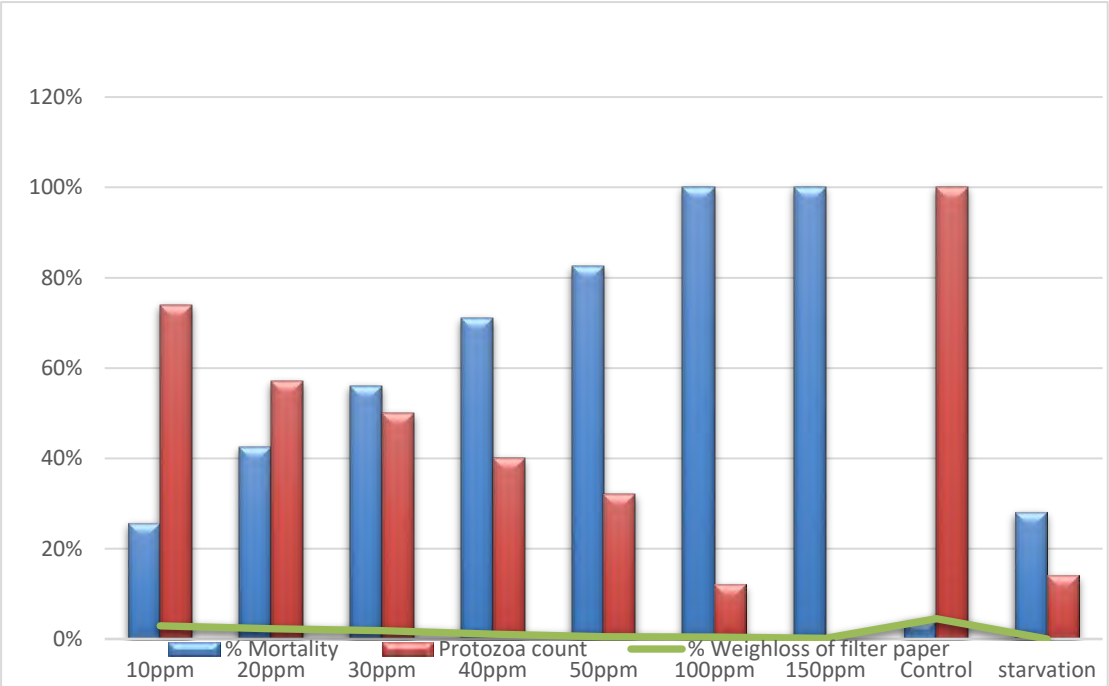

Fig. 3.4 Termite mortality (%), Protozoa count (%) per termite, and loss in filter paper weight (%) at different concentrations of phytol after 15 days.

3.3 Repellency and antifeedant activities

The repellent activity of Phytol was dose-dependent, 10 ppm concentration showed minimum repellency (17%), while 150 ppm showed maximum repellency (96%). Whereas, the control group showed 7% repellency. There was a significant difference in the number of termites observed on the treated and control halves of the filter paper. All the treatments were significantly different from the control group other than 10, 20, and 30 ppm treatments ($F (df) = 25.457 (39)$; $P < 0.001$). All the treatment groups were not significantly different from each other. The antifeedant effect of phytol ranged from 0.922% to 53.78% from 10 ppm to 150 ppm concentrations. Antifeedant activities of all the groups were not significantly different from each other but 50, 100, and 150 ppm concentration treatments were significantly different from the control treatment ($F (df) = 364.688 (39)$; $P < 0.001$).

Table 3.3 Antifeedant and repellent effect (± SE) of phytol on termites at different concentrations

Concentrations	Repellency (%)	Antifeedant indices (A)	Activity level
Control	7.00 ± 3.39	0.74 ±0.23	Minimum activity
10 ppm	17.00 ± 8.46	0.92 ± 0.19	Minimum activity
20 ppm	24.00 ± 8.72	2.19 ± 0.20	Minimum activity
30 ppm	43.00 ± 9.30	3.76 ± 0.13	Minimum activity
40 ppm	61.00 ± 6.20	5.98 ± 0.16	Minimum activity
50 ppm	74.00 ± 6.40	15.42 ± 0.94	Minimum activity
100 ppm	88.00 ± 4.64	24.15 ± 1.91	Minimum activity
150 ppm	96.00 ± 2.92	53.78 ± 1.61	Strong activity

Minimum = $0 \leq A < 25$; Moderate = $25 \leq A < 50$; Strong = $50 \leq A < 75$; Very strong = $75 \leq A < 100$ (Hassan *et al*., 2018).

3.4 Synergistic effect

Effective concentrations of *G. robusta* leaf extract (2000ppm) and phytol (50ppm) were checked for synergism and the resulting mortality was compared to their individual effect and control. All the treated groups showed significant mortality compared to the control treatment ($F (df) = 764.602 (11); P < 0.001$). The mortality in the synergism group was significantly different from the 2000 ppm plant extract and 50 ppm phytol treatment. Termite mortality observed in the synergism group was 96% whereas 2000 ppm leaf extract showed 77% mortality and 50 ppm phytol showed 85% mortality.

All the treatments showed a protozoal effect which was significantly different from the control treatment ($F (df) = 2706.163 (11); P < 0.001$). The protozoa count in the synergism treatment was significantly different from the 2000 ppm plant extract treatment and 50 ppm phytol treatment. The synergism group showed an 83% reduction in gut protozoa while 2000 ppm plant extract treatment showed 61% and 50 ppm phytol showed a 72% reduction in gut protozoa.

All the treated groups consumed significantly less percentage of filter paper compared to the control treatment ($F (df) = 294.474 (11); P < 0.001$). the reduction in filter paper weight in the synergism treatment (0.2%) was not significantly different from individual concentrations.

Table 3.4 Mean mortality, protozoa count and loss in filter paper weight (± SE) at effective concentrations of *G. robusta* leaf extract and phytol along with their synergism.

Concentrations	Mean mortality	Mean protozoa count	Mean loss in filter paper weight
Control	63.19 ± 10.2	4479.33 ± 11.57	5.37 ± 0.21
2000 ppm plant extract	74.67 ± 1.45	1740.00 ± 50.74	0.833 ± 0.12
50 ppm phytol	78.00 ± 1.15	1288.33 ± 21.67	0.70 ± 0.10
Synergism	94.00 ± 1.53	763.33 ± 29.63	0.20 ± 0.12

3.5 Wood assay

The synergistic effect of *G. robusta* leaf extract and phytol was further checked on non-durable commercial woods. All the treatments for chir pine showed significant mortality compared to the control treatment ($F (df) = 1269.118 (11); P < 0.001$). Termite mortality in synergistic treatment (97%) was significantly different from the 2000 ppm leaf extract treatment (81%) but not from the mortality in the 50 ppm phytol treatment (92%). In the case of gut protozoa, all the treatments showed a significant reduction in gut protozoa compared to the control treatment ($F (df) = 5369.058 (11); P < 0.001$). Similarly, the reduction in gut protozoa in the synergism treatment (90%) was significantly different from the 2000 ppm plant extract treatment (68%) and from the 50 ppm phytol treatment (78%). Wood weight loss of chir pine in all the treatments is significantly less than the control treatment ($F (df) = 200.642 (11); P < 0.001$). Weight loss in chir pine wood of the synergism treatment (2.9%) is significantly different from 2000 ppm plant (4.5%) and 50 ppm phytol (3.8%) treatments.

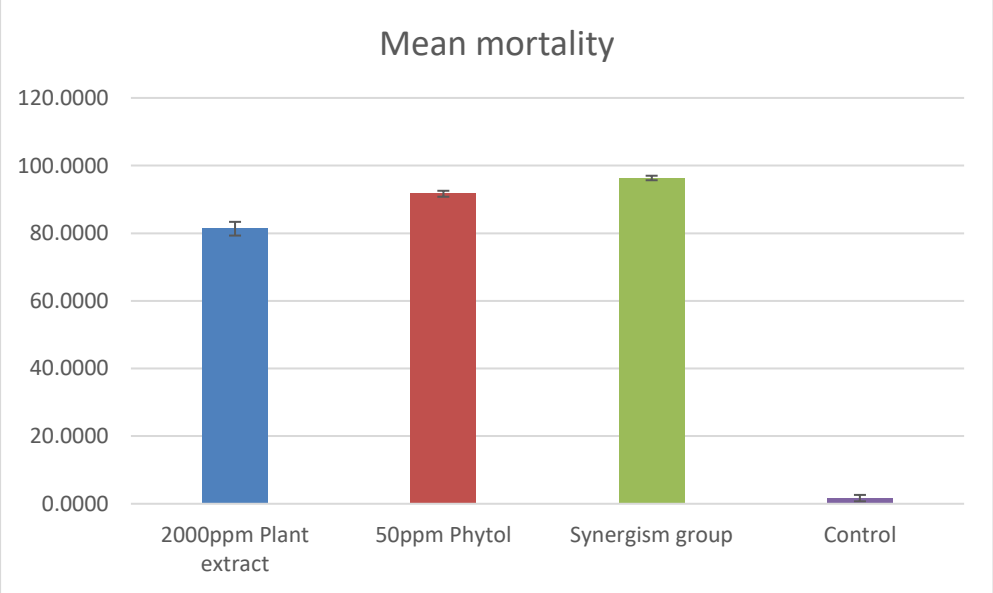

Fig. 3.5: Mean termite mortality after treating chir pine wood with effective concentrations of *G. robusta* leaf extract and phytol along with their synergism.

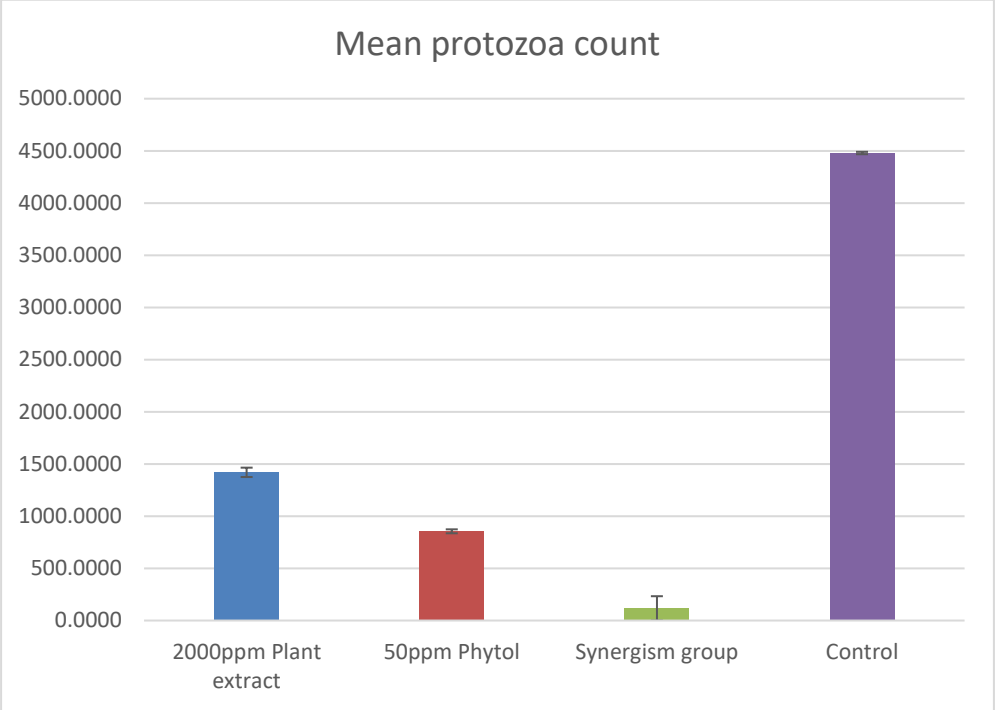

Fig 3.6: Mean protozoa count after treating chir pine wood with effective concentrations of *G. robusta* leaf extract and phytol along with their synergism.

Table 3.5 Mean mortality, protozoa count, and loss in wood weight (± SE) at effective concentrations of *G. robusta* leaf extract and phytol along with their synergism

Concentrations	Mean mortality	Mean protozoa count	Mean loss in wood weight
Control	1.67 ± 0.95	4479.33 ± 11.57	6.90 ± 0.10
2000 ppm leaf extract	81.33 ± 2.03	1460.00 ± 28.43	4.37 ± 0.13
50 ppm phytol	91.67 ± 0.88	1000.00 ± 28.87	3.73 ± 0.18
Synergism	96.33 ± 0.67	463.33 ± 25.22	2.87 ± 0.03

The synergistic effect on cottonwood was also tested and all treatments showed significant mortality compared to the control treatment ($F (df) = 366.976 (11)$; $P < 0.001$). Termite mortality in synergistic treatment (100%) was significantly different from the 2000 ppm leaf extract treatment (83%) but not from the mortality in the 50 ppm phytol treatment (97%). In the case of gut protozoa, all the treatments showed a

significant reduction in gut protozoa compared to the control treatment (F (df) = 912.207 (11); P < 0.001). Similarly, the reduction in gut protozoa in the synergism treatment (92.2%) was significantly different from the 2000 ppm plant extract treatment (68%) and from the 50 ppm phytol treatment (81%). Wood weight loss of cottonwood in all the treatments is significantly less than the control treatment (F (df) = 29.319 (11); P < 0.001). Weight loss in cottonwood of the synergism treatment (0.14%) is not significantly different from 2000 ppm plant (1%) and 50 ppm phytol (0.57%) treatments.

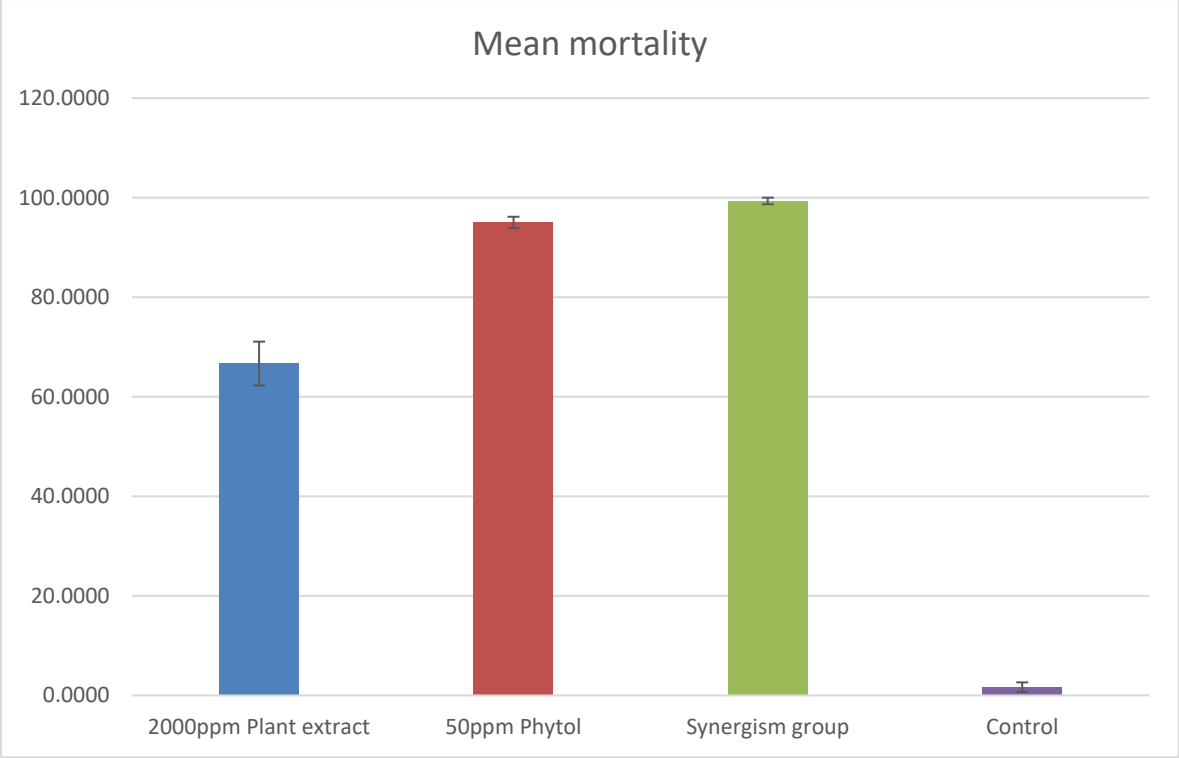

Fig 3.7: Mean termite mortality after treating cottonwood with effective concentrations of *G. robusta* leaf extract and phytol along with their synergism

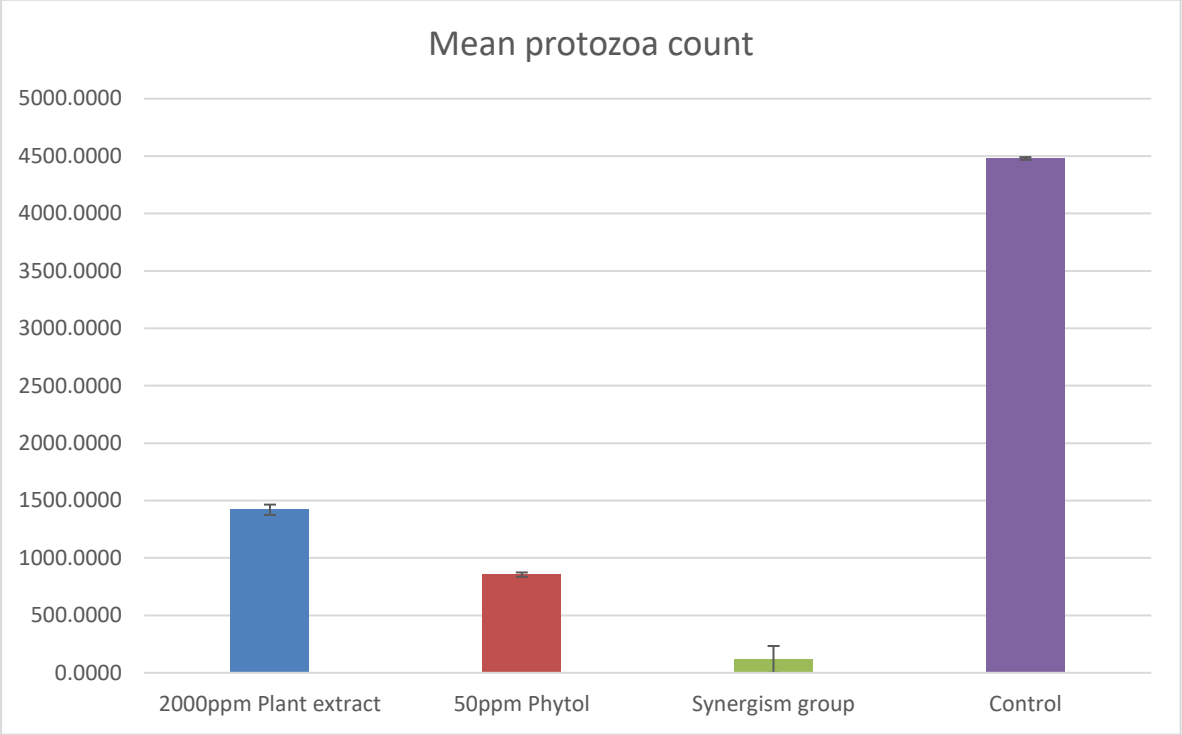

Fig 3.8. Mean protozoa count after treating cottonwood with effective concentrations of *G. robusta* leaf extract and phytol along with their synergism

Table 3.6: Mean mortality, protozoa count, and loss in wood weight (± SE) at effective concentrations of *G. robusta* leaf extract and phytol along with their synergism

Concentrations	Mean mortality	Mean protozoa count	Mean loss in wood weight
Control	1.67 ± 0.95	4479.33 ± 11.57	2.37 ± 0.29
2000 ppm plant extract	66.67 ± 4.41	1420.00 ± 44.81	1.00 ± 0.17
50 ppm phytol	95.00 ± 1.15	855.00 ± 18.93	0.57 ± 0.08
Synergism	99.33 ± 0.67	116.67 ± 116.67	0.14 ± 0.08

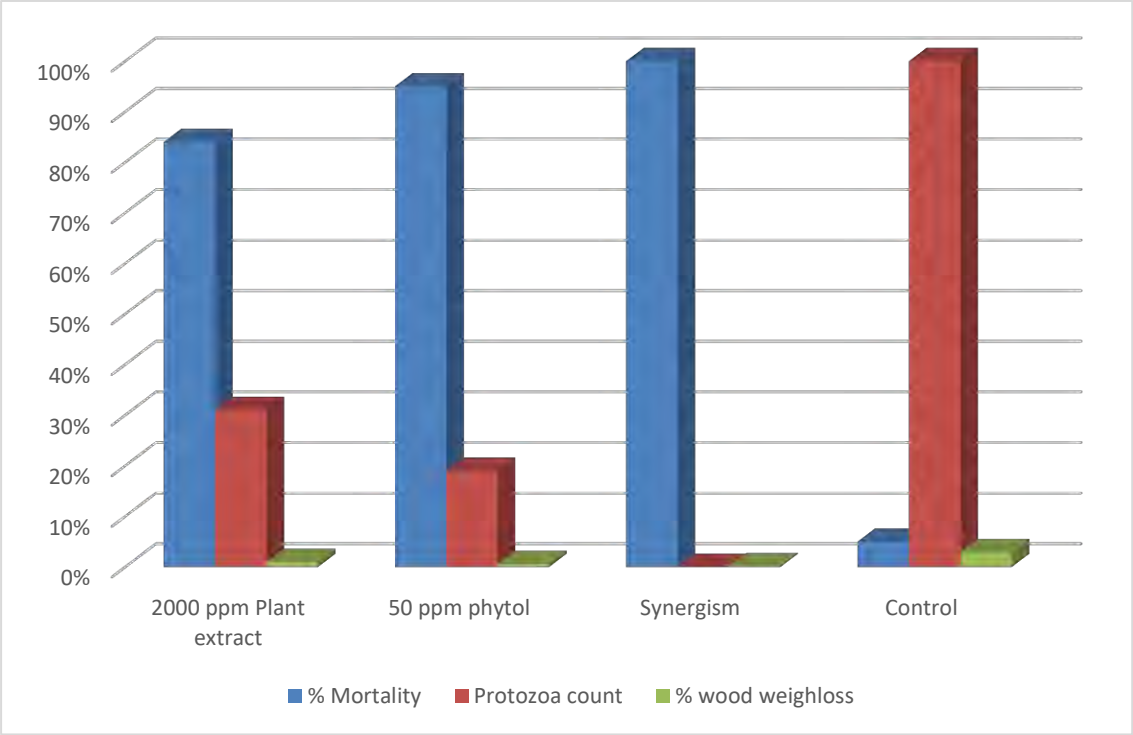

Fig. 3.9: Termite mortality (%), Protozoa count (%) per termite, and loss of cottonwood weight (%) after 15 days.

DISCUSSION

The termiticidal and protozoacidal activity of *G. robusta* leaf extract is already reported by Azal *et al*. (2019) and Ashraf *et al*. (2020). We have compared this activity with synthetic phytol. A lot of research has been conducted on plant extracts for their termiticidal properties. FTIR and GC-MS analysis of these plant extracts reveal the presence of phytol in it (Han *et al*., 2016; Khanikor *et al*., 2018; Ashraf *et al*., 2020; Sina *et al*., 2021; Tekam, 2023). Synthetic phytol has excellent termiticidal and protozoacidal potential and can be used to protect non-durable wood against termites. *G. robusta* leaf extract showed significant termiticidal potential against *H. indicola*. Maximum mortality (77%) was observed at 2000 ppm on the 15th day. Whereas, phytol showed significantly higher mortality (100%; $P < 0.001$) at low concentrations as compared to the crude extract. These results are comparable to Afzal *et al*. (2019) who reported a concentration-dependent effect of *G. robusta* leaf extract on *H. indicola* mortality. They observed >90 % mortality at their highest concentration (5 mg/ml) on the 14th day. On the other hand, Ashraf *et al*. (2020) reported the termiticidal activity of *G. robusta* leaf extract against *H. indicola* as time and dose-dependent. They observed 90% mortality (LC_{50} = 1607.95) on the 14th day at 2000 ppm concentration. In our study phytol showed 85% mortality (LC_{50} =22.072) on the 15th day at 50 ppm concentration and 98% and 100% mortality at 100 and 150 ppm concentrations on the 15th and 5th day respectively. Treatments with higher concentrations showed a reduction in termite foraging activity as a fivefold reduction in consumption of filter paper was observed. Afzal *et al*. (2019) and Ashraf *et al*. (2020) also reported the same trend. Hassan *et al*. (2018) reported the foraging rates and percentage termite mortality of *Reticulitermes flavipes* against mulberry (*Morus alba*) heartwood extract as concentration-dependent. *G. robusta* leaf extract's higher concentration causes lethargy and abdominal shrinkage in *H. indicola* workers which significantly affect foraging activity (Afzal *et al*., 2019). Leaf extracts have allelochemicals such as flavonoids, terpenoids, phenolic components, and quinones which may cause toxicity in chewing insects (Abbas *et al*., 2013; Boue and Raina, 2003; Leatemia and Isman, 2004; Ohmura *et al*., 2000). Leaf extract of *G. robusta* has six different grevillosides which are cytotoxic and has melanogenesis inhibitory activity (Yamashita-Higuchi *et al*., 2014). On the other hand, phytol is highly cytotoxic. It has insecticidal, larvicidal,

antimicrobial, antileishmanial, and antitumor activity (Pejin *et al*., 2014; Luo *et al*., 2022; Islam *et al*., 2018). It may have caused mortality in termites by inducing cytotoxicity through invading cell membranes and causing ion imbalace (Islam *et al*., 2018).

In past studies, it was reported that environmental factors such as humidity and temperature might be responsible for flux in the population of protozoa (Mannesmann, 1972a). However, another study reported that feeding of termites on toxic compounds caused a reduction in hindgut symbionts leading to increased mortality (Mannesmann, 1972b). In the present study, the mean population of gut protozoa observed in the control group was 4500 ± 30.31 which is comparable to the number 5545.0 ± 102.15 obtained by Afzal *et al*. (2019) and 3530 ± 102.15 by Hassan *et al*. (2017) in the hindgut of *H. indicola*. Lewis and Forschler (2014) reported the population of protozoa in the hindgut of *Reticulitermes* as 59000 for *R. flavipes* and 21000 for *R. hageni* which is significantly less than the protozoa population of *H. indicola*. The reason could be the size of the hindgut, ecological habitat, and wood-feeding preferences. All of these factors can alter the composition of gut protozoan in lower termites (Afzal *et al* 2019). The results of our study are comparable to Afzal *et al*. (2019) as the percentage weight loss of filter paper is positively correlated to the gut protozoa count. Our results showed a 60% reduction in gut protozoa count at 2000 ppm leaf extract concentration with 77% mortality and an 88% reduction at 100 ppm phytol concentration with 98% mortality compared to the control treatments. Afzal *et al*. (2019) reported a 62.90% reduction at 20 mg/ml (heighest) concentration of *G. robusta* leaf extract with 96.82% mortality. On the other hand, Lewis and Forschler (2010) reported 30% drop in gut protozoa count after using the treatments of chitin synthesis inhibitors. Hassan *et al*. (2017) also documented 45.7, 39.2, 36.4, and 15% reduction in the hindgut protozoa of *H. indicola* after force-feeding it on filter paper treated with 10 mg/mL *Dalbergia sissoo*, *Tectona grandis*, *Pinus roxburgii,* and *Cedrus deodara* heartwood extracts, respectively. The results of the present study are also comparable with Hassan *et al*. (2018) who reported a 55.2% decline in the gut protozoa of *R. flavipes* with 93% termite mortality after treating it with 10 mg/mL extract of white mulberry. Variance among the termite mortality rate and protozoan population suggests some physiological process involved because of the presence of antioxidants in the plant extract and phytol. In the hindgut

of termite, single redox reactions convert cellulose into acetate through the shikimate acid pathways (Raje *et al*., 2015; Hassan et a., 2017). The antioxidants of *G. robusta* leaf extract and phytol may interfere with such reactions. In the present study, an 87% reduction in gut protozoa was observed in the starved group where mortality was 27%, these results are similar to that reported by Afzal *et al*. (2019) and indicate other modes of toxicity of phytol and leaf extract. Raje *et al*. (2015) documented that turmeric extract has tumerone which is responsible for high termite mortality by targeting their respiratory and nervous systems. Hu *et al*. (2011) suggested that cannibalism can be a contributing factor in the survival of termites in the starvation controls (no extract, no phytol, and no food). Lower termites, which feed on wood cannot digest wood without the help of cellulose-producing symbiotic protozoa (Cleveland, 1925). Even the elimination of a single species of hindgut protozoa in termites can cause significant mortality (Mannesmann, 1972b). In this study, continuous no-choice feeding of *H. indicola* on leaf extract and phytol could cause digestive toxicity to the microenvironment of the gut at the cellular level by the toxicity of phytol and components of leaf extract. Phytol and components of the leaf extract could cause slow-acting toxicity in the communities of gut protozoa which could be excellent in termite control baiting technology. After foraging, termites return to their colony and hence can transfer the toxic compounds, which they acquire while feeding, to other colony members via trophallaxis (Mauldin *et al*., 1981; Hassan *et al*., 2017; Afzal *et al*., 2019).

The results of our repellency and antifeedant bioassay are comparable with Hassan *et al*. (2018) who documented that heartwood extract of white paper mulberry manifests repellent and antifeedant activities against the termite *R. flavipes*. Afzal *et al*. (2019) reported the repellent and antifeedant activities of silver oak leaf extract against *H. indicola*. In the present study, phytol also manifested repellent and antifeedant activities against *H. indicola*. Repellency and antifeedant activities were also concentration-dependent and our highest concentration (150 ppm) gives maximum repellency and antifeedant activity. The presence of antioxidants in the phytol can be the reason for the change in foraging behavior as Hassan *et al*. (2017) reported the heartwood extract of teak induces antifeedant and high mortality in *H. indicola* and *R. flavipes* may be due to the presence of quinones and phenols that have antioxidant

repellent and antifeedant properties (Ates *et al*., 2015; Abbas *et al*., 2013; Rasib *et al*., 2014).

In the synergism assay, 96% mortality was observed in the synergism group with 83% reduction in gut protozoa whereas, 2000 ppm plant extract concentration showed 77% mortality with 61% protozoan reduction, and 50 ppm phytol concentration showed 85% mortality with 72% decline in protozoan population. Phytol and *G. robusta* leaf extract both have antioxidant and cytotoxic properties which together showed synergistic activity. *G. robusta* leaf extract somehow enhanced the termiticidal and protozoacidal activity of phytol and both used in combination would be better than used separately. Phytol is an expensive chemical and if it is used in minute quantity along with *G. robusta* leaf extract to kill termites then this would be cost-effective and safe to use.

In the wood assay, non-durable cottonwood and chir pine blocks were treated with the most effective concentrations of *G. robusta* leaf extract and phytol along with their synergism group and a control. Results showed significant mortality and decline in protozoan population in all treated groups which confirms the successful penetration of leaf extract and phytol in both non-durable woods. This final bioassay confirms the termiticidal and protozoacidal activity of phytol and its synergism and comparison to the *G. robusta* leaf extract. It interferes with the cellulose digestion and cellulase production in the termite gut (Ragon *et al*., 2008; Abe *et al*., 2000). Cottonwood showed more good results than chir pine for all the treated groups. These results are comparable to Hassan *et al*. (2018) who observed minimum foraging activity and 100% mortality in *R. flavipes* by exposing them to white mulberry heartwood extract treated non-durable wood for two weeks. Afzal *et al*. (2019) also observed 100% mortality in *H. indicola* after exposing them to non-durable wood treated with *G. robusta* leaf extract. Hence, phytol can be used as a termiticidal compound in the preservation of non-durable wood against pests. Benelli *et al*. (2020) documented the insecticidal activity of phytol; however, to the best of my knowledge, its termiticidal activity has not been reported before.

Conclusion

This study illustrated that Phytol has a significant ($P < 0.001$) termiticidal activity against *H. indicola*. Phytol showed repellent, antifeedant activity along with significantly reducing the hindgut protozoa of *H. indicola* in no-choice bioassays. The termiticidal activity of phytol was compared to the activity of *G. robusta* leaf extract and the synergism of both was also evaluated. The *G. robusta* leaf extract in synergism with phytol resulted in marked protozoacidal and termiticidal potential.

References

Abbas, M., Shahid, M., Iqbal, M., Anjum, F., Sharif, S., Ahmed, S., & Pirzada, T. (2013). Antitermitic activity and phytochemical analysis of fifteen medicinal plant seeds. *Journal of Medicinal Plants Research*, *7*(22), 1608-1617.

Abe, T., Bignell, D. E., & Higashi, M. (Eds.). (2000). *Termites: evolution, sociality, symbioses, ecology*. Springer Science & Business Media.

Afzal, M., Farman, M., Rasib, K. Z., & Qureshi, N. A. (2019). Biocidal action of silver oak (Grevillea robusta) leaf extract on the termite Heterotermes indicola Wasmann (Blattodea: Rhinotermitidae). *International Biodeterioration & Biodegradation*, *139*, 1-10.

Aihetasham, A., Rasib, K. Z., Hasan, S. R., & Bodlah, I. (2017). Effect of Carica papaya, Helianthus annus and Bougainvillea glabra aqueous extracts against termite, Heterotermes indicola (Isoptera: Rhinotermitidae). *Punjab Univ. J. Zool*, *32*(1), 51-56.

Ashraf, A., Qureshi, N. A., Shaheen, N., Iqbal, A., Fatima, H., Afzal, M., ... & Qureshi, M. Z. (2020). Termiticidal and protozocidal potentials of eight tropical plant extracts evaluated against Odontotermes obesus Rambur (Blattodea; Termitidae) and Heterotermes indicola Wasmann (Blattodea; Rhinotermitidae). *Polish Journal of Environmental Studies*, *29*(5), 3493-3507.

Ateş, S., Gür, M., Özkan, O. E., Akça, M., Olgun, Ç., & Güder, A. (2015). Chemical contents and antifungal activity of some durable wood extractives vs. Pleurotus ostreatus. *BioResources*, *10*(2), 2433-2443.

Baggio, A. J., Caramori, P. H., Androcioli Filho, A., & Montoya, L. (1997). Productivity of southern Brazilian coffee plantations shaded by different stockings of Grevillea robusta. *Agroforestry systems*, *37*, 111-120.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and chemical toxicology*, *46*(2), 446-475.

Barbehenn, R. V. (2002). Gut-based antioxidant enzymes in a polyphagous and a graminivorous grasshopper. *Journal of chemical ecology*, *28*, 1329-1347.

Benelli, G., Pavela, R., Drenaggi, E., Desneux, N., & Maggi, F. (2020). Phytol, (E)-nerolidol and spathulenol from Stevia rebaudiana leaf essential oil as effective and eco-friendly botanical insecticides against Metopolophium dirhodum. *Industrial Crops and Products*, *155*, 112844.

Bhuvaneswari, S., Sripriya, N., Balamurugan, A., Siril, A., Kasinathan, V., Karthikeyan, P. T., & Prakash, N. U. (2014). Studies on the Phytochemistry and Bioefficacy of Industrial Crops Coffea canephora and Gravillea robusta from Kolli Hills. *International Journal of Research in Pharmaceutical Sciences*, *5*, 147-151.

Bignell, D. E., Roisin, Y., & Lo, N. (Eds.). (2010). *Biology of termites: a modern synthesis*. Springer Science & Business Media.

Bonachela, J. A., Pringle, R. M., Sheffer, E., Coverdale, T. C., Guyton, J. A., Caylor, K. K., ... & Tarnita, C. E. (2015). Termite mounds can increase the robustness of dryland ecosystems to climatic change. *Science*, *347*(6222), 651-655.

Boué, S. M., & Raina, A. K. (2003). Effects of plant flavonoids on fecundity, survival, and feeding of the Formosan subterranean termite. *Journal of Chemical Ecology*, *29*, 2575-2584.

Bourguignon, T., Lo, N., Cameron, S. L., Šobotník, J., Hayashi, Y., Shigenobu, S., ... & Evans, T. A. (2014). The evolutionary history of termites as inferred from 66 mitochondrial genomes. *Molecular biology and evolution*, *32*(2), 406-421.

Brauman, A., Majeed, M. Z., Buatois, B., Robert, A., Pablo, A. L., & Miambi, E. (2015). Nitrous oxide (N2O) emissions by termites: does the feeding guild matter. *PloS one*, *10*(12), e0144340.

Breznak, J. A. (2000). Ecology of prokaryotic microbes in the guts of wood-and litter-feeding termites. *Termites: evolution, sociality, symbioses, ecology*, 209-231.

Brune, A. (2009). Symbionts aiding digestion. In *Encyclopedia of insects* (pp. 978-983). Academic Press.

Brune, A. (2014). Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology*, *12*(3), 168-180.

Buczkowski, G., & Bertelsmeier, C. (2017). Invasive termites in a changing climate: A global perspective. *Ecology and evolution*, *7*(3), 974-985.

Cairo, J. F., Carazzolle, M. F., Leonardo, F. C., Mofatto, L. S., Brenelli, L. B., Gonçalves, T. A., ... & Squina, F. M. (2016). Expanding the knowledge on lignocellulolytic and redox enzymes of worker and soldier castes from the lower termite Coptotermes gestroi, Front. Microbiol. 7 (2016) 1518.

Chang, S. T., Wang, S. Y., Wu, C. L., Su, Y. C., & Kuo, Y. H. (1999). Antifungal compounds in the ethyl acetate soluble fraction of the extractives of Taiwania (Taiwania cryptomerioides Hayata) heartwood.

Chaudry, M. I., Ahmad, M., Malik, N. K., Akhtar, M. S., & Arshad, M. (1972). *Termites of Pakistan: Identify, distribution, & ecological relationship*. Final technical report, PL-480 Project No. A17-fs-12. Peshawar.

Cheng, S. S., Chang, H. T., Wu, C. L., & Chang, S. T. (2007). Anti-termitic activities of essential oils from coniferous trees against Coptotermes formosanus. *Bioresource technology*, *98*(2), 456-459.

Cleveland, L. R. (1925). The feeding habit of termite castes and its relation to their intestinal flagellates. *The Biological Bulletin*, *48*(5), 295-308.

Cock, I. E. (2008). Antibacterial activity of selected Australian native plant extracts. *Internet J Microbiol*, *4*(2), 1-8.

Cock, I. E., & Ruebhart, D. R. (2008). Assessment of the toxicity of selected Australian native plant extracts using the Artemia franciscana nauplii bioassay. *Internet Journal of Toxicology*, *5*(2), 2.

Cosme Jr, L., Haro, M. M., Guedes, N. M. P., Della Lucia, T. M. C., & Guedes, R. N. C. (2018). Tropical wood resistance to the West Indian drywood termite Cryptotermes brevis: If termites can't chew…. *Pest management science*, *74*(4), 914-924.

da Silva, J. M., Antinarelli, L. M. R., Ribeiro, A., Coimbra, E. S., & Scio, E. (2015). The effect of the phytol-rich fraction from Lacistema pubescens against Leishmania amazonensis is mediated by mitochondrial dysfunction. *Experimental parasitology*, *159*, 143-150.

de Moraes, J., de Oliveira, R. N., Costa, J. P., Junior, A. L., de Sousa, D. P., Freitas, R. M., ... & Pinto, P. L. (2014). Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease Schistosomiasis mansoni. *PLoS neglected tropical diseases*, *8*(1), e2617.

de Souza, N. J., & Nes, W. R. (1969). The presence of phytol in brown and blue-green algae and its relationship to evolution. *Phytochemistry*, *8*(5), 819-822.

Delgarde, S., & Rouland-Lefevre, C. (2002). Evaluation of the effects of thiamethoxam on three species of African termite (Isoptera: Termitidae) crop pests. *Journal of Economic Entomology*, *95*(3), 531-536.

Doi, S., Aoyama, M., Ohara, S., & Omura, W. (2001). Insect repellent containing flavonoids and extraction of them from plants. *Patent Application JP*, *169175*.

Du, H., Chouvenc, T., & Su, N. Y. (2017). Development of age polyethism with colony maturity in Coptotermes formosanus (Isoptera: Rhinotermitidae). *Environmental Entomology*, *46*(2), 311-318.

Dungani, R., Bhat, I. U. H., Khalil, H. P. S., Naif, A., & Hermawan, D. (2012). Evaluation of antitermitic activity of different extracts obtained from Indonesian teakwood (Tectona grandis Lf). *BioResources*, *7*(2).

Elango, G., Rahuman, A. A., Kamaraj, C., Bagavan, A., Zahir, A. A., Santhoshkumar, T., ... & Rajakumar, G. (2012). Efficacy of medicinal plant extracts against Formosan subterranean termite, Coptotermes formosanus. *Industrial Crops and Products*, *36*(1), 524-530.

Engel, M. S., Grimaldi, D. A., Nascimbene, P. C., & Singh, H. (2011). The termites of Early Eocene Cambay amber, with the earliest record of the Termitidae (Isoptera). *ZooKeys*, (148), 105.

Faix, Š., Juhas, Š., & Faixová, Z. (2007). The effect of essential oil intake on changes of plasma antioxidant status in mice. *Acta Veterinaria Brno*, *76*(3), 357-361.

Freymann, B. P., Buitenwerf, R., Desouza, O., & Olff, H. (2008). The importance of termites (Isoptera) for the recycling of herbivore dung in tropical ecosystems: a review. *European Journal of Entomology*, *105*(2), 165.

Ghaneian, M. T., Ehrampoush, M. H., Jebali, A., Hekmatimoghaddam, S., & Mahmoudi, M. (2015). Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. *Environmental Health Engineering and Management Journal*, *2*(1), 13-16.

Govorushko, S. (2019). Economic and ecological importance of termites: A global review. *Entomological Science*, *22*(1), 21-35.

Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of applied microbiology*, *86*(6), 985-990.

Han, M., Luo, L., & Yuan, Z. (2016). Biological Activity of Lantana camara L. Leaf Petroleum Ether Extract against the Termite, Reticulitermes flaviceps (Oshima)(Isoptera: Rhinotermitidae). *Egyptian Journal of Biological Pest Control*, *26*(4).

Harit, A., Shanbhag, R., Chaudhary, E., Cheik, S., & Jouquet, P. (2017). Properties and functional impact of termite sheetings. *Biology and Fertility of Soils*, *53*, 743-749.

Harwood, C. E., & Booth, T. H. (1992). Status of Grevillea robusta in forestry and agroforestry. In *HARWOOD, CE (Ed.). Grevillea robusta in agroforestry and forestry: proceedings of an international workshop. Nairobi: ICRAF* (pp. 9-16).

Hassan, B., Ahmed, S., Kirker, G., Mankowski, M. E., & Misbah ul Haq, M. (2020). Synergistic effect of heartwood extracts in combination with linseed oil as wood preservatives against subterranean termite Heterotermes indicola (Blattodea: Rhinotermitidae). *Environmental science and pollution research*, *27*, 3076-3085.

Hassan, B., Ahmed, S., Kirker, G., Mankowski, M. E., & Misbah-ul-Haq, M. (2018). Antioxidant effects of four heartwood extractives on midgut enzyme activity in Heterotermes indicola (Blattodea: Rhinotermitidae). *Environmental entomology*, *47*(3), 741-748.

Hassan, B., Ahmed, S., Mehmood, N., Mankowski, M. E., & Misbah-ul-Haq, M. (2019). Toxicity potential of heartwood extractives from two mulberry species against Heterotermes indicola. *Maderas. Ciencia y tecnología*, *21*(2), 153-162.

Hassan, B., Mankowski, M. E., Kirker, G. T., Clausen, C. A., & Ahmed, S. (2018). Effects of white mulberry (Morus alba) heartwood extract against Reticulitermes

flavipes (Blattodea: Rhinotermitidae). *Journal of Economic Entomology*, *111*(3), 1337-1345.

Hassan, B., Mankowski, M. E., Kirker, G., & Ahmed, S. (2017). Effects of heartwood extractives on symbiotic protozoan communities and mortality in two termite species. *International Biodeterioration & Biodegradation*, *123*, 27-36.

Hassan, B., Mankowski, M. E., Kirker, G., & Ahmed, S. (2017). Effects of heartwood extractives on symbiotic protozoan communities and mortality in two termite species. *International Biodeterioration & Biodegradation*, *123*, 27-36.

Honigberg, B. M. (1970). Protozoa associated with termites and their role in digestion. *The biology of termites*, 1-36.

Hu, X. P., Song, D., & Gao, X. (2011). Biological changes in the Eastern subterranean termite, Reticulitermes flavipes (Isoptera, Rhinotermitidae) and its protozoa profile following starvation. *Insectes sociaux*, *58*, 39-45.

Husseneder, C., Simms, D. M., Delatte, J. R., Wang, C., Grace, J. K., & Vargo, E. L. (2012). Genetic diversity and colony breeding structure in native and introduced ranges of the Formosan subterranean termite, Coptotermes formosanus. *Biological Invasions*, *14*, 419-437.

Ikeda-Ohtsubo, W., Desai, M., Stingl, U., & Brune, A. (2007). Phylogenetic diversity of 'Endomicrobia'and their specific affiliation with termite gut flagellates. *Microbiology*, *153*(10), 3458-3465.

Ischebeck, T., Zbierzak, A. M., Kanwischer, M., & D. rmann, P. (2006). A salvage pathway for phytol metabolism in Arabidopsis. *Journal of Biological Chemistry*, *281*(5), 2470-2477.

Islam, M. T., Ali, E. S., Uddin, S. J., Shaw, S., Islam, M. A., Ahmed, M. I., ... & Atanasov, A. G. (2018). Phytol: A review of biomedical activities. *Food and chemical toxicology*, *121*, 82-94.

Islam, M. T., de Alencar, M. V. O. B., da Conceição Machado, K., da Conceição Machado, K., de Carvalho Melo-Cavalcante, A. A., de Sousa, D. P., & de Freitas, R. M. (2015). Phytol in a pharma-medico-stance. *Chemico-Biological Interactions*, *240*, 60-73.

Jones, R., Silence, P., & Webster, M. (2015). Preserving History: Subterranean Termite Prevention in Colonial Williamsburg. *Colonial Williamsburg Foundation, USA*.

Jouquet, P., Chaudhary, E., & Kumar, A. R. V. (2018). Sustainable use of termite activity in agro-ecosystems with reference to earthworms. A review. *Agronomy for Sustainable Development*, *38*, 1-11.

Jouquet, P., Traoré, S., Choosai, C., Hartmann, C., & Bignell, D. (2011). Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *European Journal of Soil Biology*, *47*(4), 215-222.

Kadir, R., Ali, N. M., Soit, Z., & Khamaruddin, Z. (2014). Anti-termitic potential of heartwood and bark extract and chemical compounds isolated from Madhuca utilis Ridl. HJ Lam and Neobalanocarpus heimii King PS Ashton. *Holzforschung*, *68*(6), 713-720.

Khanikor, B., Barman, J., Sarma, R., Mahanta, S., & Adhikari, K. (2018). Evaluation of efficacy of three essential oils against Odontotermes feae (Isoptera: Termitidae). *Journal of Environment Pollution and Human Health*, *6*(2), 68-76.

Komiya, T., Kyohkon, M., Ohwaki, S., Eto, J., Katsuzaki, H., Imai, K., ... & Hibasami, H. (1999). Phytol induces programmed cell death in human lymphoid leukemia Molt 4B cells. *International journal of molecular medicine*, *4*(4), 377-457.

Krishna, K., & Grimaldi, D. A. (2003). The first Cretaceous Rhinotermitidae (Isoptera): a new species, genus, and subfamily in Burmese amber. *American Museum Novitates*, *2003*(3390), 1-10.

Krishna, K., Grimaldi, D. A., Krishna, V., & Engel, M. S. (2013). Treatise on the Isoptera of the world.(Bulletin of the American Museum of Natural History, no. 377).

Krishna, K., Grimaldi, D. A., Krishna, V., & Engel, M. S. (2013). Treatise on the Isoptera of the world.(Bulletin of the American Museum of Natural History, no. 377).

Kubota, S., Shono, Y., Matsunaga, T., & Tsunoda, K. (2007). Response of the subterranean termite Coptotermes formosanus (Isoptera: Rhinotermitidae) to soil treated with microencapsulated fenobucarb. *Pest Management Science: formerly Pesticide Science*, *63*(12), 1224-1229.

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., ... & Rossi, J. P. (2006). Soil invertebrates and ecosystem services. *European journal of soil biology*, *42*, S3-S15.

Leatemia, J. A., & Isman, M. B. (2004). Toxicity and antifeedant activity of crude seed extracts of Annona squamosa (Annonaceae) against lepidopteran pests and natural enemies. *International Journal of Tropical Insect Science*, *24*, 150-158.

Legendre, F., Whiting, M. F., Bordereau, C., Cancello, E. M., Evans, T. A., & Grandcolas, P. (2008). The phylogeny of termites (Dictyoptera: Isoptera) based on mitochondrial and nuclear markers: implications for the evolution of the worker and pseudergate castes, and foraging behaviors. *Molecular phylogenetics and evolution*, *48*(2), 615-627.

Lewis, J. L., & Forschler, B. T. (2004). Protist communities from four castes and three species of Reticulitermes (Isoptera: Rhinotermitidae). *Annals of the Entomological Society of America*, *97*(6), 1242-1251.

Li, H., Xu, Z., Deng, T., Chen, L., Li, J., Wei, J., & Mo, J. (2010). Species of termites (Isoptera) attacking trees in China. *Sociobiology*, *56*(1), 109-120.

Li, Y., Dong, Z. Y., Pan, D. Z., Pan, C. H., & Chen, L. H. (2017). Effects of termites on soil pH and its application for termite control in Zhejiang Province, China. *Sociobiology*, *64*(3), 317-326.

Little, N. S., Schultz, T. P., & Nicholas, D. D. (2010). Termite-resistant heartwood. Effect of antioxidants on termite feeding deterrence and mortality.

Lo, N., Tokuda, G., Watanabe, H., Rose, H., Slaytor, M., Maekawa, K., ... & Noda, H. (2000). Evidence from multiple gene sequences indicates that termites evolved from wood-feeding cockroaches. *Current Biology*, *10*(13), 801-804.

Löffler, E., & Kubiniok, J. (1996). Landform development and bioturbation on the Khorat Plateau, Northeast Thailand. *Natural History Bulletin of the Siam Society*, *44*, 199-216.

Lukasik, I. (2007). Changes in activity of superoxide dismutase and catalase within cereal aphids in response to plant o-dihydroxyphenols. *Journal of applied entomology*, *131*(3), 209-214.

Luo, D. Y., Yan, Z. T., Che, L. R., Zhu, J. J., & Chen, B. (2022). Repellency and insecticidal activity of seven Mugwort (Artemisia argyi) essential oils against the malaria vector Anopheles sinensis. *Scientific reports*, *12*(1), 5337.

Maiti, P. K. (2006). A taxonomic monograph on the world species of termites of the family Rhinotermitidae (Isoptera: Insecta). Memoirs of the Zoological Survey of India 20(4): 1-272.

Mannesmann, R. (1972). Relationship between different wood species as a termite food source and the reproduction rate of termite symbionts 1. *Zeitschrift für Angewandte Entomologie*, *72*(1-4), 116-128.

Manzoor, F. (2011). Preliminary evaluation of Ocimum sanctum as toxicant and repellent against termite, Heterotermes indicola (Wasmann)(Isoptera: Rhinotermitidae). *Pakistan Journal of Science*, *63*(1).

Manzoor, F., & Mir, N. (2010). Survey of termite infested houses, indigenous building materials and construction techniques in Pakistan. *Pakistan Journal of Zoology*, *42*(6).

Manzoor, F., Sayyed, A. H., Rafique, T., & Malik, S. A. (2012). Toxicity and repellency of different insecticides against Heterotermes indicola (Isoptera: Rhinotermitidae). *J. Anim. Plant Sci*, *22*(1), 65-71.

Mauldin, J. K., FL, C., & NM, R. (1981). Protozoan populations of Reticulitermes flavipes (Kollar) exposed to heartwood blocks of 21 American species.

Misbah-ul-Haq, M., Khan, I. A., Farid, A., & Ullah, M. (2015). Dose response relationship of subterranean termite, Heterotermes indicola (Wasmann) and two insect growth regulators, hexaflumuron and lufenuron. *Journal of Entomology and Zoology Studies*, *3*(4), 86-90.

Mitchell, J. D. (2002). Termites as pests of crops, forestry, rangeland and structures in southern Africa and their control. *Sociobiology*, *40*(1), 47-69.

Morimoto, M., Fukumoto, H., Hiratani, M., Chavasiri, W., & Komai, K. (2006). Insect antifeedants, pterocarpans and pterocarpol, in heartwood of Pterocarpus macrocarpus Kruz. *Bioscience, biotechnology, and biochemistry*, *70*(8), 1864-1868.

Mugerwa, S. (2015). Magnitude of the termite problem and its potential anthropogenic causes in N akasongola district of U ganda. *Grassland science*, *61*(2), 75-82.

Negassa, W., & Sileshi, G. W. (2018). Integrated soil fertility management reduces termite damage to crops on degraded soils in western Ethiopia. *Agriculture, ecosystems & environment*, *251*, 124-131.

Neil, A. S., Kamble, S. T., Warriner, R. A., & Davis, R. W. (2008). Influence of temperature on rate of uptake and subsequent horizontal transfer of fipronil by eastern subterranean termites (Isoptera: Rhinotermitidae). *J. Econ. Entomol*, *101*(3), 902-908.

Ohkuma, M. (2008). Symbioses of flagellates and prokaryotes in the gut of lower termites. *Trends in microbiology*, *16*(7), 345-352.

Ohmura, W., Doi, S., Aoyama, M., & Ohara, S. (2000). Antifeedant activity of flavonoids and related compounds against the subterranean termite Coptotermes formosanus Shiraki. *Journal of Wood Science*, *46*, 149-153.

Ordonez, A. A. L., Gomez, J. D., & Vattuone, M. A. (2006). Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. *Food chemistry*, *97*(3), 452-458.

Osbrink, W. L., & Lax, A. R. (2002). Effect of tolerance to insecticides on substrate penetration by Formosan subterranean termites (Isoptera: Rhinotermitidae). *Journal of Economic Entomology*, *95*(5), 989-1000.

Pejin, B., Ciric, A., Glamoclija, J., Nikolic, M., & Sokovic, M. (2015). In vitro anti-quorum sensing activity of phytol. *Natural product research*, *29*(4), 374-377.

Pejin, B., Kojic, V., & Bogdanovic, G. (2014). An insight into the cytotoxic activity of phytol at in vitro conditions. *Natural product research*, *28*(22), 2053-2056.

Pejin, B., Savic, A., Sokovic, M., Glamoclija, J., Ciric, A., Nikolic, M., ... & Mojovic, M. (2014). Further in vitro evaluation of antiradical and antimicrobial activities of phytol. *Natural Product Research*, *28*(6), 372-376.

Pinniger, D. B. (2012). *Managing pests in paper-based collections*. Preservation Advisory Centre, The British Library.

Poulsen, M., Hu, H., Li, C., Chen, Z., Xu, L., Otani, S., ... & Zhang, G. (2014). Complementary symbiont contributions to plant decomposition in a fungus-farming termite. *Proceedings of the National Academy of Sciences*, *111*(40), 14500-14505.

Prabuseenivasan, S., Jayakumar, M., & Ignacimuthu, S. (2006). In vitro antibacterial activity of some plant essential oils. *BMC complementary and alternative medicine*, *6*(1), 1-8.

Proteau, P. J. (1998). Biosynthesis of phytol in the cyanobacterium Synechocystis sp. UTEX 2470: utilization of the non-mevalonate pathway. *Journal of natural products*, *61*(6), 841-843.

Qasim, M., Lin, Y., Fang, D., & Wang, L. (2015). Termites and microbial biological control strategies. *South Asia Journal of Multidisciplinary Studies*, *1*(6).

Ragon, K. W., Nicholas, D. D., & Schultz, T. P. (2008). Termite-resistant heartwood: the effect of the non-biocidal antioxidant properties of the extractives (Isoptera: Rhinotermitidae). *Sociobiology*, *52*(1), 47-54.

Ragon, K. W., Nicholas, D. D., & Schultz, T. P. (2008). Termite-resistant heartwood: the effect of the non-biocidal antioxidant properties of the extractives (Isoptera: Rhinotermitidae). *Sociobiology*, *52*(1), 47-54.

Raje, K. R., Hughes, G. P., Gondhalekar, A. D., Ginzel, M. D., & Scharf, M. E. (2015). Toxicity of turmeric extracts to the termite Reticulitermes flavipes (Blattodea: Rhinotermitidae). *Journal of economic entomology*, *108*(4), 1479-1485.

Rao, A. N., Samatha, C., & Sammaiah, C. (2012). Bio-diversity of termites in Bhadrachalam forest region, Khammam District, Andhra Pradesh. *Journal of Biodiversity*, *3*(1), 55-59.

Rao, M. R., Singh, M. P., & Day, R. (2000). Insect pest problems in tropical agroforestry systems: Contributory factors and strategies for management. *Agroforestry Systems*, *50*, 243-277.

Rasib, K. Z., Ashraf, H., & Afzal, M. (2014). Feeding preferences of Odontotermes obesus (Rambur)(Isoptera: Termitidae) on different commercial and non-commercial woods from Lahore, Pakistan, under laboratory and field conditions. *Zoology and Ecology*, *24*(4), 369-379.

Ripa, R., Luppichini, P., Su, N. Y., & Rust, M. K. (2007). Field evaluation of potential control strategies against the invasive eastern subterranean termite (Isoptera: Rhinotermitidae) in Chile. *Journal of Economic Entomology*, *100*(4), 1391-1399.

Rontani, J. F., & Volkman, J. K. (2003). Phytol degradation products as biogeochemical tracers in aquatic environments. *Organic Geochemistry*, *34*(1), 1-35.

Rust, M. K., & Saran, R. K. (2006). Toxicity, repellency, and transfer of chlorfenapyr against western subterranean termites (Isoptera: Rhinotermitidae). *Journal of Economic Entomology*, *99*(3), 864-872.

Salihah, Z., Sattar, A., & Khatoon, R. (1994). Detection of nesting system of termites, Heterotermes indicola at NIFA campus. *NIFA Ann. Rep*, *94*, 96-104.

Saljoqi, A. U. R., Khan, M. A., Sattar, A., Ullah, M., & Khan, F. (2012). Behavioral changes of Heterotermes indicola (Isoptera: Rhinotermitidae) against some natural products. *Pakistan Journal of Zoology*, *44*(6).

Saljoqi, A. U. R., Khan, M. A., Sattar, A., Ullah, M., & Khan, F. (2012). Behavioral changes of Heterotermes indicola (Isoptera: Rhinotermitidae) against some natural products. *Pakistan Journal of Zoology*, *44*(6).

SALJOQI, A. U. R., MUHAMMAD, N., KHAN, I. A., NADEEM, M., & SALIM, M. (2014). Effect of different insecticides against termites, Heterotermes indicola L.(Isoptera: Termitidae) as slow acting toxicants. *Sarhad Journal of Agriculture*, *30*(3).

Samarth, R. M., & Krishna, V. (2007). Evaluation of radical scavenging activity of certain plant extracts using cell free assays. *Pharmacologyonline*, *1*, 125-137.

Santos, A., Santos, J. C., Cardoso, J. R., Serrão, J. E., Zanuncio, J. C., & Zanetti, R. (2016). Sampling of subterranean termites Syntermes spp.(Isoptera: Termitidae) in a eucalyptus plantation using point process and geostatistics. *Precision Agriculture*, *17*, 421-433.

Saran, R. K., & Rust, M. K. (2014). Toxicity, uptake, and transfer efficiency of fipronil in western subterranean termite (Isoptera: Rhinotermitidae). *Journal of economic entomology*, *100*(2), 495-508.

Sattar, A., Salihah, Z., Farid, A., & Traniello, J. F. A. (2002). Determining different parameters of Co-60 bait as a radiotracer for three species of subterranean termites. *Nucleus (Islamabad)*, *39*(1-2), 89-94.

Scheffrahn, R. H. (2008). Termites (Isoptera) Ed. John L. Capinera. Encyclopedia of Entomology.

Shelton, T. G., & Grace, J. K. (2003). Effects of exposure duration on transfer of nonrepellent termiticides among workers of Coptotermes formosanus Shiraki (Isoptera: Rhinotermitidae). *Journal of Economic Entomology*, *96*(2), 456-460.

Sina, I., Simbolon, S., Sutoro, M., Sugiyarto, S., & Ahmad, A. (2021, January). Assessments: toxicity efficacy of Tinospora Crispa (family: Menispermaceae) against Macrotermes gilvus (family: Termitidae) in oil palm plantation. In *Proceedings of the 1st International Conference on Economics Engineering and Social Science, InCEESS 2020, 17-18 July, Bekasi, Indonesia*.

Skolmen, R. G. (1974). *Natural Durability of Some Woods Used in Hawaii... Results of 9 1/2 Years' Exposure* (Vol. 292). Pacific Southwest Forest and Range Experiment Station.

Smith, D. M., Jackson, N. A., Roberts, J. M., & Ong, C. K. (1998). Root quantity, activity and below-ground competition in Grevillea robusta agroforestry systems. *Final report to the Forestry Research Programme, DFID, Institute of Hydrology report IH*, *98*(7), 92.

Smith, J. L., & Rust, M. K. (1990). Tunneling response and mortality of the western subterranean termite (Isoptera: Rhinotermitidae) to soil treated with termiticides. *Journal of Economic Entomology*, *83*(4), 1395-1401.

Sotannde, O. A., Yager, G. O., Zira, B. D., & Usman, A. (2011). Termiticidal effect of neem extracts on the wood of Khaya senegalensis. *Research Journal of Forestry*, *5*(3), 128-138.

Sroka, Z., & Cisowski, W. H. P. S. (2003). Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. *Food and Chemical Toxicology*, *41*(6), 753-758.

Stingl, U., Maass, A., Radek, R., & Brune, A. (2004). Symbionts of the gut flagellate Staurojoenina sp. from Neotermes cubanus represent a novel, termite-associated lineage of Bacteroidales: description of 'Candidatus Vestibaculum illigatum'. *Microbiology*, *150*(7), 2229-2235.

Stingl, U., Radek, R., Yang, H., & Brune, A. (2005). "Endomicrobia": cytoplasmic symbionts of termite gut protozoa form a separate phylum of prokaryotes. *Applied and Environmental Microbiology*, *71*(3), 1473-1479.

Su, N. Y., & Scheffrahn, R. H. (2000). Termites: Evolution, Sociality, Symbioses, Ecology. Chapter 20. Pg 437-453. *Termites as Pests of Buildings Editor: Bignell, DE Dordrecht, Netherlands: Kluwer Academic Publishers. 466pp*.

Su, N. Y., Chew, V., Wheeler, G. S., & Scheffrahn, R. H. (1997). Comparison of tunneling responses into insecticide-treated soil by field populations and laboratory groups of subterranean termites (Isoptera: Rhinotermitidae). *Journal of economic entomology*, *90*(2), 503-509.

Su, N. Y., Scheffrahn, R. H., & Ban, P. M. (1993). Barrier efficacy of pyrethroid and organophosphate formulations against subterranean termites (Isoptera: Rhinotermitidae). *Journal of Economic Entomology*, *86*(3), 772-776.

Takahashi, M., Fuchino, H., Satake, M., Agatsuma, Y., & Sekita, S. (2004). In vitro screening of leishmanicidal activity in Myanmar timber extracts. *Biological and Pharmaceutical Bulletin*, *27*(6), 921-925.

Tasaki, E., Kobayashi, K., Matsuura, K., & Iuchi, Y. (2017). An efficient antioxidant system in a long-lived termite queen. *PLoS One*, *12*(1), e0167412.

Tekam, U. S. (2023). Review on Toxic and Repellent Properties of Plant Extracts Against Termite. *Scientific Approach for Self Reliant India*, 78.

Tsunoda, K. (2003). Economic importance of Formosan termite and control practices in Japan (Isoptera: Rhinotermitidae). *Sociobiology*.

Ullah, M. S., Sikder, M. A. A., Sharmin, T., & Rashid, M. A. (2014). Pharmacological activities of Grevillea robusta, a medicinal plant of Bangladesh. *Bangladesh Pharmaceutical Journal*, *17*(2), 135-137.

Van Den Brink, D. M., & Wanders, R. J. A. (2006). Phytanic acid: production from phytol, its breakdown and role in human disease. *Cellular and Molecular Life Sciences*, *63*, 1752-1765.

Van Huis, A. (2017). Cultural significance of termites in sub-Saharan Africa. *Journal of Ethnobiology and Ethnomedicine*, *13*(1), 1-12.

Vasconcellos, A., & Policarpo, I. S. (2015). Edible and medicinal termites: a global overview. *Journal of Ethnobiology and Ethnomedicine*, *11*, 29-29.

Waqas, W., Muhammad, A., Farhat, N., Muhammad, I., Muhammad, Y., & Muhammad, T. (2011). Evaluation of Grevillea robusta, diatomaceous earth and K-Obiol for the control of Mung Dhora (Callosobruchus chinensis L.)(Insecta: Coleoptera: Bruchidae). *Pakistan Entomologist*, *33*(2), 105-111.

Watanabe, D., Gotoh, H., Miura, T., & Maekawa, K. (2014). Social interactions affecting caste development through physiological actions in termites. *Frontiers in physiology*, *5*, 127.

Yamashita-Higuchi, Y., Sugimoto, S., Matsunami, K., Otsuka, H., & Nakai, T. (2014). Grevillosides J–Q, arbutin derivatives from the leaves of Grevillea robusta and their melanogenesis inhibitory activity. *Chemical and Pharmaceutical Bulletin*, *62*(4), 364-372.

Yeoh, B. H., & Lee, C. Y. (2007). Tunneling responses of the Asian subterranean termite, Coptotermes gestroi in termiticide-treated sand (Isoptera: Rhinotermitidae). *Sociobiology*, *50*(2), 457-468.

Yii, J. E., Bong, C. F. J., King, J. H. P., & Kadir, J. (2015). Synergism of entomopathogenic fungus, Metarhizium anisopliae incorporated with fipronil against oil palm pest subterranean termite, Coptotermes curvignathus. *Plant Protection Science*, *52*(1), 35-44.

Zubair, A. R., Shahzad, M. A., & Saleem, A. (2007). Efficacy of insecticides against sugarcane termites. *J. Agric. Res*, *45*(3), 215-219.

termite

ORIGINALITY REPORT

17%	15%	14%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	prr.hec.gov.pk Internet Source	6%
2	onlinelibrary.wiley.com Internet Source	1%
3	Submitted to Higher Education Commission Pakistan Student Paper	1%
4	Muhammad Afzal, Muhammad Farman, Khalid Zamir Rasib, Naveeda Akhtar Qureshi. "Biocidal action of silver oak (Grevillea robusta) leaf extract on the termite Heterotermes indicola Wasmann (Blattodea: Rhinotermitidae)", International Biodeterioration & Biodegradation, 2019 Publication	1%
5	hdl.handle.net Internet Source	1%
6	academic.oup.com Internet Source	1%
7	www.thejaps.org.pk	